Deep-sea field experiments on the biological impacts of direct deep-sea CO_2 injection

J.P. Barry, B.A. Seibel, J.C. Drazen, M.N. Tamburri, K.R. Buck, C. Lovera, L. Kuhnz, E.T. Peltzer, K. Osborn, P.J. Whaling, P. Walz, P.G. Brewer

Monterey Bay Aquarium Research Institute, 7700 Sandholdt Road, Moss Landing, CA 95039

ABSTRACT

Direct injection of CO_2 into the ocean, a radical idea suggested 25 years ago (Marchetti 1977), is among several carbon sequestration alternatives under consideration to offset the accelerating rise in anthropogenic greenhouse gases (Reichle et al 1999, Brewer et al. 1999). This issue raises important questions concerning the impacts of pH changes and elevated CO_2 levels for marine ecosystems and the role, if any, ocean sequestration should play in a national or global carbon management strategy. While there is uncertainty concerning physical responses to greenhouse gas forcing (Caldeira et al 2003), there is no doubt that oceanic CO_2 levels have risen significantly (Keeling and Whorf 2002, Barnola et al. 2003) and will continue to do so (Marland et al. 2001). Roughly $1/3^{rd}$ of current fossil fuel CO_2 emissions (~7 $GtCO_2y^{-1}$) enter the sea surface through air-sea exchange (Houghton et al. 1990, McNeil et al 2003), thereby acidifying the upper ocean (Sabine et al. 2002). Continued acidification by air/sea CO_2 exchange (Haugan and Drange 1992) or direct ocean CO_2 sequestration (Drange et al. 2001, Harvey 2003) will challenge the physiological tolerances of species inhabiting both shallow (Kleypas et al. 1999, Knowlton 2001) and deep (Tamburri et al. 2000, Seibel and Walsh 2003) marine ecosystems. Here we present the initial results of *in situ* deep-sea CO_2 release experiments off Central California, showing that various deep-sea taxa are sensitive to short-term (~ 1 mo.) exposure to CO_2-rich, low pH plumes emanating from deep-sea CO_2 pools.

INTRODUCTION

Warming of 0.75 ^{o}C over the Earth during the last century (Mann et al. 1999) has been accompanied by broad changes in marine and terrestrial ecosystems (Parmesan and Yohe 2003, Root et al. 2003). In this century, however, Earth's climate is expected to warm more rapidly; global CO_2 emissions are expected to increase from present rates near 7 GTCy-1 to 15 GtCy-1 by 2050 (Marland et al. 2001). Simultaneously, acidification of the surface ocean (-0.3 pH units by 2100; Haugan and Drange 1992) may place coral reefs and other shallow marine ecosystems in peril (Kleypas et al 1999, Knowlton 2001). Ocean sequestration would reduce atmospheric emissions, but would add to the accumulating burden of fossil fuel CO_2 in the ocean. And while "dangerous anthropogenic interference" with climate has been debated widely, no such debate has taken place over acceptable oceanic CO_2 levels. Thus, although direct deep-sea CO_2 injection is technically feasible (IPCC 2001), the environmental consequences of large-scale CO_2 sequestration remain unknown and may be substantial (Seibel and Walsh 2003).

Immersion in CO_2-laden, acidic seawater from CO_2 injection poses physiological challenges to marine animals that respond by tolerance, compensation, or death. Responses are based on physiological repertoires that have evolved over thousands of generations to tolerate the range of natural environmental variability encountered. Animals that have evolved in highly stable conditions typical of deep-ocean waters are, in general, more sensitive to a variety of environmental perturbations than shallow-water animals, including those associated with CO_2 injection (Seibel and Walsh 2003). The main CO_2-related stresses can include acidosis of intra- and extra-cellular fluids, requiring pH compensation and inducing respiratory stress, and metabolic suppression, associated with hypercapnia (Pörtner and Reipschläger 1996). Changes in ocean pH caused by direct sequestration or air/sea exchange that fall within the range of normal environmental variation are expected to be less stressful than more extreme perturbations. Over the world ocean, seawater pH varies today from ~7.3 to ~8.5, (www.nodc.noaa.gov), and differs among ocean basins. pH varies most in the upper ocean; the mean (SD) pH in depths <1000 m for the Atlantic and North Pacific Oceans are 8.2 (0.15) and 7.9 (0.22), respectively, representing variation of 0.6 and 0.9 pH units). Deep-sea environments are less variable; pH between 3000-4000 m for these areas is

8.0 (0.02) and 7.8 (0.05), variation of 0.1 and 0.2 pH units. Individuals and populations are likely to experience even less natural pH variability.

