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Lethality of Increasing CO_2 Levels on Deep-Sea Copepods in the Western North Pacific

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The first CO_2 exposure experiments on several species of pelagic copepods inhabiting surface and deep layers in the western North Pacific were conducted. Living organisms were collected from two layers between the surface and 1,500 m between latitudes of 11 and 44°N, and they were exposed aboard ship to various pCO_2 up to about 98,000 μatm. Mortality of copepods from both shallow and deep layers in subarctic to subtropical regions increased with increasing pCO_2 and exposure time. Deep-living copepods showed higher tolerance to pCO_2 than shallow-living copepods. Furthermore, deep-living copepods from subarctic and transitional regions had higher tolerances than the subtropical copepods. The higher tolerances of the deep-living copepods from subarctic and transitional regions may be due to the adaptation to the natural pCO_2 conditions in the subarctic ocean.

Keywords:
· Lethality,
· pCO_2,
· epipelagic,
· mesopelagic,
· copepod,
· pH.

1. Introduction

Sequestration of carbon dioxide (CO_2) in the deep ocean, which has a potentially large storage capacity (Hoffert *et al*., 1979; Lackner, 2003), has been proposed as one of the mitigation options for slowing down the increase in atmospheric CO_2—the most influential greenhouse gas (Houghton *et al*., 2001). Several options have been considered for CO_2 storage in oceans (Haugan and Drange, 1992; Herzog and Edmond, 1994; Handa and Ohsumi, 1995; Ormerod and Angel, 1996), such as storing in deep-sea basins as hydrates and in the bathypelagic zone as dissolved CO_2. The option of storing in a deep-sea basin should enable storage of CO_2 in a narrow area for a long period of time and localizes the impact (Omori *et al*., 1998); however, the organisms living there will be exterminated because of the effects of high CO_2 concentrations. The option of dissolving CO_2 in the bathypelagic zone, such as 1,000 to 3,000 m, may possibly minimize the impact, if the CO_2 is adequately diluted (Handa and Ohsumi, 1995; Drange *et al*., 2001).

At present only limited information about the effect of CO_2 on marine organisms is available (Shirayama, 1997; Omori *et al*., 1998). Auerbach *et al*. (1997) used data from experiments of lowering pH by hydrochloric or sulfuric acids to estimate the effects of CO_2 ocean sequestration on marine organisms. Some CO_2 exposure experiments have been conducted recently. Effects of CO_2 on the phytoplankton (Riebesell *et al*., 2000; Riebesell, 2004), fishes (Kikkawa *et al*., 2003), invertebrates including copepods (Kurihara *et al*., 2004) and foraminifera (Takeuchi *et al*., 1997) have been reported; however, those organisms were from coastal or epipelagic regions and not from the deep sea. Bacteria isolated from the deep sea (Takeuchi *et al*., 1997) have been examined for the

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Table 1. Sampling data and oceanographic background.

Station	Date	Time	Location	Sampling layer*	Sampling layer (m)	Net	No. of tows	Oceanographic characteristics (min–max)**					
								Depth (m)	Layer (m)	Temperature (°C)	pCO_2 (μatm)	pH	TCO_2 (μmol kg^{-1})
1	Aug. 12. 1999	18:00–19:00	43°N, 155°E	shallow	0–150	NORPAC	1	5358	0–500	3.1–11.5	500–2020	7.43–8.05	2070–2420
				deep	0–1000	NORPAC	1		500–1500	2.1–3.1	1450–2040	7.44–7.55	2420–2440
2	Aug. 17. 1999	18:00–20:00	36°N, 155°E	shallow	0–500	NORPAC	3	5575	0–500	6.7–24.3	480–1010	7.76–8.24	2000–2250
		12:30–14:00		deep	500–1500	VMPS	1		500–1500	2.4–6.7	1010–1850	7.51–7.76	2250–2440
3	Oct. 1. 1999	9:30–11:00	25°26′ N, 144°50′ E	shallow	0–500	NORPAC	4	5713	0–500	11.3–28.9	500–760	7.92–8.27	1980–2170
		11:00–13:00		deep	500–1500	VMPS	1		500–1500	2.5–11.3	760–1670	7.57–7.92	2170–2420
4	Aug. 25. 1999	13:00–15:00	24°N, 155°E	shallow	0–500	NORPAC	3	5486	0–500	10.3–29.0	470–870	7.88–8.31	1980–2200
		8:00–10:00		deep	500–1500	VMPS	1		500–1500	2.4–10.3	870–1640	7.55–7.88	2200–2420
5	Sep. 10. 1999	9:00–10:00	14°N, 155°E	shallow	0–500	NORPAC	3	5911	0–500	7.4–29.4	490–1420	7.66–8.31	1980–2310
		7:00–9:00		deep	500–1500	VMPS	1		500–1500	2.8–7.4	1140–1480	7.64–7.66	2310–2400

*Shallow: collected from 500 m depth to surface, Deep: collected from 1500 m to 500 m.
**Oceanographic data depicted for stations 1, 3 and 5 are those from 44°N 155°E, 25°N 147°E and 12°N 155°E, respectively, because no oceanographic data were available for the sampling points.

growth effects of increasing CO_2, but they were not planktonic species and were subcultured under laboratory conditions. Although *in situ* studies (Tamburri *et al*., 2000; Barry *et al*., 2004; Carman *et al*., 2004; Ishida *et al*., 2005) have been performed in the deep-sea, these were limited to the benthic community and did not include plankton.

