

# Simulated sequestration of anthropogenic carbon dioxide at a deep-sea site: Effects on nematode abundance and biovolume

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## Abstract

One proposal for ameliorating global warming is to sequester large amounts of carbon dioxide in the deep ocean, but the environmental consequences of sequestration for sediment-dwelling animals are poorly known. In a previous publication, we reported that ~80% of benthic copepods were killed in an experimental release of CO<sub>2</sub> off northern California at 3262 m. The effects of this release on nematodes are reported here. We examined samples of nematodes taken inside two 'corrals' into which CO<sub>2</sub> was directly injected (providing an extreme endpoint for CO<sub>2</sub> exposure) and taken near to and far from this CO<sub>2</sub> source. After 30 days, pore-water pH was unchanged (~7.8) at the sediment–water interface far (~40 m) from corrals, but pH profiles were reduced by ~0.75 near (~2 m) corrals. Corral pH was highly acidic (5.4 in a measurement from a subsequent experiment). Fifty randomly selected nematodes from each of four vertical layers from the 14 cores were photographed. They were assigned to a tail group (based on morphology), and individual biovolume was estimated from measurements of body length and width. Although nematode abundance (expressed as total nematodes and by tail group) was not affected, length, width, and individual biovolume significantly differed between near and far samples. Median nematode biovolume examined across tail group and core layer increased by ~48% inside and near corrals. Differences between near and corral samples were always less than differences between near and far samples. However, nematode length:width ratio did not differ between near and far, and the shapes of length, width, and biovolume frequency distributions were similar in all samples. We postulate that the nematode community throughout the upper 3 cm suffered a high rate of mortality after exposure to CO<sub>2</sub>, and that nematodes were larger because *postmortem* expansions in body length and width occurred. Decomposition rates were probably low and corpses did not disintegrate in 30 days. The observable effects of a reduction in pH to about 7.0 after 30 days were as great as an extreme pH reduction (5.4), suggesting that 'moderate' CO<sub>2</sub> exposure, compared to the range of exposures possible following CO<sub>2</sub> release, causes high mortality rates in the two most abundant sediment-dwelling metazoans (nematodes and copepods).

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## 1. Introduction

Since the beginning of the industrial revolution, the concentration of carbon dioxide in the atmosphere has increased from 275 to 370 ppm (Hoffert et al., 2002) largely as a consequence of the burning of fossil fuel (Keeling and Whorf, 1998). This rate of increase is unprecedented in the past 400,000 years and is a potential contributor to global warming (Reichle et al., 1990). Given that most fossil fuel CO<sub>2</sub> will eventually diffuse into the ocean, one proposal to reduce atmospheric concentration is to collect it at point sources (e.g. power plants) and inject it directly into the deep ocean, where it would remain for hundreds of years (Marchetti, 1977; Ormerod et al., 2002). Sequestration of CO<sub>2</sub> by direct injection in the ocean in amounts that would contribute meaningfully to decreasing the rate of global warming appears to be possible (IPCC, 2001), but before this approach is adopted the environmental consequences must be well understood.

CO<sub>2</sub> can cause direct impairment of animal physiological function, for example, by respiratory distress (Tamburri et al., 2000). CO<sub>2</sub> can also affect organisms indirectly because increasing concentration decreases the pH of seawater. Resulting acidosis may cause a variety of severe physiological consequences, perhaps leading to death (Pörtner et al., 2004). Relatively little is known about the tolerance of CO<sub>2</sub> or lowered pH by deep-sea biota. Ordinarily, they experience a relatively constant physicochemical environment and are thought to be poorly adapted to environmental change (Shirayama, 1995; Shirayama, 1997; Seibel and Walsh, 2001; Seibel and Walsh, 2003); relevant research, however, is limited (Tamburri et al., 2000; Barry et al., 2004).

We previously reported on an experiment in which CO<sub>2</sub> was released on the seafloor. Carman et al. (2004) found that the abundances of several micro-metazoan taxa (including nematodes and benthic copepods) were not reduced at or near (2 m from) the source of CO<sub>2</sub>, compared to a distant (40 m) site, after 29 days. Thistle et al. (2005) noted that, in the same experiment, sediment pH was reduced and that significantly more dead copepods were collected near the CO<sub>2</sub> source. These observations were reconciled by assuming that copepod corpses exposed to CO<sub>2</sub>-rich water had not decayed away at the time of collection. The fate of nematodes exposed to CO<sub>2</sub> may or may not be

comparable but this fate has proven difficult to quantify from our samples. Microscopic examination revealed that nematodes exposed to CO<sub>2</sub> were not obviously or consistently damaged, and we did not have an independent method to calibrate a condition index as did Thistle et al. (2005). Previous research using a DNA stain showed that nematode mortality in a different CO<sub>2</sub> release experiment was ~90% in an area 0.5 m from a CO<sub>2</sub> source, although other release experiments did not find similarly high levels of nematode mortality (Barry et al., 2005). DNA stains of our specimens yielded inconsistent results (Carman, unpublished). We, therefore, examined alternative methods to elucidate the effects of CO<sub>2</sub> exposure on nematodes. The responses of nematodes and copepods may yield insight into CO<sub>2</sub> effects because, taken together, they more adequately represent a broad range of marine animals. Nematodes are typically tolerant of chemical contamination, while copepods are sensitive (Peterson et al., 1996). They have different physiologies regarding oxygen tolerance (Wetzel et al., 2001), and exposure potentials may differ as copepods have a relatively impermeable chitinous exoskeleton and nematodes a more permeable proteinaceous cuticle.