We evaluated the biological impacts of direct CO_2 injection on deep-sea animals *in situ* during two experiments (E1, E2) exposing deep-sea animals to the dissolution plume from pools of liquid CO_2 released into PVC "corrals" on the seafloor at 3600 m depth off California (Fig. 1). Our experiments were designed to investigate the potential effects of direct ocean CO_2 sequestration and develop deep-sea experimental techniques for controlled ecosystem CO_2 enrichment (e.g. DeLucia et al. 1999). Liquid CO_2 is heavier than seawater at this depth, but dissolves slowly, producing a CO_2-rich, low-pH dissolution plume. We measured the survival of various groups of deep-sea organisms exposed to these plumes. Creation of a dissolving pool of CO_2 on the seafloor[0], selected here because it is experimentally tractable, is only one of many variants of proposed ocean CO_2 injection strategies (Haugan and Drange 1992, Drange et al 2001, Caldeira and Rau 2000).

METHODS

An ROV-mounted CO_2-release system (Brewer et al. 1999) developed by the Monterey Bay Aquarium Research Institute was used to inject liquid CO_2 into PVC corrals placed on the seafloor on the continental rise in 3600 m depth, 85 nm off Moss Landing, CA (36° 42' 33.4" N, 123° 31' 22.0"W). The CO_2 persisted in liquid form, with a hydrate skin, throughout the study. We did not observe large volume changes from massive hydrate formation (Brewer et al. 1999).

In the first experiment (E1), 3 small (48 cm diameter x 15 cm high) PVC corrals were filled with ~twenty liters of liquid CO_2 (Fig. 1), and study animals were held in mesh cages (46 x 46 x 20 cm) placed nearby (<1m). The survival rates of megafauna held in cages and organisms inhabiting sediments adjacent to CO_2 corrals were compared with control groups near three empty corrals. Several individuals each of urchins (*Cystechinus* sp.) and holothurians (*Abyssocucumis* sp.) were collected from the seafloor nearby using a suction sampler and placed carefully in each mesh cage adjacent to CO_2 corrals (n=3) and control corrals (n=3). Sediment cores (7.5 cm diameter x 20 cm deep) were collected to obtain mud samples for microbial, meiofaunal, and macrofaunal counts and analyses (n=6 per corral). Sets of sediment cores were collected prior to dispensing the CO_2 and after 35d exposure. Macrofaunal samples were sieved (300 μm). Meiofaunal analyses were based on percol-gradient centrifugation technique. Microbial counts performed under epifluorescence microscopic inspection of DAPI-stained samples. Abundance, biovolume, or indices of mortality (e.g. tissue degradation) were compared among treatments at the beginning and end of the experiment.