It is therefore necessary to study the effects of CO_2 on planktonic organisms living in deep environments for the assessment of the impact of CO_2 ocean sequestration into the deep sea. Copepods are the most dominant group in the pelagic zooplankton community of the North Pacific and other oceans (e.g. Parsons *et al*., 1983; Mauchline, 1998; Yamaguchi *et al*., 2000). In this study, we examined the lethal effects of CO_2 on living copepods collected from the bathypelagic zone.

2. Materials and Methods

2.1 Sampling

The experiments were carried out on board ship during the oceanographic cruise of WEST-COSMIC (Western Pacific Environment Study on CO_2 Ocean Sequestration for Mitigation of Climate Change) (Ishizaka, 1999). We conducted the zooplankton exposure experiments onboard just after the sampling, because it is difficult to keep most pelagic zooplankton, especially those that inhabit the deep-sea, for an extended period. Zooplankton were sampled by vertical tows of two layers at five points on cruise NH-99 of the R/V Hakurei-Maru No. 2 (Metal Mining Agency of Japan) from August 4, 1999 to October 13, 1999 (Fig. 1). We chose a vertical tow for the sampling because sampled animals were in much better condition than those obtained from horizontal or oblique tows. The sampling conditions are given in Table 1.

Shallow-living copepods were collected in a tow from 150 m to the surface at Station 1 and from 500 m to the surface at the other stations, respectively, with a modified NORPAC net (mesh size 300 μm) equipped with a large volume (3 L) cod-end. Deep-living copepods were collected from 1,500 m to 500 m depth, where increases of CO_2 concentration would be expected if the moving ship ocean sequestration method (Ozaki, 1997) were used. However, we collected deep-living animals at Station 1 by a vertical tow of the NORPAC net from 1,000 m and sorted out the deep-living animals. It was expected that adequate numbers of meso- and bathypelagic zooplankters would be collected by a vertical tow, although the population density of organisms decreases exponentially with depth (e.g. Vinogradov, 1968; Koppelmann and Weikert, 1992). For the deep tow, VMPS-6000 (Vertical Multiple Plankton Sampler Tsurumi Seiki Co.) (Terazaki and Tomatsu, 1997) was used with a mesh size of 300 μm and a larger opening (1 m^2) because of the lower densities of zooplankton reported in the deep-sea. A sample was taken

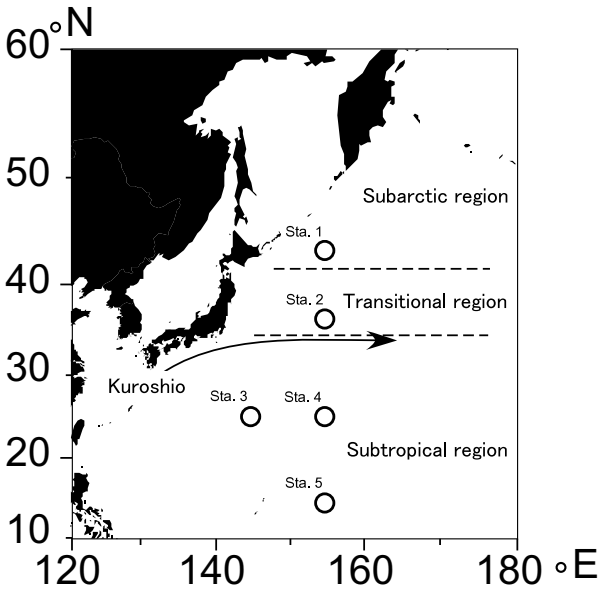

Fig. 1. Sampling stations for zooplankton used in the experiments. Experiments were performed onboard during cruise NH-99 for the oceanographic research of WEST-COSMIC.