There are several ways in which CO<sub>2</sub> may possibly affect nematodes without causing a change in total abundance. For example, nematode recruitment after exposure to CO<sub>2</sub> could offset reductions in abundance caused by lethal effects. Such an effect could alter nematode species composition without a change in abundance. However, an analysis of nematode species composition would be demanding with the highly diverse and poorly known fauna typical of the deep sea. Therefore, we chose to use a simpler but informative functional-group approach, through the examination of tail morphology, to refine abundance data. Thistle et al. (1995) showed that nematode tail groups respond to environmental influences and that relative abundances of tail groups are as sensitive as feeding groups to environmental influences. We also examined nematode length, width, length:width ratio, and biovolume spectra because individual body size and shape have been found to be sensitive gauges of ecological impact (Vanaverbeke et al., 2003). For example, changes in nematode body size due to disturbance by sand removal (Vanaverbeke et al., 2003) and with changing food availability (Soltwedel et al., 1996; Soetaert et al., 2002), sediment type (Tita et al., 1999), and oxygen conditions have been

detected. Conversely, organic pollution (Duplisea and Hargrave, 1996) had no effect on nematode biomass spectra. Vanaverbeke et al. (2004a, b) found that sedimentation from a spring phytoplankton bloom influenced both nematode community composition and biomass spectra, suggesting similar sensitivities. Finally, laboratory fixation studies have shown that nematode body size after death may vary with the cause of death (Fagerholm, 1979) suggesting that body size may convey information regarding nematode morbidity. Therefore, we tested for CO<sub>2</sub> effects on nematode tail-group abundance and body size, shape, and biovolume spectra, and considered the potential for these measurements to more generally detect change in deep-sea nematode communities.

## 2. Methods

The study site for this experiment (Barry et al., 2005, CO<sub>2</sub>-4) was located in Monterey Canyon off Monterey, CA, USA (36° 22.8'N, 122° 40.7'W) at a depth of 3262 m (Carman et al., 2004). All sampling and experimental manipulations were conducted with the ROV *Tiburion*, operated from the R/V *Western Flyer*. In October 2002, approximately 20 l of liquid CO<sub>2</sub> was pumped into each of three 48 cm diameter by 15 cm tall PVC pipes that had been set into the seabed such that each extended ~10 cm into the water. The CO<sub>2</sub> slowly dissolved in these 'corrals', producing a CO<sub>2</sub>-rich, dense, low-pH dissolution plume that dispersed from the corrals and contaminated surrounding sediment. Corrals were positioned at the apexes of an equilateral triangle ~4 m on a side. Approximately 29 days later, we collected six 7 cm inner diameter cores from an area 1–2 m away from a corral and six additional cores from an area ~40 m away from the nearest corral. Single cores were also collected from within two of the corrals. Upon retrieval, cores from the corrals were sectioned and preserved as described below. Remaining cores were held at 4 °C until a pH profile of the overlying water and upper 8 mm of sediment was made with a Unisense microelectrode and a Knick Portamess 913 pH meter (data presented in Thistle et al., 2005). On a subsequent cruise (fall, 2003), a core from a CO<sub>2</sub> corral in the Monterey Canyon at 3600 m was collected for shipboard pH measurement. Corrals used in the 2003 experiment were identical to those used in 2002, and similar amounts of CO<sub>2</sub> were added with the same equipment and ROV (Barry

et al., 2005). The core was collected after about 30 days exposure to CO<sub>2</sub>, and a profile was measured with the same pH meter and technique as described above.

Overlying water from all cores was collected by aspiration and concentrated on a 32 µm sieve. After a 1.9 cm-diameter subcore was inserted, each core was extruded, and the sediment outside the subcore was sectioned at 0–5, 5–10, 10–20, and 20–30 mm intervals with a precision extruder (Fuller and Butman, 1988). Sediment was fixed in 4% formaldehyde solution, made up in 35‰ artificial seawater and buffered to neutrality with borax, and stained with Rose Bengal. In the laboratory, each core layer was extracted with LUDOX (following Somerfield and Warwick, 1996), rinsed through a 32-µm sieve, and nematodes from both the supernatant and pellet were enumerated with the use of a stereodissection microscope.

All nematodes from each sample were transferred to glycerin-filled, 15 mm diameter etched wells on microscope slides. Fifty nematodes were placed in each well, except the last well into which any remaining nematodes,  $n \leq 50$  ind, were placed. Cover slips (25 × 25 mm) were carefully placed over the wells and sealed with clear fingernail polish. Digital photographs were taken of a sub-sample of nematodes, a maximum of 50 if available, from each sample-depth combination at appropriate magnification ranging from 5–450 ×, on an Olympus BX50 microscope. An approximately equal fraction of the total number of nematodes was selected for photography from each microscope slide well. To select nematodes to photograph from a well, the slide was first placed on the microscope stage. At 20 × magnification and starting in the center of the left side of the well, the slide was then moved by stage manipulators slowly to the right side of the well. Each nematode in the field of view was photographed with a SPOT RT (model 7.2 Color Mosaic) camera using SPOT RT Software, v. 3.5. If on this pass the appropriate number of nematodes was not found, a second pass was made. This second pass started at the center of the top of the well and ended at the center of the bottom of the well. This pass was made slowly and carefully to ensure that duplicate photographs were not taken. All nematodes were photographed in samples containing fewer than 50 nematodes.

IPLab, v. 3.6 software was used as an aid to measure the length and width of each nematode. A hand-operated computer mouse was used to

transcribe the length (excluding filiform tails) and greatest width of each nematode, and, based on calibrated equivalency, measured values were generated and entered into a spreadsheet by software programming functions. Biovolume for each nematode was calculated following Feller and Warwick (1988) in which biovolume ( $\mu\text{L}$ ) = length (mm)  $\times$  width (mm)<sup>2</sup>  $\times$  530. In addition, each nematode was categorized into one of four tail morphology groups following Thistle et al. (1995). Tail groups were (1) rounded (blunt end), (2) clavate-conicocylindrical (initially conical but with an extension to the tip), (3) conical (pointed tip with a tail length less than 5 body widths) and (4) elongate (with a tail longer than five body widths).

Data were analyzed using SAS, v 9. Estimated abundance of each of the four tail morphology groups was calculated using tail-morphology-group frequencies from nematodes subsampled for photography. The proportion of the subsample classified as tail group 1, 2, 3, or 4 was multiplied by the observed total nematode abundance for that layer.