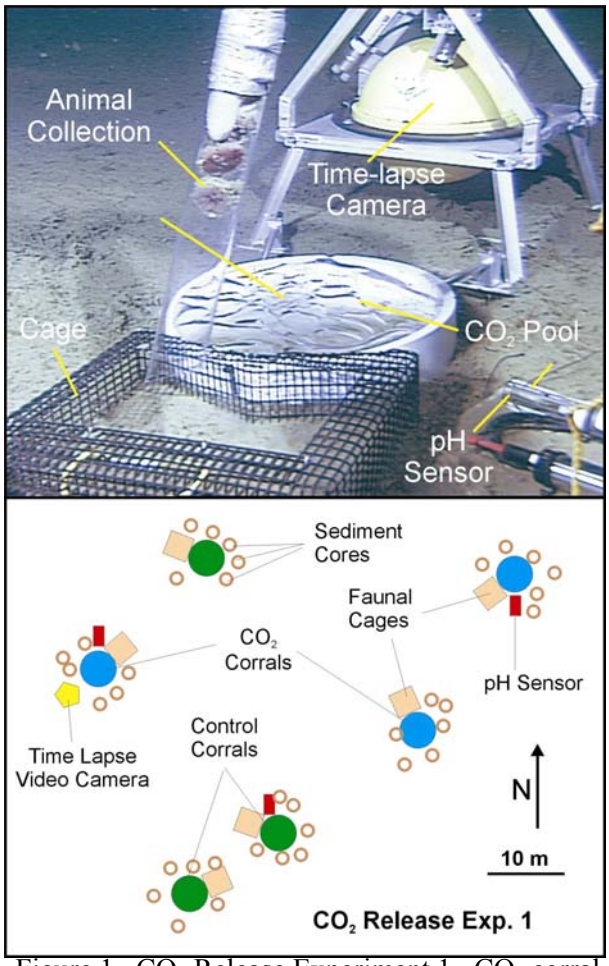

Figure 1. CO_2 Release Experiment 1. CO_2 corral filled with liquid CO_2, animal cage used to hold megafauna, pH sensor, and time-lapse video camera used in E1 (top). Sea urchins and holothurians are visible in acrylic tube, used as suction sampler to collect and deploy megafauna. Experimental layout of CO_2 release E1 at 3600 m depth shown in bottom image.

A second experiment (E2) was similar, but used a single, larger (93 cm diameter x 30 cm high) corral containing ~75 l of liquid CO_2. Study organisms included urchins and infaunal organisms used in E1, and common fishes (eelpout, *Pachycara sp.*; rattail, *Coryphaenoides armatus*). Fishes were collected in baited traps prior to CO_2 release. Fish traps and urchin cages were positioned 1, 5, 10, and 50 m from the central CO_2 pool. Infaunal organisms were sampled from sediment cores at these distances. CO_2 in

experimental corrals was replenished after ~2 weeks in each experiment to ensure continued CO_2 dissolution. Both experiments were terminated after ~1 month.

The intensity of plume exposure was estimated from pH sensors positioned 3-5 cm above the seafloor and located 1 m (E1), 5 m, and 50 m (E2) from CO_2 corrals. Time-series records of pH were obtained from 1 m away from a CO_2 pool during E1, and from 5 m, and 50 m from the central CO_2 corral during E2.

The direction and speed of near-bottom currents at the site were measured using an acoustic doppler current meter deployed 2 m above the bottom during E1. Currents 15 m above the bottom were used for analyses. Current records were not obtained during E2.

CO_2 corrals used in E1 were 15 cm high, and filled completely with liquid CO_2, leading to fairly rapid dissolution, likely due to the direct exposure of the CO_2 surface to near bottom currents. During E2, a single larger PVC corral (33 cm high x 94 cm diameter) was filled only 2/3 full, partially insulating the CO_2 surface from bottom currents. This appears to have resulted in a slower dissolution rate and perhaps smaller pH excursions around the corral.

Because all animals collected from 3600 m depths died upon ascent to the surface, mortality caused by CO_2 exposure was distinguished from death during ascent by assessing amphipod tissue condition. A rating system from 1 (intact, "recent death") to 5 (nearly entirely degraded, tissues translucent to transparent, exoskeleton fragile) was used for tissue condition. Ratings of $\geq$4 had been dead for $\geq$2 weeks, based on comparisons with tissue degradation rates of amphipods measured at the site in separate assays. Mortality (% individuals dead) was calculated as the percentage of all individuals with tissue ratings of $\geq$4.

Natural variation in ocean pH was determined from inspection of pH measurements throughout the world ocean available from the National Ocean Data Center (http://www.nodc.noaa.gov/). Estimates of pH changes in the deep-sea caused by 100 y of CO_2 sequestration using injection rates of 0.25 and 4 gtCy-1 were calculated assuming no outgassing of injected CO_2 from the volume of the bottom 1 km of the ocean (~3.6 x 108 km^{-3}), alkalinity = 2400 $\mu m \cdot kg^{-1}$, depth = 3500 m, T = 1.5 ^{o}C, initial ΣCO_2 = 2350 $\mu m \cdot kg^{-1}$, and ending ΣCO_2 = 2356 and 2443 $\mu m \cdot kg^{-1}$, respectively.

RESULTS

In the first experiment (E1) changes in seawater pH around corrals were highly variable owing to changes in current direction with the tides, leading to large peak pH perturbations (ΔpH ~ –1.5 units were observed within 1 m of the CO_2 corrals) during periods when currents were flowing over pH sensors, and little or no pH change when currents carried the CO_2 dissolution plume away from pH sensors. Excursions in pH greater than 1 unit were rare (<5% of the time) even near CO_2 pools, and reductions of $\geq$ –0.2 units occurred only 25% of the time.