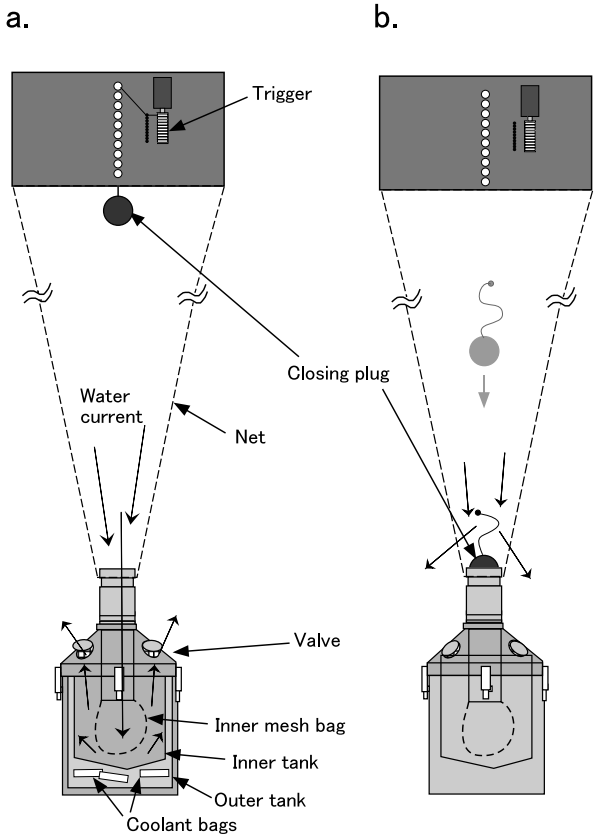

Fig. 2. Diagram of cod-end fitted to the Vertical Multiple Plankton Sampler (VMPS) for sampling of living copepods from the deep sea. (a) Mouth of cod-end is left open during towing through the sampling layer. The inner mesh bag weakens the impact of water flow on the captured zooplankton. (b) Plug drops down upon a signal being sent from the control unit and falls into the mouth of the cod-end. Samples in the cod-end are separated from the external seawater.

from one layer from each tow with a closing mechanism. A special thermal cod-end was used, which kept the internal temperature of the cod-end below 20°C, as the surface temperature was about 30°C (Fig. 2). We conducted the majority of sampling in daytime since most copepods are known to exhibit diel vertical migration behavior, but some samplings extended to nighttime (Table 1).

Animals collected in the cod-ends were cooled immediately in an iced seawater bath, and healthy animals were selected from the samples and kept in incubators set at each experimental temperature for a maximum of half a day until each experiment was performed.

Seawater from a depth of 1,000 m was collected by Niskin bottles on a CTD-rosette multiple sampler (Sea-Bird SBE-9 and General Oceanics GO-1016). The depth was chosen because the water contains little dissolved organic matter and lower bacterial activity than the surface layer (e.g. Millero, 1996; Nagata *et al.*, 2000), which might cause deterioration of water quality. The water was filtered using a GF/F glass fiber filter to remove other plankters and was refrigerated at the target temperatures until the experiments.

2.2 Exposure experiment and calculations

Five or fewer healthy animals were placed with a pipette into a 500 ml glass bottle equipped with a polyethylene stopper, filled with the experimental seawater adjusted to various concentrations of pCO_2. Experimental seawater with high pCO_2 was made by bubbling various concentrations of CO_2 (1,000–90,000 μatm) with N_2 and O_2 gases. Filtered seawater, which was not treated with CO_2 bubbling, was used in the control experiments. Total carbonate was measured with a Capni-Con Total CO_2 Analyzer (Cameron Instrument Company, Inc.) and pH was measured with an Orion ion meter 920A (Thermo Electron Corporation). Partial pressures of experimental seawater were calculated using pH and total carbonate with the "CO2SYS" program (Lewis and Wallace, 1997).

Each bottle was incubated in the dark at approximate *in situ* temperatures for each assemblage:—3, 10, or 17°C for shallow-living copepods and 3°C for deep-living copepods. Animals were not fed during the experiment to minimize effects from the decomposition of food. The behavior and survival of animals were observed at 6 and 12 h and thereafter at 12 h intervals after the initial exposure.

Table 2. Experimental conditions at each station. Exposure pCO_2 values were calculated from total carbonate and pH at the beginning. Incubation temperatures were approximate values at the average depth of each sampling layer.

Location	Experiment No.	Depth type	Total number of animals	Exposure pCO_2 (μatm)	Incubation temperature (°C)
Station 1	1	shallow	25	860, 3500, 4400, 13000, 22000	3
	2	shallow	25	860, 3500, 4400, 13000, 22000	3
	3	deep	25	860, 3500, 4400, 13000, 22000	3
Station 2	4	shallow	25	1100, 1500, 7100, 11000, 27000	10
	5	deep	25	800, 1100, 5500, 8800, 21000	3
	6	deep	15	800, 1100, 8800	3
	7	deep	15	800, 1100, 8800	3
Station 3	8	shallow	70	1200, 5500, 6900, 14000, 98000	17
	9	deep	50	650, 3400, 4200, 9000, 62000	3
	10	deep	20	650, 4200, 9000, 62000	3
	11	deep	20	650, 4200, 9000, 62000	3
Station 4	12	shallow	24	1300, 2900, 7000, 10000, 30000	17
	13	shallow	22	1300, 2900, 7000, 10000, 30000	17
	14	deep	40	940, 2200, 5400, 7900, 23000	3
Station 5	15	shallow	73	1600, 12000, 14000, 18000, 47000	17
	16	deep	71	530, 7200, 8600, 11000, 29000	3

We judged the animals to be dead when they showed no motion and did not react to artificial stimulus by a fine needle, or when they settled on the bottom and the body color became opaque. The animals that died during the course of the experiment were fixed with formalin and taxonomically classified.

Experimental conditions are shown in Table 2. Partial pressures of CO_2 in the seawater used for the controls varied from 530 to 1,600 μatm. The exposure groups kept at high pCO_2 ranged from 1,100 to 98,000 μatm, which correspond to pH 7.65 to 6.02. Five to three dose groups were set for each experiment, the number of the dose groups depending on the numbers of healthy individuals obtained by sampling and identified on board. The number of animals for each dose was also varied from 4 to 15 individuals. Fewer than five animals were put into the bottle, and if more than five animals could be prepared for each dose experiment, several bottles were used.