Abundant data were standardized to a count of  $\text{ind mm}^{-1}$  of core layer (i.e., 0–5, 5–10, 10–20, and 20–30 mm), weighted in the analysis by the number of millimeter represented by the core layer and expressed as  $\text{ind } 10\text{ cm}^{-2}$  for all layers. We applied a generalized linear mixed-model ANOVA with a Poisson error distribution for samples from areas near and far from corrals (samples from inside corrals were not included in any statistical tests because only two cores were available). In order to account for the non-independence of observations at each layer within a sediment core, covariance for the repeated measures on each core was modeled with an exponential semi-variogram. The Type 3 tests of fixed effects and least squares means were calculated with the SAS glimmix macro (<http://ftp.sas.com/techsup/download/stat/glimm800.html>). Multiple comparisons of least squares means were conducted using the Tukey–Kramer procedure. Analyses were performed for the effects of CO<sub>2</sub> treatment, core layer, and their interaction on nematode abundance of each tail group type (note that total nematode abundance from the 0–20 mm layers in the same cores was analyzed by Carman et al., 2004).

Nematode length, width, and biovolume estimates were  $\log_e(\ln)$  transformed. We employed a two-way factorial ANOVA design to test for the effects of proximity to corrals and core layer and their interaction on nematode length, width,

length:width ratio, and biovolume. Because not all tail groups were found in all treatment combinations, tail group was used as a co-variate in the analysis. Multiple comparisons of least squares means were conducted using the Tukey–Kramer procedure.

### 3. Results

#### 3.1. pH

pH at the sediment surface from samples taken far from corrals was equivalent to the expected background pH ( $\sim 7.78$ ) of this area of the deep sea and to measurements taken before CO<sub>2</sub> was released (Barry et al., 2005). pH values through overlying water and the upper 8 mm from cores taken near corrals were on average 0.75 pH units lower than from cores far from corrals (data presented in Thistle et al. 2005). The pH at the sediment surface measured from a single core from a CO<sub>2</sub> corral sampled in 2003 was 5.4.

#### 3.2. Abundance

The depth distribution of total nematodes (pooled across tail groups) was similar in corral, near, and far samples through 3 cm (Fig. 1). Lowest total nematode abundance was found in the 0–5 mm layer and highest abundance reached about 100 individuals  $10\text{ cm}^{-2}$  in the 10–20 mm layer, regardless of distance from corrals. Total nematode abundance (to a depth of 2 cm) from these samples was previously shown to be unaffected by CO<sub>2</sub> exposure (Carman et al., 2004).

Tail groups 1 (rounded) and 2 (clavate-conicocylindrical) were relatively rare, and each comprised about 8% of nematodes photographed. Tail group 3 (conical) comprised about 22%, and tail group 4 (elongate) about 50% of all nematodes collected. Abundance ranged from 50  $\text{ind } 10\text{ cm}^{-2}$  in tail group 4 (elongate) to about 5  $\text{ind } 10\text{ cm}^{-2}$  in tail group 1 (rounded) (Fig. 1). Nematode abundance in each tail group was similar in far, near, and corral samples (Fig. 1), and all tail groups displayed their lowest abundance in the 0–5 mm core layer and highest abundance in the 10–20 mm core layer (data not shown). Separate analyses of the estimated abundance for each tail group only in samples near and far from corrals were conducted with two-way ANOVA (Table 1). Core layer significantly affected abundance of tail groups 1, 3, and 4. Proximity to

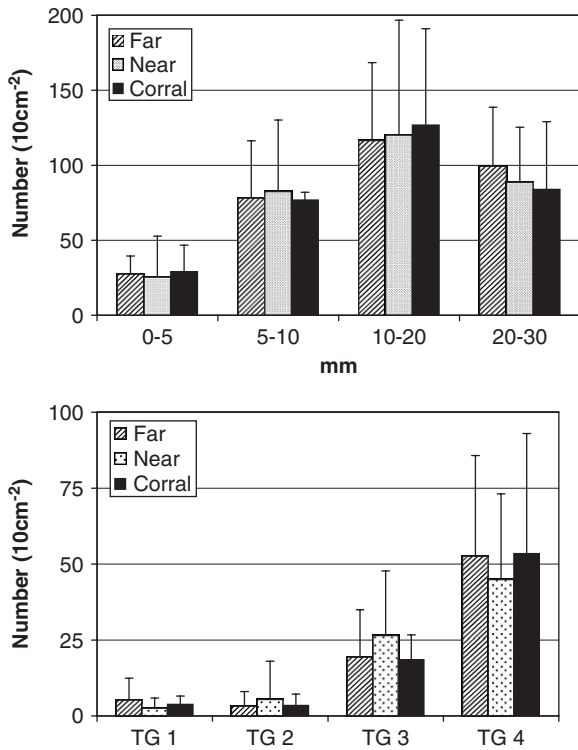


Fig. 1. Nematode abundance (ind 10 cm<sup>-2</sup>; error bars = 1 standard deviation) in samples from inside coralls (*n* = 2), near (*n* = 6) coralls, and far (*n* = 6) from coralls. Upper figure, mean abundance in each core layer, pooled across tail groups. Lower figure, mean abundance of each tail group, pooled across core layer.

Table 1  
Results (expressed as *p* values with values <0.05 in bold) of a two-way ANOVA testing the effect of proximity to coralls and core layer (and their interaction) on abundance of each nematode tail group

Effect	Distance from coralls (near vs. far)	Core layer	Distance*Core layer interaction
Tail group 1	0.3264	<b>0.0075</b>	0.5864
Tail group 2	1.0000	1.0000	1.0000
Tail group 3	0.1532	<b>0.0032</b>	0.6404
Tail group 4	0.2854	<b>0.0138</b>	0.7366

coralls did not influence abundance in any tail group, and all interaction terms were non-significant.

### 3.3. Nematode body length and width

A total of 2682 nematodes was measured. The range in length of nematodes was 172–3951 μm.