In the second experiment (E2) maximum pH shifts recorded 5 and 50 m from the CO_2 pool showed moderate (-0.2 pH units) to minor (-0.05 units) peak pH changes, and small average pH changes (-0.008, -0.003 units), respectively. Shifts of $\geq$ -0.2 units were recorded less than 2% of the time 5 m from the CO_2 pool. Unfortunately, the pH sensor adjacent (1 m) to the central CO_2 corral failed during E2.

Due to the rotary character of inertial and tidal currents at the site, pH perturbations were cyclical, exposing organisms to elevated CO_2 levels during short periods when they were in the path of the dissolution plume. Adoption of more complex experimental techniques analogous to terrestrial ecosystem studies (DeLucia et al. 1999) may be necessary to create steady pH fields. Near-bottom currents during E1 averaged 4.4 $cm \cdot s^{-1}$, with net transport to the SE at 1.7 $cm \cdot s^{-1}$. Fourier analysis of currents and variation in pH 1m (E1) and 5 m (E2) from CO_2 corrals all indicated strong periodicity near 12.4 h, associated with the major semidiurnal lunar tidal constituent (M2). In effect, organisms 1m from CO_2 pools (E1) were bathed in CO_2-rich waters (ΔpH –1.0 or greater) for ~30 minutes, twice per day. The CO_2 plume was an order of magnitude weaker at 5 m during E2, where pH shifts of $\geq$0.1 unit persisted for ~15 minutes, twice per day.

Most organisms were sensitive to large pH changes in CO_2 dissolution plumes very near CO_2 pools. Urchins and holothurians mortality near (<1m) CO_2 corrals was high during exposure and dissolution of skeletal elements was observed in several urchins. Urchins in control cages appeared unharmed, and all holothurians in control cages were absent, and presumably escaped. Survival of the amphipod, *Haploops lodo*, was low after intense CO_2 exposure during E1. Its abundance and tissue

TABLE 1. Summary of CO_2 Impacts

	1 m (E1)	1 m (E2)	5 m (E2)	10 m (E2)	50 m (E2)
Change in pH units: Max (mean)	-1.0 (-0.2)		-0.1 (-0.008)		-0.01 (-0.003)
Bacteria	-2 ns	0	0	0	0
Meiofauna					
Flagellates	64 **	65	33	23	0
Amoebae	67 *	68	34	24	0
Nematodes	63 **	0	0	0	0
Macrofauna					
Amphipod (*Haploops lodo*)	95 ***	15 *	2 ns	3 ns	3 ns
Epibenthic Megafauna					
Urchin – *Cystechinus* sp.	100 **	100	80	0	0
Holothurian – *Abyssocucumis* sp.	100 **				
Near-Bottom Deep-sea Fishes					
Zoarcid – *Pachychara* sp.		0	0		0
Macrourid - *Coryphaenoides armatus*		100	100		100

Table 1. Summary of faunal impacts during CO_2 release experiments. Changes in pH represent the maximum and (mean) perturbations to ambient pH levels during each experiment. Values for each taxon are percentage mortality estimates based on comparisons of CO_2 vs. Control treatments (E1) or initial vs. end samples (E2). CO_2 impacts were high for samples within areas of large pH shifts, and undetectable or non-significant for small pH shifts. Negative mortality listed for bacteria indicates an increase in cell counts. Failure of pH sensors prevented measurements of pH shifts near (1m) the CO_2 pool during E2. All faunal groups except bacteria exhibited high rates of mortality near CO_2 pools in E1. E1, E2 indicate Experiment 1, and 2, respectively. Blanks indicate no data. ns, *, **, *** indicate non-significant, $p<0.05$, $p<0.01$, $p<0.001$ for t-tests.

condition were initially similar among treatments, but differed greatly after one month, indicating high mortality rates near CO_2 pools, and very low mortality near control corrals. Sediment-dwelling meiofauna showed similar declines in population density or condition after exposure to intense CO_2 stress. The abundance of flagellates and amoebae were similar near CO_2 and control corrals before CO_2 injection, but declined near CO_2 pools by the end of the experiment. Reduced densities of both groups probably reflect the death and decay of individuals impacted by CO_2. Nematodes, the most prevalent meiofaunal taxon declined only slightly in biovolume near CO_2 pools, apparently due the slow degradation of their chitinous cuticle. Detailed inspection of individuals stained with DAPI using epifluorescence microscopy (indicating the presence / absence of intact cell nuclei), however, indicated that most nematodes near CO_2 had died compared to low mortality near control stations. Unexpectedly, cell counts of sediment bacteria were similar between CO_2 and control corrals, despite the large pH shift and mortality of other groups, and even increased during the study near CO_2 pools.