Pelagic copepods can be divided into two groups that can be separated by habitat depth (Sewell, 1948); one is epipelagic and the other is meso- or bathypelagic. Furthermore, the faunal structure in the western North Pacific differs between the subarctic and subtropical regions (Yamaguchi *et al*., 2002). These broad distributions could be verified from the taxonomic classification of experimental animals (Table 3). Therefore, we compared surface-living copepods with deep-living ones between northern (subarctic and transitional regions) and southern species (subtropical region).

2.3 Calculation of half lethal time

Since the mortality rate under acutely toxic conditions is generally approximated by a sigmoid curve, the conversion of the rate to the probit value resulted in a linear relation with the dose. The half lethal concentration is equal to five times the probit value (e.g. Sakuma, 1964; Stephan, 1977). In this study, we considered the elapsed time as a dose instead of the concentration and calculated the half lethal time (LT_{50}) by the probit method (e.g. Stephan, 1977) for each group after probit conversion of the mortality rates at each observation.

3. Results

3.1 Species composition

Large copepods were dominant in the subarctic and transitional region of north the Kuroshio extension. We were therefore able to identify the species prior to each experiment and conducted the experiments with single species. *Calanus pacifica*, *Metridia pacifica* and *Euchaeta marina*, known to be epipelagic species, were taken from the shallow layer samples and used to represent shallow-living copepods. *Paraeuchaeta birostrata*, *Gaidius variabilis* and *Heterostylites major*, known to be meso- or bathypelagic species, were taken from the deep layer samples and used to represent deep-living copepods (Table 3). *Neocalanus cristatus* , which is known to migrate seasonally and inhabits the deep layer in late summer–mid autumn (Kobari and Ikeda, 1999), were sorted from

Table 3. Species composition of the experiments. Animals used in Exp. 1–7 were identified before the experiment and the others were identified after the experiment.

Station	Experiment No.	Sampling layer	No. of species	Depth type*	Species
1	1	shallow	1	Epi	*Calanus pacificus*
	2	shallow	1	Epi	*Metridia pacifica*
	3	deep	1	Eury	*Neocalanus cristatus*
2	4	shallow	1	Meso/Bathy	*Euchaeta marina*
	5	deep	1	Meso/Bathy	*Paraeuchaeta birostrata*
	6	deep	1	Meso/Bathy	*Heterostylites major*
	7	deep	1	Meso/Bathy	*Gaidius variabilis*
3	8	shallow	12	Epi	*Candacia bipinnata, C.* sp., *Euchaeta indica*, *E. marina*, *Heterorhabdus papilliger*, *H. spinifrons*, *Scolecithrix danae*
				Meso/Bathy	*Acrocalanus longicornis*, *Pleuromamma abdominalis*, *Undeuchaeta major*
				Eury	*Eucalanus subtenuis*, *Neocalanus robustior*
	9	deep	4	Eury	*Eucalanus bungii*, *E. hyalinus*, *Rhincalanus nasutus*, *R. cornutus*
	10	deep	3	Meso/Bathy	*Pleuromamma abdominalis*, *P. gracilis*, *P. xiphias*
	11	deep	9	Meso/Bathy	*Chiridiella brachydactyla*, *Chirundina strsstsii*, *Euchirella curticauda*, *E. messinensis*, *E. pulchra*, *E. venusta*, *Pseudochirella polyspina*, *Undeuchaeta major*, *U. plumosa*
4	12	shallow	14	Epi	*Calanus sinicus*, *Euchaeta marina*, *Labidocera acutifrons*, *L. minuta*
				Meso/Bathy	*Euchirella curticauda*, *Phaenna spinifera*, *Pleuromamma abdominalis*, *P. gracilis*, *P. xiphias*, *Undeuchaeta plumosa*
				Eury	*Eucalanus attenuatus*, *Neocalanus gracilis*, *N. robustior*, *Rhincalanus nasutus*
	13	shallow	11	Epi	*Canthocalanus pauper*, *Centropages elongatus*, *Clausocalanus furcatus*, *Euchaeta marina*, *Heterorhabdus papilliger*, *Mesocalanus tenuicornis*, *Nannocalanus minor*, *Scolecithrix danae*
				Meso/Bathy	*Pleuromamma abdominalis*, *P. gracilis*
				Eury	*Neocalanus robustior*
	14	deep	18	Meso/Bathy	*Aetideopsis rostrata*, *Euchirella curticauda*, *E. venusta*, *Euchirella* sp., *Gaetanus kruppii*, *Metridia curticauda*, *Onchocalanus affinis*, *Paraeuchaeta elongata*, *Pleuromamma abdominalis*, *P. xiphias*, *Scaphocalanus brevicornis*, *S. affinis*, *Scolecithricella dentata*, *Scottocalanus securifrons*
				Eury	*Eucalanus attenuatus*, *Neocalanus robustior*, *Rhincalanus cornutus*, *R. nasutus*
5	15	shallow	14	Epi	*Calanoides philippinensis*, *Cosmocalanus darwini*, *Euchaeta marina*, *Mesocalanus tenuicornis*, *Nannocalanus minor*, *Scolecithrix danae*
				Meso/Bathy	*Euchirella messinensis indica*, *Euchirella* sp., *Phaenna spinifera*, *Phyllopus helgae*, *Pleuromamma abdominalis*, *P. gracilis*, *P. xiphias*
				Eury	*Neocalanus robustior*
	16	deep	22	Epi	*Heterorhabdus papilliger*
				Meso/Bathy	*Aetideus* sp., *Chiridiella brachydactyla*, *Chirundina streetsii*, *Euchirella messinensis*, *E. pulchra*, *E. venusta*, *Euchirella* sp., *Gaetanus* sp., *Lucicutia flavicornis*, *Phaenna spinifera*, *Pleuromamma abdominalis*, *P. gracilis*, *Pleuromamma xiphias*, *Pseudochirella polyspina*, *Phyllopus helgae*, *Scaphocalanus affinis*, *S. echinatus, S.* sp., *Scottocalanus securifrons*, *Undeuchaeta plumosa*, *Undinella* sp.