Median nematode length was similar in the uppermost core layers but increased with increasing sediment depth in the 10–20 and 20–30 mm layers (Table 2). Nematode length distributions pooled across core layer and tail group were bimodal in corral, near, and far samples (Fig. 2). In samples far from coralls, a larger peak occurred at about 500–600 μm, and a smaller peak occurred at 900–1000 μm. Frequencies gradually decreased with increasing size above 1000 μm; the distribution was thus skewed toward longer nematodes (Fig. 2). Length distributions of nematodes from inside and near coralls were shifted toward a longer shape compared to far nematodes. A peak in nematode length from samples inside and near coralls was also found at about 500–600 μm, but this peak was smaller than the peak from far samples. The second but larger peak in samples inside coralls was at about 800 μm. For nematodes collected near coralls, this peak was also higher and was shifted to the right of far samples. Median nematode length was 704.3 μm far from coralls, 826.1 μm near coralls and 796.2 μm inside coralls; a difference of 17% between near and far nematodes. Two-way ANOVA comparing only far- and near-corral samples was conducted using ln-transformed length measurements. Nematodes far from coralls differed significantly in length from nematodes near coralls (*p* = 0.0090). Core layer (*p* < 0.0001) also influenced

Table 2  
Nematode median length (μm ind<sup>-1</sup>), width (μm ind<sup>-1</sup>), length/width ratio and biovolume (μL ind<sup>-1</sup>), pooled across tail groups, in far, near, and corral samples by layers

	0–5 mm	5–10 mm	10–20 mm	20–30 mm
<b>Length</b>				
Far	676.3	623.3	663.2	859.8
Near	697.2	653.5	892.1	938.7
Corral	673.3	734.4	813.5	903.8
<b>Width</b>				
Far	24.3	23.3	22.8	26.3
Near	26.1	27.3	28.0	27.1
Corral	26.9	26.7	28.1	30.5
<b>Length:width ratio</b>				
Far	26.7	26.7	27.9	29.4
Near	26.2	23.2	28.2	31.1
Corral	26.4	25.3	26.2	28.7
<b>Biovolume</b>				
Far	0.213	0.178	0.177	0.313
Near	0.254	0.241	0.349	0.380
Corral	0.225	0.285	0.345	0.429

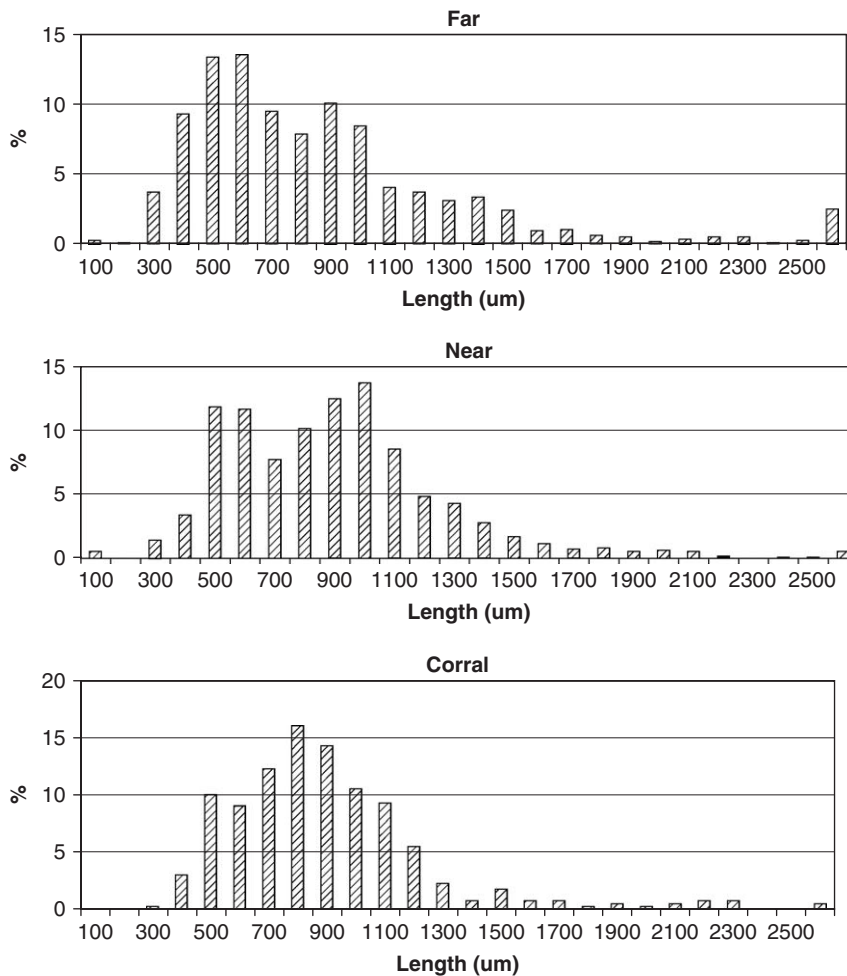


Fig. 2. Nematode length frequency distribution pooled across core layer and tail groups. Upper figure is from samples taken far from coralls, middle figure is from samples taken near to coralls, and lower figure is from samples taken inside coralls.

nematode length, but the interaction term was not significant ( $p = 0.0888$ ).

The range in nematode width was 6–147  $\mu\text{m}$ . Median nematode widths were generally similar in the uppermost core layers but increased in the 10–20 and 20–30 mm core layers (Table 2). In all samples, width distributions were unimodal with a peak at  $\sim 30 \mu\text{m}$  (Fig. 3). The frequency of size class widths  $> 30 \mu\text{m}$  gradually decreased. However, the frequency of nematodes in the range of widths from 40 to 60  $\mu\text{m}$  from near and inside coralls exceeded that in samples far from the  $\text{CO}_2$  source. When pooled across all tail groups and layers, the median width of nematodes collected far from coralls was 24.0 and 27.2  $\mu\text{m}$  near coralls, and 27.6  $\mu\text{m}$  inside coralls. This represents a 13% increase in the width of nematodes near and inside coralls compared to

far from coralls. Two-way ANOVA on ln-transformed nematode width (comparing only near and far samples) revealed that proximity to coralls ( $p = 0.0001$ ) and core layer ( $p < 0.0001$ ) both influenced width. Although the interaction term was significant ( $p = 0.0133$ ), increases in width near and inside coralls were generally similar and consistent in all tail groups and layers. For example, Tukey–Kramer tests show that near and far samples differed significantly in all layers ( $p = 0.0002$  in the 5–10 mm layer,  $p < 0.0001$  at the 10–20 mm layer and  $p = 0.0105$  at the 20–30 mm layer) except 0–5 mm ( $p = 0.8662$ ).