Faunal responses to the apparently milder CO_2 plume produced during the second experiment were less severe than observed during E1, and decreased at distances of 5 m or greater where pH shifts were very small (Table 1). Urchins held in cages within 1m of the central CO_2 pool died during E2, but no obvious skeletal degradation was observed. Most urchins 5 m from CO_2 also died after exposure to pH reductions of only 0.1 to 0.3 units for less than 2% of the time during E2 and an average pH shift of only –0.008. No CO_2 effects were detectable for urchins held in distant cages (10, 50 m) where pH changes were small ($\Delta pH =< -0.05$ units less than 1% of E2). The mortality rate for amphipods (*Haploops lodo*) near (1m) the CO_2 pool was much lower during E2 than measured in E1, but was greater than before CO_2 release (Table 1). Densities of the smallest meiofaunal groups (flagellates and amoebae) declined near the CO_2 pool, with detectable changes up to 10 m from the pool. Nematode mortality was low, however, suggesting that they were somewhat more tolerant to the milder pH changes during E2 than smaller taxa.

DISCUSSION

Our results support the expectation (Seibel and Walsh 2003) that deep-sea species may be sensitive to pH stress that will accompany a direct CO_2 injection sequestration program. CO_2-related physiological stress, if not lethal, will convey higher "costs of living" through the energetic costs of acid / base balance, restricted aerobic capacity, and inhibition of protein synthesis. These costs may be highest for deep-sea organisms, which typically have limited metabolic capacity. Physiological responses of individuals to increased CO_2 levels may translate into changes in the survival, growth, and reproduction rates of populations, and shifts in the ecosystem dynamics of deep-sea communities.

The scale of ecosystem impacts from a direct CO_2 sequestration program depends on the depths, locations, and certainly the volume of CO_2 injected. Since any CO_2 released will result in CO_2 dissolution plumes from pH ~4 in the boundary layer to background values, animals in close proximity to disposal sites are at risk. Plume effects over larger scale may be estimated coarsely from expected pH fields. For example, if 0.25 to 4 $GtCy^{-1}$ as CO_2 is injected for 100 y beneath 3000 m and disperses worldwide (see methods), the pH of the deep-waters of the entire world ocean will shift by –0.02 to –0.3 units. These levels are comparable to the pH changes observed ~5 m from our CO_2 pools, and overlap or exceed the present range of natural deep-ocean pH variability. Even larger pH perturbations will occur in mixing zones that may extend 10s to 100s of km around disposal sites (Haugan and Drange 1992, Caldeira and Wickett 2002).

Direct deep-sea CO_2 sequestration could partially mitigate the anthropogenic rise in atmospheric pCO_2 that will almost certainly accelerate through this century. Although fossil fuel conservation and alternative energy sources should be primary carbon management strategies, the decision to implement a direct ocean CO_2 sequestration program hinges on the balance between the lesser of two evils – the unabated effects of climate warming or acidification, or both, on terrestrial and shallow marine ecosystems, or damage to deep-sea ecosystems by CO_2 sequestration. Moreover, because most climate stabilization scenarios assume that CO_2 emissions will be balanced by removal, where the ocean is the largest CO_2 sink, it is likely that ocean pH will continue to decrease, with consequences that are currently not understood. Ongoing research should provide guidance concerning the risks of direct CO_2 injection, and may mandate other methods or more environmentally benign CO_2 sequestration approaches (e.g. accelerated carbonate dissolution; Caldeira and Rau 2000). Clearly, an ocean carbon sequestration program will be successful only if its intended benefits – a stabilization of atmospheric CO_2 and mitigation of climate warming consequences for terrestrial and shallow water ocean systems, outweigh its liabilities – energy expended on sequestration and damage to deep-sea ecosystems. Lacking presently is sufficient information on both sides of this balance.

REFERENCES

Barnola, J.-M., Raynaud, D., Lorius, C., & Barkov, N.I. Historical CO_2 record from the Vostok ice core. In *Trends: A Compendium of Data on Global Change*. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tenn., U.S.A (2003).

Brewer, P.G., Friederich, G., Peltzer, E.T., & Orr, F.M. Jr. Direct experiments on the ocean disposal of fossil fuel CO_2. *Science* 284, 943-945 (1999).