*Epi: Epipelagic, Meso: Mesopelagic, Bathy: Bathypelagic, Eury: Eurybathic.

Table 4. Proportion of individuals assigned to each depth type. The depth types are classified as epipelagic, meso- and bathypelagic, and wide inhabitant type according to Brodsky (1950) and Chihara and Murano (1997).

Station	Experiment No.	Sampling layer	Depth type*		
			Epi	Meso/Bathy	Eury
1	1	shallow	100		
	2	shallow	100		
	3	deep			100**
2	4	shallow	100		
	5	deep		100	
	6	deep		100	
	7	deep		100	
3	8	shallow	60	30	10
	9	deep			100
	10	deep		100	
	11	deep		100	
4	12	shallow	42	29	29
	13	shallow	59	36	5
	14	deep		75	25
5	15	shallow	70	22	8
	16	deep	1	99	

*Epi: Epipelagic, Meso: Mesopelagic, Bathy: Bathypelagic, Eury: Eurybathic.
**_N. cristatus_ seasonally migrate from surface waters to near 2,000 m and inhabits the deep layer in this season.

a deep layer sample and were used to represent deep-living copepods.

In the subtropical region, most of the zooplankters were small in size and the samples had a higher diversity. Since we were unable to isolate monotypic species samples prior to each experiment, the zooplankton species assemblages used for CO_2 exposure experiments in the subtropical region were critically examined taxonomically after the experiments (Table 3). As a result of this classification, 42–70% of the individuals in the "surface" copepod assemblages were true epipelagic species, while 75–100% of the individuals in the "deep" copepod assemblages were considered meso-/bathypelagic species (Table 4). Only a few species at low numbers of individuals were captured from both shallow and deep layers simultaneously.

3.2 Survival time

Copepods in the control experiments survived for 4–7 days, except for the shallow layer experiment in the subtropical region (Exp. 15), where the increase of mortality started earlier (Fig. 3). Mortality generally increased with exposure time and increase of pCO_2 in all the experiments, and similar trends were observed in the other experiments (not shown in Fig. 3). Mortality was generally lower in the deep layer experiments than in the shallow layer experiments.

3.3 Half lethal time

Because integrated mortality increased with exposure time, half lethal time (LT_{50}) could be calculated from the time series of mortality data. Estimated LT_{50} values are shown in Table 5. There were several experiments where LT_{50} was estimated to be longer than the experimental period, because at the end of the experiments dead animals comprised less than half the number of total animals used during each experiment, such as the 1,500 μatm exposure group of *E. marina* (Table 5, Exp. 4). Higher pCO_2 generally shortened the survival time of zooplankton.

In the subarctic and transitional regions, the LT_{50} decreased with increasing pCO_2, and the LT_{50} values for shallow-living copepods were much smaller than for deep-living copepods, especially under high pCO_2 exposure conditions (Fig. 4(a)). These results indicate that the deep-living copepods were less sensitive to increasing pCO_2