Soetaert et al. (2002) demonstrated that a large peak in the distribution of length:width ratio in deep-sea nematodes occurs at  $\sim 30 \times$  longer than wide, and that long and slender nematodes become

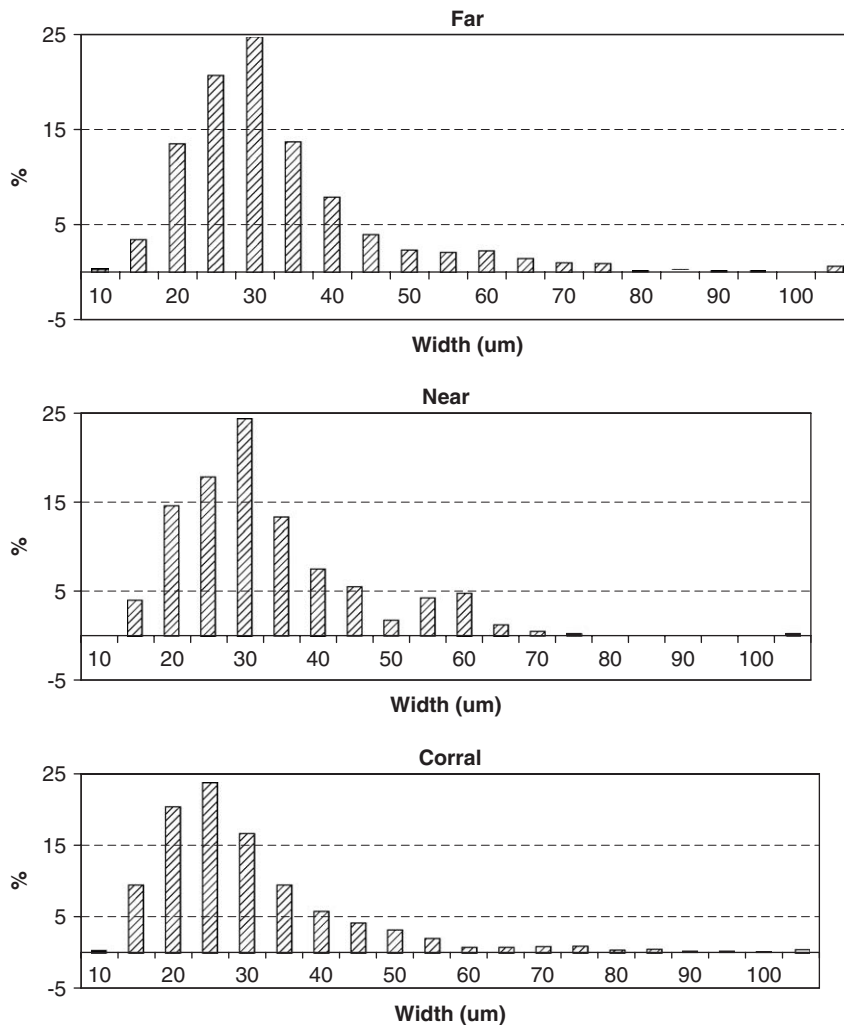


Fig. 3. Nematode width frequency distribution pooled across core layer and tail groups. Upper figure is from samples taken far from coralls, middle figure is from samples taken near to coralls, and lower is figure from samples taken inside coralls.

more common with increasing depth in the sediment. The length:width ratio of nematodes in our study displayed similar trends with the highest ratios in the 20–30 mm core layer (Table 2). When pooled across core layer and tail group, length:width ratios displayed unimodal distributions with a peak  $\sim 27$  (i.e., a body length  $27 \times$  longer than wide, Fig. 4) in all samples. Median length:width ratios were similar for all samples; 28.1 from far, 27.1 from near, and 26.7 from inside coralls. Two-way ANOVA compared only near and far samples and revealed that distance from coralls did not influence length:width ratio ( $p = 0.0815$ ). Core layer influenced length:width ratio ( $p = 0.0004$ ). The interaction term was not significant ( $p = 0.0871$ ). Thus, body shape of nematodes, as measured by

length:width ratio, favored longer nematodes relative to width at deeper sediment depths but did not differ between near and far samples (Fig. 4).

### 3.4. Nematode biovolume

Overall, nematode biovolume ranged from  $0.0047\text{--}34.9 \mu\text{L ind}^{-1}$ . Median nematode biovolume was similar among core layers throughout the upper 20 mm, but biovolume increased markedly in the 20–30 mm layer (Table 2). Within each core layer, nematode biovolume was largest in samples near and inside coralls; differences in medians between near and far samples averaged about  $0.06 \mu\text{L ind}^{-1}$  (Table 2) and about 0.04 between near and inside corral samples. Median biovolume also varied