Caldeira, K. & Wickett, M.E. Comparing pH impacts of oceanic CO_2 injection and atmospheric CO_2 release. *Eos Trans. AGU,* 83(1), Spring Meet. Suppl., Abstract OS51F-01 (2002).

Caldeira, K., & Rau, G.H. Accelerating carbonate dissolution to sequester carbon dioxide in the ocean: Geochemical implications. *Geophys. Res. Let*. 27, 225-228 (2000).

Caldeira, K., Jain, A.K., & Hoffert, M.I. Climate sensitivity uncertainty and the need for energy without CO_2 emission. *Science*, 299, 2052-2054 (2003).

DeLucia, E. H., J.G. Hamilton, S.L. Naidu, R.B. Thomas, J.A. Andrews, A. Finzi, M. Lavine, R. Matalama, J.E. Mohan, G.R. Hendrey, and W.H. Schlesinger. (1999) Net primary production of a forest ecosystem with experimental CO_2 enrichment. *Science*, 284, 1177- 1179.

Drange, H., Alendal, G., & Johannessen, O.M. Ocean release of fossil fuel CO_2: A case study. *Geophys. Res. Lett*. 28, 2637-2640 (2001).

Harvey, L.D.D. Impact of deep-ocean carbon sequestration on atmosphereic CO_2 and on surface-water chemistry. *Geophys. Res. Lett.* 30, 1237-1240 (2003).

Haugan, P.M., & Drange, H. Sequestration of CO_2 in the deep ocean by shallow injection. *Nature* 357, 318-320 (1992).

Houghton, J.T. et al., *Climate Change: The IPCC Scientific assessment. Intergovernmental Panel on Climate Change*, Cambridge University Press (1990).

IPCC. In *Climate Change 2001: The Scientific Basis* (eds Houghton, J.T., et al., Cambridge University Press, Cambridge, 896 p, 2001).

Keeling, C.D. & Whorf, T.P. Atmospheric CO_2 records from sites in the SIO air sampling network. In *Trends: A Compendium of Data on Global Change*. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tenn., U.S.A. (2002)

Kleypas, J.A. *et al*., Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. *Science* 284, 118-120 (1999).

Knowlton, N. The future of coral reefs. *Proc. Nat. Acad. Sci.* 98, 5419-5425 (2001).

Mann, M.E., Bradley, R.S. & Hughes, M.K. Northern hemisphere temperatures during the past millennium: interferences, uncertainties, and limitations. *Geophys. Res. Lett*. 26, 759-762 (1999).

Marchetti, C. On geoengineering and the CO_2 problem. *Climate Change* 1, 59-69 (1977).

Marland, G., Boden, T.A., & Andres, R.J. Global, Regional, and National CO_2 Emissions. In *Trends: A Compendium of Data on Global Change*. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy (2001; http://cdiac.esd.ornl.gov/trends/trends.htm).

McNeil, B., Matear, R.J., Key, R.M., Bullister, J.L., & Sarmiento, J.L. Anthropogenic CO_2 uptake by the ocean based on the global chlorofluorocarbon data set. *Science* 299, 235-239.

Parmesan, C. & Yohe, G. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421, 37-42 (2003).

Pörtner, H.-O., Reipschläger, A. In, *Ocean Storage of CO_2. Environmental, Workshop 2: Environmental Impact* (eds Ormerod, B., Angel, M.) 57-81 (IEA Green house and Gas R & D Programme, Southampton Oceanography Centre, UK, 1996).

Reichle, D. et al. *Carbon Sequestration: Research and Development* (Office of Science, Office of Fossil Energy, U.S. Dept. of Energy, 1999).

Root, T.L., et al. Fingerprints of global warming on wild animals and plants. *Nature* 421, 57-60 (2003).

Sabine, C.L., et al. Distribution of anthropogenic CO_2 in the Pacific Ocean. *Global Biogeochemical Cycles* 16, 1083-1099 (2002).

Seibel, B.A. & Walsh, P.J. Biological impacts of deep-sea carbon dioxide injection inferred from indices of physiological performance. *J. Exp. Biol.* 206, 641-650 (2003).

Tamburri, M.N., Peltzer, E.T., Friederich, G.E., Aya, I., Yamane, K., & Brewer, P.G. A field study of the effects of CO_2 ocean disposal on mobile deep-sea animals. *Mar. Chem.* 72, 95-101 (2000).

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