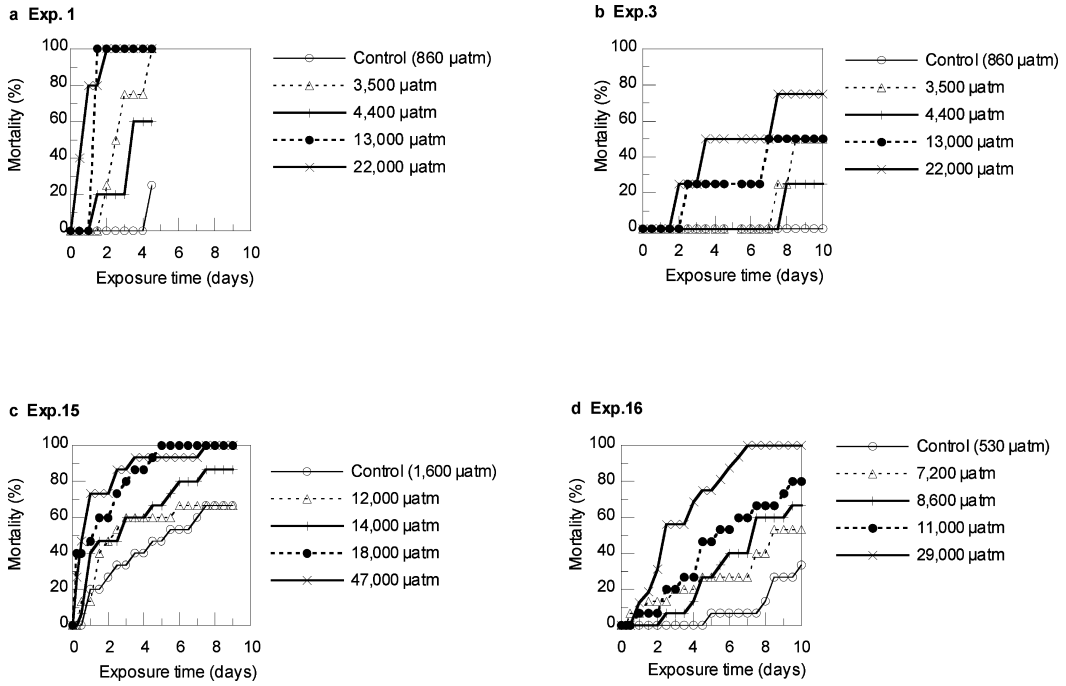

Fig. 3. Mortality curve of zooplankton exposed to various concentrations of CO_2. (a) Exp. 1, *Calanus pacificus* collected from surface layer at 43°N, 155°E, (b) Exp. 3, *Neocalanus cristatus* collected from deep-layer at 43°N, 155°E, (c) Exp. 15, epipelagic copepods collected from surface layer at 24°N, 155°E, and (d) Exp. 16, mesopelagic copepods collected from deep layer at 24°N, 155°E.

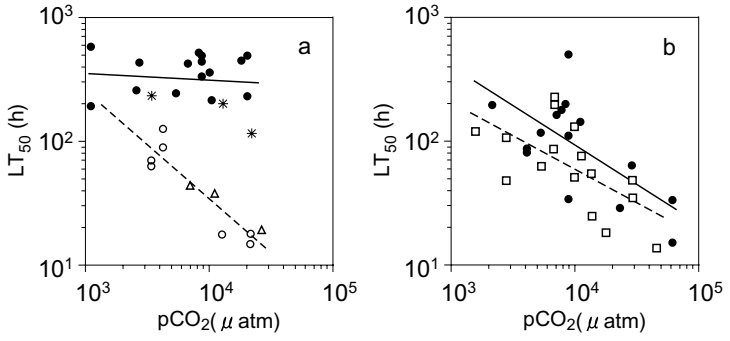

Fig. 4. Estimated half-lethal time (LT_{50}) of copepods with respect to increased partial pressure of CO_2. LT_{50} values plotted against exposed pCO_2 in the subarctic and transitional (a) and in the subtropical regions (b). LT_{50} of shallow-living copepods are shown by open symbols. LT_{50} of deep-living copepods are shown by closed circles and asterisks. *Neocalanus cristatus* is distinguished by asterisks because they are in the dormant phase. Other symbols refer to the incubation temperature, in which circles, triangles and squares represent 3°C, 10°C and 17°C, respectively. The regression lines for each group are shown by solid or dotted lines.

and consequently more tolerant of higher CO_2 concentrations than the shallow-living copepods.

In the subtropical region, the LT_{50} evidently shortened with increasing pCO_2, for not only the shallow-living copepods but also the deep-living ones (Fig. 4(b)). Moreover, the slopes of both regression curves for the subtropical region were nearly equivalent, in contrast to those of the subarctic copepods. Although the LT_{50} ranges overlapped, the LT_{50} values of the deep-living copepods were significantly longer than those of the shallow-living ones ($p = 0.05$, by ANCOVA).

Table 5. Half lethal time of copepods exposed to high CO_2 concentrations.

Station (Location)	Surface-living Groups				Deep-living Groups			
	Experiment	pCO_2 (μatm)	pH	LT_{50}	Experiment	pCO_2 (μatm)	pH	LT_{50}
1	1	860[†]	7.92	—	3	860[†]	7.92	—
(43°N, 155°E)		3,500	7.36	62		3,500	7.36	240
		4,400	7.25	89		4,400	7.25	240–250
		13,000	6.81	24–35		13,000	6.81	200
		22,000	6.54	15		22,000	6.54	120
	2	860[†]	7.92	—				
		3,500	7.36	70				
		4,400	7.25	130				
		13,000	6.81	18				
		22,000	6.54	17				
2	4	1,100[†]	7.78	—	5	800[†]	7.88	—
(36°N, 155°E)		1,500	7.65	>140[‡]		1,100	7.74	580
		7,100	7.04	43		5,500	7.11	240
		11,000	6.83	38		8,800	6.89	450
		27,000	6.52	19		21,000	6.58	230
					6	800[†]	7.88	—
						1,100	7.74	>410[‡]
						8,800	6.89	490
					7	800[†]	7.88	—
						1,100	7.74	190
						8,800	6.89	330
3	8	1,200[†]	7.78	—	9	650[†]	7.96	—
(25°26′ N, 144°50′ E)		5,500	7.17	62		3,400	7.30	>350[‡]
		6,900	7.08	85		4,200	7.21	>350[‡]
		14,000	6.77	25		9,000	6.89	110
		98,000	6.02	<6[§]		62,000	6.14	33
					10	650[†]	7.96	—
						4,200	7.21	48–53
						9,000	6.89	34
						62,000	6.14	15
					11	650[†]	7.96	—
						4,200	7.21	81
						9,000	6.89	>200[‡]
						62,000	6.14	6–12