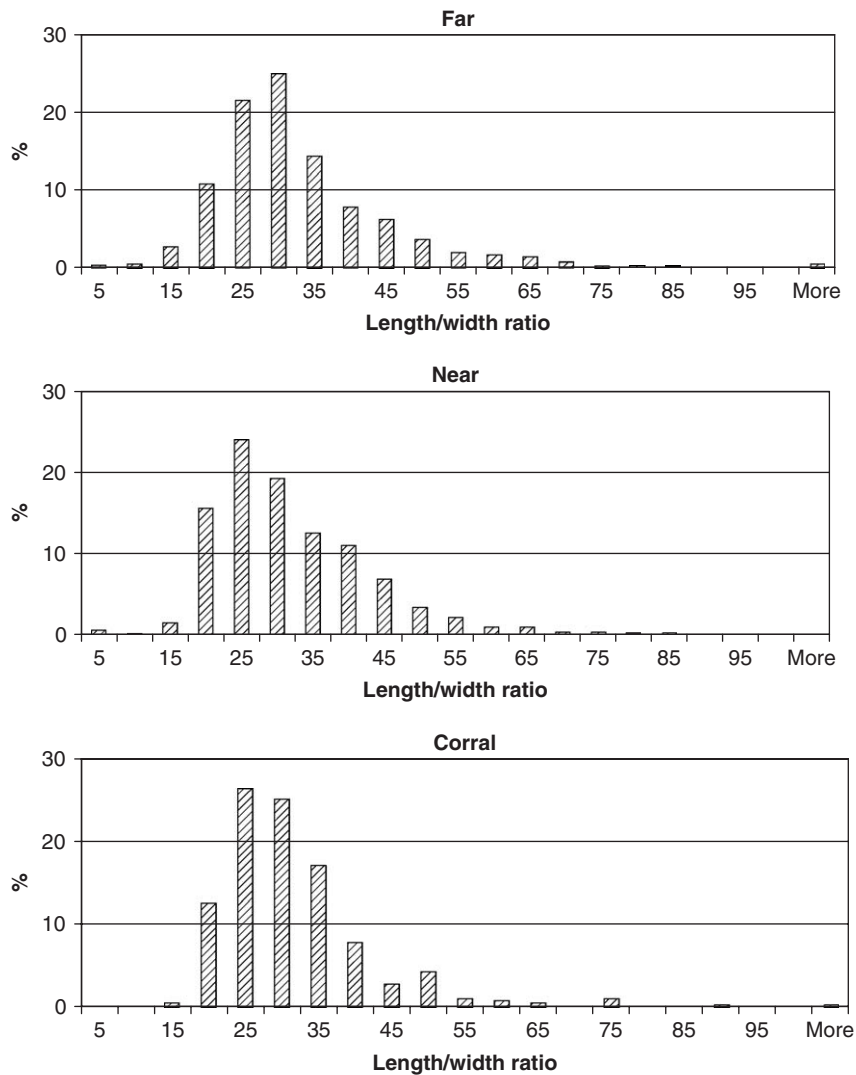


Fig. 4. Nematode length/width ratio frequency distribution pooled across core layer and tail groups. Upper figure is from samples taken far from corral, middle figure is from samples taken near to corral, and lower figure is from samples taken inside corral.

among nematode tail groups in samples taken far from the CO<sub>2</sub> source, ranging from a low of about 0.107  $\mu\text{L ind}^{-1}$  for tail group 1 (bluntly rounded) to a high of 0.245  $\mu\text{L ind}^{-1}$  in nematodes with elongate tails (group 4) (Table 3). Nematode biovolume in all tail groups from near and inside corral was larger than in far samples (Table 3). The largest difference was observed in tail group 3, which increased from a median of 0.21  $\mu\text{L ind}^{-1}$  in far samples to a median of 0.32  $\mu\text{L ind}^{-1}$  for nematodes collected near corral. The difference in biovolume between near and inside corral in all tail groups was always smaller than the difference between near and far samples (Table 1).

Table 3

Nematode median biovolume ( $\mu\text{L ind}^{-1}$ ) in far, near, and corral samples for each nematode tail group (see text for definitions of tail group designations), pooled across core layers

	Tail group 1	Tail group 2	Tail group 3	Tail group 4
Far	0.107	0.159	0.209	0.245
Near	0.207	0.254	0.316	0.331
Corral	0.233	0.226	0.252	0.347

Biovolume frequency distributions were bimodal, with a large and small peak for nematodes in all samples (Fig. 5). The large peak was at 0.2  $\mu\text{L ind}^{-1}$