4. Discussion

4.1 Lethality to copepods

With CO_2 injection into the deep-sea for oceanic CO_2 sequestration, it is inevitable that organisms near the injection site will be exposed to higher pCO_2. To investigate the biological impacts of higher CO_2 concentrations, several studies have been performed on fish (Kikkawa *et al*., 2003), nematodes, bacteria (Takeuchi *et al*., 1997), sea urchins and copepods (Kurihara *et al*., 2004); however, most of these studies were on coastal and epipelagic species. Since copepods are one of the most dominant groups in the ocean from the surface to the deep sea (Raymont, 1983; Yamaguchi *et al*., 2000) and play important roles in the biogeochemistry of the ocean, it is important to know the effect that CO_2 injection may have on pelagic copepods.

This study showed that copepods exposed to high pCO_2 had a higher mortality rates, as with other taxa (Takeuchi *et al*., 1997; Riebesell *et al*., 2000; Kikkawa *et*

Table 5. (continued).

Station (Location)	Surface-living Groups					Deep-living Groups			
	Experiment		pCO_2 (μatm)	pH	LT_{50}	Experiment	pCO_2 (μatm)	pH	LT_{50}
4	1	2	1,300†	7.72	—	14	940†	7.81	—
(24°N, 155°E)			2,900	7.40	48		2,200	7.47	200
			7,000	7.03	230		5,400	7.10	120
			10,000	6.86	51		7,900	6.92	180
			30,000	6.42	34		23,000	6.48	29
		13	1,300†	7.72	—				
			2,900	7.40	110				
			7,000	7.03	200				
			10,000	6.86	130				
			30,000	6.42	48				
5	1	5	1,600†	7.64	—	16	530†	8.02	—
(14°N, 155°E)			12,000	6.84	75		7,200	6.96	163
			14,000	6.76	55		8,600	6.88	201
			18,000	6.64	18		11,000	6.76	142
			47,000	6.23	14		29,000	6.35	64

†Seawater for control experiments.
‡LT_{50} were not calculated because of few instances of mortality.
§LT_{50} were not calculated because of drastic mortality changes between the observation intervals.

al., 2003), and longer exposures resulted in higher mortalities. Similar results were found for fishes (Kikkawa *et al*., 2003); however, the results from fishes showed less dependency on exposure time than with copepods. As fishes have ion-transporting chloride cells in the gills, it has been speculated that they regulate intra- and inter-cellular pH by the active excretion of excess protons through them (Ishimatsu *et al*., 2004). Similar cells that exchange respiratory gases through the body surface have not been found in copepods. The active regulation by such chloride cells may lead to the differences in dependences on exposure period between fishes and copepods.

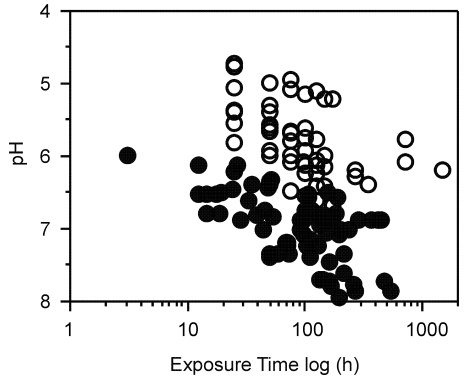

Fig. 5. Mortality of animals exposed to pH lowered by acids and CO_2. Open circles: LT_{50} (Auerbach *et al*., 1997; Yamada and Ikeda, 1999) of littoral and pelagic copepods at each pH controlled by acids. Closed circles: LT_{50} of copepods exposed to a high partial pressure of CO_2, indicated by pH observed in the experiment.

4.2 Comparison with effect of lowered pH

When constructing an assessment model of the biological impacts of CO_2 ocean sequestration, Auerbach *et al*. (1997) speculated on the lethal effects on marine organisms of high concentrations of CO_2 due to the acute toxicity of low pH induced by hydrochloric or sulfuric acid because of the lack of experimental data using CO_2. Yamada and Ikeda (1999) reported the acute toxicity of low pH to pelagic zooplankton, including copepods. However, the influence of increasing CO_2 concentration is not limited to a lowering of pH but also results in an increase of pCO_2. The influence of an increase of pCO_2 on organisms can be more severe than a lowering of the pH (Seibel and Walsh, 2001). Because CO_2 can permeate the cell membrane more easily than hydrogen ions (Ishimatsu *et al*., 2004), CO_2 permeating into the body results in a lowering of internal pH. Comparison of LT_{50} of copepods exposed to higher pCO_2 and to lowered pH caused by the addition of hydrochloric or sulfuric acid indicated that

copepods died under significantly higher pH conditions when combined with CO_2 exposure (Fig. 5). Similar results have been reported for fishes (Kikkawa *et al*., 2004). Therefore, in order to assess the biological effects of CO_2 ocean sequestration, the accumulation of data through experiments using CO_2 itself is necessary.

4.3 Characteristics of deep-living copepods

Only few species were found living in both shallow and deep layers from the sampling stations. Percentage dominance of species classified as "shallow" and "deep" assemblage members were 42–70% and 75–100%, respectively. Therefore, the pCO_2-LT_{50} relationships found for each layer reflect the difference of CO_2 sensitivity between the copepod assemblages of different layers.

A comparison between the results of experiments on the "deep" and "shallow" copepod assemblages suggests that the deep-living copepods were more resistant to higher CO_2 concentrations than the shallow-living ones. Such higher CO_2 tolerance was most conspicuous in the deep-living copepods that were caught in the subarctic and transitional regions. These results somewhat contradict our common understanding that deep-living organisms are more vulnerable to high CO_2 concentrations than shallower-living ones (Shirayama, 1997; Seibel *et al*., 1997; Seibel and Walsh, 2001).

Some subarctic copepod species are known to have a dormant stage in their life cycle (Kobari and Ikeda, 1999). Since animals in a dormant stage reduce their metabolic activity, it might be suspected that their dormancy makes them insensitive to high pCO_2 levels. However, the tolerance level of *N. cristatus*, which is dormant during this season, was similar to that of species that lack a dormant stage in their life cycle, and the pCO_2-LT_{50} relationship (Fig. 4(a)) of deep-living copepods in the subarctic region was quite similar, even if the *N. cristatus* results are excluded. The question as to whether the dormant stage has higher tolerance to increased pCO_2 still remains, until a comparison between the dormant and non-dormant stages of the same species can be conducted. Nevertheless, this study indicates that dormancy does not seem to be an important factor determining the sensitivity of deep-living copepods to increasing pCO_2.

Temperature is known to be a major environmental factor that affects various biological processes (Randall *et al*., 1997). Correction for temperature differences is required for physiological comparisons, especially for poikilothermic animals. The toxicity of most chemicals generally increases with increasing temperature (Wakabayashi, 2003). Various physiological rates are influenced by temperature, such as respiration, uptake, excretion, and detoxification, which affects toxicity. For example, the lethal concentration half number (LC_{50}) of mercury chloride to *Oncorhynchus mykiss* was reduced with increasing temperature (Macleod and Pessah, 1973). Since metabolic depression is caused by increasing CO_2 through the internal acid-base imbalance caused by CO_2 diffusion and its dissociation into the body (Seibel and Walsh, 2001; Pörtner *et al*., 2004), it is probable that the toxic strength of high concentrations of CO_2 is influenced by temperature as with other toxic chemicals. Deep-living copepods may display an apparent tolerance because of the low temperature used in the experiments. The deep-living copepods were evidently tolerant of higher pCO_2, although both the surface- and deep living copepods were incubated at the same temperature at Station 1 (Table 2). Furthermore, the slopes of the regression curves in the pCO_2-LT_{50} relationship (Fig. 4(a)) are different between the deep-living and the surface living copepods. These facts suggest that the insensitivity to pCO_2 observed in deep-living copepods is not explainable by temperature effects, but that other characteristics or factors may cause the insensitivity.

The mesopelagic and bathypelagic zones have naturally higher pCO_2 than the epipelagic zone. In particular, a maximum in pCO_2 is observed at approximately 1,000 m depth and pCO_2 decreases towards the surface, where concentrations approximate those of atmospheric pCO_2 (e.g. Millero, 1996). Since the higher pCO_2 observed in the mesopelagic zone depends on the decomposition of sinking organic matter produced by primary production at the ocean surface, pCO_2 in the mesopelagic zone is higher in the subarctic area, because of the higher level of primary production compared to that in subtropical areas (Table 1). It is presumed that the tolerances of meso- or bathypelagic copepods may have been affected by adaptation to such naturally higher pCO_2 conditions.

4.4 Effect of CO_2 ocean sequestration

Dispersion of discharged CO_2 to levels of several tens of thousands of times the natural concentration, representing a pCO_2 increase of 100–200 μatm (Sato and Sato, 2002), has been suggested for the moving-ship ocean CO_2 sequestration project (Ozaki, 1997). From the results of this study, pelagic copepods are presumed not to be vulnerable to this level of increased pCO_2 caused by the dispersed CO_2. However, when liquid CO_2 is discharged from the pipe, pCO_2 is estimated to reach approximately 200,000 μatm near the nozzle before dispersion. Extrapolation of our results indicates that deep-living copepods in subtropical regions and in subarctic/transitional regions can survive under such concentrations for 2–3 and 24 hours, respectively. As a next step, it is necessary to verify this extrapolation to higher CO_2 concentrations. Furthermore, it is not likely that an individual animal would stay at any single concentration during the actual CO_2 sequestration, and it is therefore necessary to investigate the influence of CO_2 when concentrations vary with time.

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