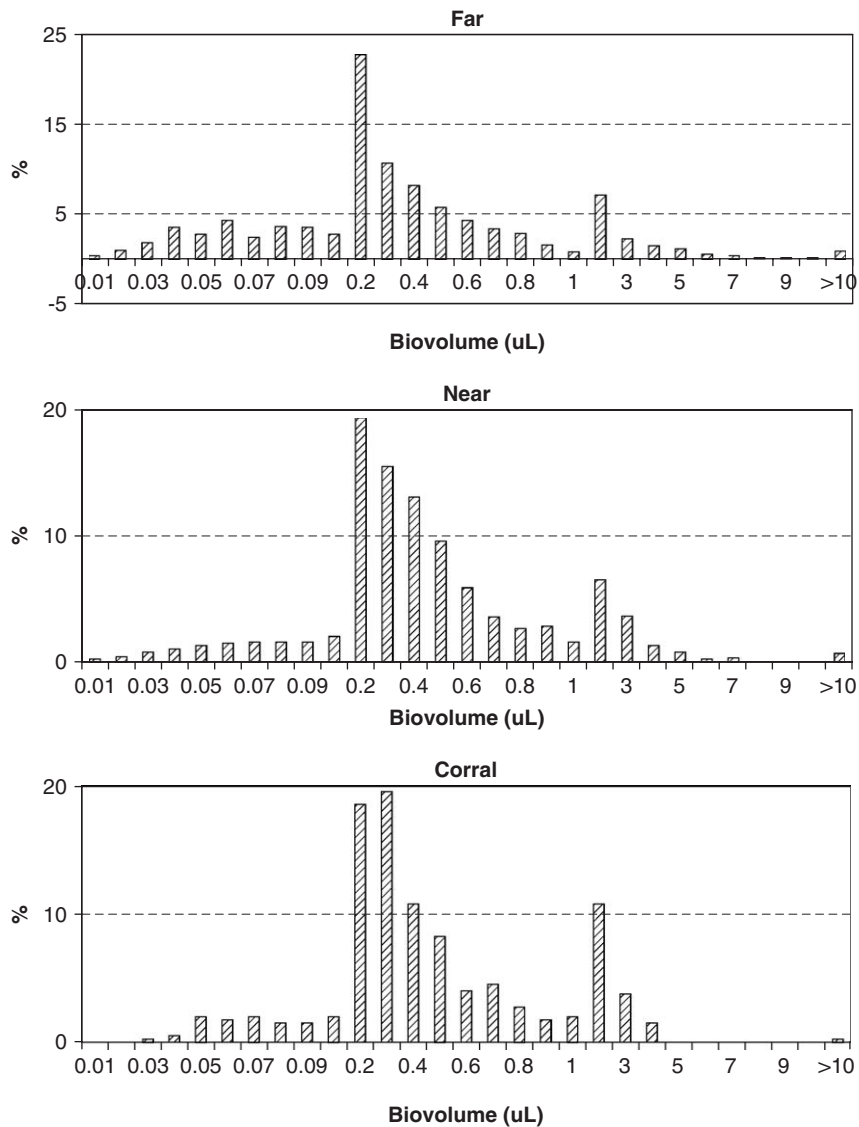


Fig. 5. Nematode biovolume frequency distribution pooled across core layer and tail groups. Biovolume is plotted on a logarithmic scale. Upper figure is from samples taken far from coralls, middle figure is from samples taken near to coralls, and lower figure is from samples taken inside coralls.

in all sample types but the frequency of biovolumes from 0.3 to 0.7  $\mu\text{L ind}^{-1}$  was much higher in nematodes from near and inside coralls. A second, much smaller peak occurred at  $\sim 2 \mu\text{L ind}^{-1}$  in far samples and at 3  $\mu\text{L ind}^{-1}$  in near and inside corral samples. In general, smaller size classes of nematodes were more frequent in far samples, and larger size classes were more common in near and inside corral samples. Pooled across all tail groups and layers, the median nematode biovolume was 0.21  $\mu\text{L ind}^{-1}$  in samples taken far from coralls, 0.31  $\mu\text{L ind}^{-1}$  near coralls and 0.30  $\mu\text{L ind}^{-1}$  in

samples taken inside coralls. This difference (a 48% relative increase in median nematode biovolume in sediments near coralls) is consistent with increases in length and width in near samples. In other words, the measured increases in nematode length and width would be expected to increase estimated biovolume by the amounts observed. Two-way ANOVA on ln-transformed nematode biovolume (excluding coralls) revealed that near and far samples differed ( $p = 0.0002$ ), and that core layer ( $p < 0.0001$ ) influenced biovolume. Although the interaction term was significant ( $p = 0.0178$ ),

increases in biovolume near and inside corrals were generally similar and consistent in all tail groups and core layers. For example, Tukey–Kramer tests show that near and far samples differed significantly in all layers ( $p = 0.0024$  in the 5–10 mm layer,  $p < 0.0001$  at the 10–20 mm layer and  $p = 0.0218$  at the 20–30 mm layer) except 0–5 mm ( $p = 0.9999$ ).

#### 4. Discussion

The release of carbon dioxide in the deep ocean strongly influences local environmental conditions including pH (Brewer et al., 2004; Barry et al., 2005). We released CO<sub>2</sub> into open-topped containers that confined liquid CO<sub>2</sub>. After dissolution, CO<sub>2</sub>-rich seawater flowed over the surrounding seafloor. CO<sub>2</sub> in this experiment did not persist visibly (liquid CO<sub>2</sub> is visible in seawater due to its refractive index) after 30 days, and we therefore used sediment pore water pH as a surrogate of exposure. We observed that acidity in the sediment of a core taken inside a corral in a later experiment was greatly increased resulting in a pH of 5.4, representing an extreme endpoint for CO<sub>2</sub> exposure. However, in this later experiment, dissolution was incomplete and visible CO<sub>2</sub> persisted for the duration of the experiment (Barry et al., 2005). At the far site, pH at the sediment–water interface was typical of the surrounding deep sea (~7.8) and was unchanged compared to samples taken before CO<sub>2</sub> was released. Pore-water pH in the upper 8 mm were lowered by ~0.75 at the near-coral site (Thistle et al., 2005). In addition, the near site probably experienced a lower pH than we observed for some unknown time immediately after the CO<sub>2</sub>-rich plume of seawater moved over the site. Natural variation in pH in the deep sea is ~0.1 pH unit (Ormerod et al., 2002). Therefore, we argue that infauna inside corrals were continuously exposed to very high concentrations of CO<sub>2</sub> and/or acidified pore water for 29 days and that infauna near corrals experienced increased CO<sub>2</sub> exposure and/or acidification for an extended period (Thistle et al., 2005).

Our previous research concluded that total nematode abundance was unaffected by proximity to corrals and that abundance in samples from inside corrals was very similar to samples taken near and far from corrals. Further, total nematode abundance differed with core layer, but the core-layer effect did not vary with CO<sub>2</sub> exposure, suggesting that nematodes did not migrate to deeper depths to avoid CO<sub>2</sub> exposure (Carman et al., 2004).

We have extended these findings here and show the same results with a nematode functional group; CO<sub>2</sub> exposure did not influence the abundance or composition of any of the four tail-group types in any of the core layers. Although not as sensitive as a species analysis, the lack of a response in tail-group composition suggests that nematodes did not respond to CO<sub>2</sub> exposure by differential recruitment, mortality, or migration.

We also report here that nematode length, width, and individual biovolume spectra were significantly affected by CO<sub>2</sub> exposure. Nematodes from all tail groups from inside and near corrals were longer (by 17% based on medians) and wider (13%) than nematodes in far samples, and these differences in body dimensions resulted in a large increase (48%) in estimated individual nematode biovolume. Nematode length, width and biovolume also varied significantly down core, but the statistical interaction between CO<sub>2</sub> exposure and core layer was not significant for body length. Although interactions between core layer and CO<sub>2</sub> exposure were significant for width and biovolume, near and far samples differed in all core layers except 0–5 mm. Nematode length:width ratio was unaffected by CO<sub>2</sub> exposure, and the shapes of frequency distributions of length, width and biovolume were similar in corral, near, and far samples.

Changes in nematode body dimensions and individual biovolume may have been the result of changing nematode demography or community composition following CO<sub>2</sub> exposure (e.g., juvenile nematodes could have recruited or disturbance-exploiting species colonized). Although observed changes in body dimension (except length:width ratio) were statistically significant, they were small in magnitude relative to the great range of nematode body sizes observed in the study. Length, width, and biovolume distributions in near and corral samples were shifted to larger values compared to far samples without a change in distribution shape (e.g., biovolume distributions from all samples were bimodal). Overall, length and width increases associated with CO<sub>2</sub> exposure were small compared to those reported by investigators who study environmental effects on nematode body size. For example, Tita et al. (1999) found that median nematode width varied from muddy to sandy intertidal sediments by a factor of about 3. Soltwedel et al. (1996) found seasonal variation in mean nematode length of about 23%, presumably associated with a recruitment event, at a deep-sea

site that exceeded length variation (17%) in our samples. Nematodes far from corrals were smaller than nematodes from inside and near corrals, suggesting that recruitment by juveniles did not occur in areas exposed to CO<sub>2</sub>. The relatively small but consistent changes in nematode dimensions and biovolume (but not body shape as measured by length:width ratio) found near and inside corrals suggest that a large proportion of the individual nematodes present at the time of exposure to CO<sub>2</sub> were similarly affected without major shifts in nematode demography or community composition, which is consistent with a major mortality event.

If substantial mortality occurred, 29 days may have been insufficient for nematode corpses to completely decompose. Carman et al. (2004) found that dead copepods required more than 30 days to completely disintegrate while exposed in situ to bottom water at this site. In a similar experiment conducted at 3600 m depth in Monterey Canyon, Barry et al. (2004) used a DNA stain to conclude that nematodes suffered high mortality, without complete decomposition, when exposed to CO<sub>2</sub>. Tietjen (1967) found that dead marine nematodes from shallow water held at room temperature in the laboratory did not disintegrate for more than 2 months in cultures with bacteria but without flagellated protists, but in cultures with protists, decomposition was completed in less than 2 days. Barry et al. (2004) found that flagellates and amoebae also suffered high levels of mortality after CO<sub>2</sub> exposure in Monterey Canyon. Perhaps CO<sub>2</sub> exposure has a strong influence on bacterial or protist decomposer communities, further slowing the already slow rates of decomposition typical of the deep sea (Jannasch, 1979).

Nematodes could colonize sites exposed to CO<sub>2</sub> either from surrounding sediment (by errant burrowing) or by settlement of individuals entrained in the water column. We sampled only the upper portion (0–3 cm) of the probable vertical range ( $\geq 6$  cm) of nematodes at our study site. Nematode length:width ratio has been shown to increase with increasing sediment depth (Soetaert et al., 2002), and length:width ratio of nematodes varied similarly with core layer in our study. However, we found no evidence that CO<sub>2</sub> exposure influenced variation in length:width ratio with sediment depth. This result suggests that nematodes living below 3 cm did not migrate toward the surface after CO<sub>2</sub> exposure. Furthermore, emergence trap data (Carman et al., 2004 and unpublished) suggest that

nematodes are poor colonists from the water column; only one nematode was found in two emergence traps while 46 harpacticoid copepods were found after a 30-day recruitment period. Other studies have shown that macroinfauna take longer than 30 days to colonize in the deep sea (Kukert and Smith, 1992; Levin and Dibacco, 1995). Thus, nematodes probably did not migrate in sufficient numbers to alter the community in 29 days (even if colonists perished when they reached sediments with increased acidity).

Nematodes have a proteinaceous cuticle and generate positive internal pressure in a pseudocoelom by contraction of longitudinal muscles (Brusca and Brusca, 1990). Upon death, these muscles may relax and, in the absence of circular muscles, body dimensions may expand and influence estimates of biovolume (Riemann, 1988). Fagerholm (1979) demonstrated that nematode body dimensions vary with preservation technique and that some fixatives increase body length by 20% (preservation with formalin did not alter length). These observations suggest that exposure to CO<sub>2</sub> led to *postmortem* increases in individual nematode body dimension and biovolume. If so, biovolume could be used more generally as a way to distinguish living from dead nematode communities (e.g., in samples to determine the impact of pollutant release collected quickly before changes in abundance by decomposition can occur) in deep sea or polar regions where decomposition is slow. Vital stains (such as Cell Tracker Green) released in situ on the deep-sea floor have recently been used to visually distinguish living and dead organisms (Bernhard, personal communication), and could be used to relate changes in nematode biovolume with percent mortality after an environmental insult. Biovolume estimation from preserved samples may be useful in situations in which vital staining is impractical.

Given the extreme acidity in corral sediments, high rates of nematode mortality caused by acidosis or hypercapnia seems likely (Seibel and Walsh, 2001; Carman et al., 2004; Pörtner et al., 2004; Barry et al., 2005), and most, if not all, nematodes collected from corrals were probably dead at the time of sample collection. Median nematode length, width, and individual biovolume in all tail groups and core layers were more similar to each other in corral and near samples than in near and far samples. We contend that observed changes in body dimension and biovolume were caused by CO<sub>2</sub>-induced mortality. If so, nematodes near corrals

must have suffered high rates of mortality throughout the upper 3 cm that were equivalent to mortality rates in corrals. Corral nematodes were probably exposed to a  $\text{pH} \leq 5.4$  during the 30 days of the experiment, and nematodes near corrals were exposed to a  $\text{pH} \leq 7.0$  over the same time. Unfortunately, we cannot generate a dose–tolerance relationship for nematodes because we could not follow the change in pH over short-time intervals and do not know exactly when nematode mortality occurred. The conclusion that ‘moderate’  $\text{CO}_2$  exposure near corrals, compared to the extreme environment in corrals, caused substantial mortality to infaunal nematodes is consistent with the conclusions of Thistle et al. (2005), who found that  $\text{CO}_2$  exposure caused high mortality in meiobenthic copepods in the same samples. Thus,  $\text{CO}_2$  exposure in this experimental release appears to have caused high mortality in the two most abundant metazoan taxa of the deep sea. This similar level of mortality in two taxa with different physiologies and tolerances to toxicants suggests additional taxa will be impacted at similar exposure intensities.

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