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**Review**

# Sub-Lethal Effects of Elevated Concentration of $CO_2$ on Planktonic Copepods and Sea Urchins

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**Data concerning the effects of high $CO_2$ concentrations on marine organisms are essential for both predicting future impacts of the increasing atmospheric $CO_2$ concentration and assessing the effects of deep-sea $CO_2$ sequestration. Here we review our recent studies evaluating the effects of elevated $CO_2$ concentrations in seawater on the mortality and egg production of the marine planktonic copepod, *Acartia steueri*, and on the fertilization rate and larval morphology of sea urchin embryos, *Hemicentrotus pulcherrimus* and *Echinometra mathaei*. Under conditions of +10,000 ppm $CO_2$ in seawater (pH 6.8), the egg production rates of copepods decreased significantly. The survival rates of adult copepods were not affected when reared under increased $CO_2$ for 8 days, however longer exposure times could have revealed toxic effects of elevated $CO_2$ concentrations. The fertilization rate of sea urchin eggs of both species decreased with increasing $CO_2$ concentration. Furthermore, the size of pluteus larvae decreased with increasing $CO_2$ concentration and malformed skeletogenesis was observed in both larvae. This suggests that calcification is affected by elevated $CO_2$ in the seawater. From these results, we conclude that increased $CO_2$ concentration in seawater will chronically affect several marine organisms and we discuss the effects of increased $CO_2$ on the marine carbon cycle and marine ecosystem.**

Keywords:
· Rising atmospheric $CO_2$ concentration,
· $CO_2$ ocean sequestration,
· biological impact,
· sub-lethal effects,
· egg production,
· fertilization,
· morphology,
· copepods,
· sea urchins.

## 1. Introduction

For about 50 million years, the atmospheric concentration of $CO_2$ has been fluctuating between 180 ppm to 280 ppm. However, since the industrial revolution, the atmospheric $CO_2$ concentration has rapidly increased and recently it has exceeded 365 ppm (Bazzaz, 1990; Houghton *et al*., 1992; Keeling and Whorf, 1994). Human activity produces approximately 5.0–7.0 Gt C $y^{-1}$, about 2.0 Gt $y^{-1}$ of which is absorbed into the ocean and 3.3 Gt $y^{-1}$ of which accumulates continuously in the atmosphere (Keeling *et al*., 1996; Takahashi *et al*., 1997; Battle *et al*., 2000; Sarmiento *et al*., 2000).

$CO_2$ absorption by the ocean is driven by differences in the partial pressure of $CO_2$ ($PCO_2$) between the atmosphere and the ocean surface, which is mainly controlled by a "biological pump". The pump may be defined as the movement of $CO_2$ that enters into the ocean from the atmosphere to the deep-ocean floor through biological processes, i.e. photosynthetic fixation of $CO_2$ by phytoplankton, passive export of organic carbon (e.g. fecal pellets of zooplankton, detritus, and dead organisms) and carbonates (e.g. shells and bones) by gravitation, or through vertical migration of zooplankton to the deep ocean (Fowler and Knauer, 1986; Zhang and Dam, 1997; Rivkin and Legendre, 2002).

In the surface ocean, there exists a continuous sea-air gas exchange (Takahashi *et al*., 1997); thus, the $CO_2$ concentration is in equilibrium in respect with the ocean surface and the atmosphere. However, $CO_2$ in the deep ocean is isolated from the atmosphere because the pycnocline prevents free gas exchange between the atmosphere and the deep ocean. The deep sea serves, there-

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fore, as a carbon reservoir. Further increases in temperature, due to increased atmospheric $CO_2$ (Houghton *et al*., 2001), are predicted to increase sea surface stratification, which will in turn reduce the downward flux of carbon to the deep ocean (Sarmiento *et al*., 1998; Arrigo *et al*., 1999).

In addition to global warming, the elevation of atmospheric $CO_2$ also leads to increased $CO_2$ concentration in seawater, which in turn drives an increase in concentration of dissolved inorganic carbon (DIC i.e. $CO_{2(aq)}$, $HCO_3^-$, and $CO_3^{2-}$) and a decrease in pH at the ocean surface. By the end of this century, the concentration of $CO_2$ in the air is expected to rise to 750 ppm, which would reduce the pH of the surface seawater from 8.0 to 7.8 (Wolf-Gladrow *et al*., 1999). Between the years 2100 and 2200, atmospheric $CO_2$ concentration is expected to be as high as 1,500 to 2,100 ppm, which will cause an additional decline in pH of approximately 0.5 (Wigley *et al*., 1996). Therefore, in addition to the indirect effects of global warming, the direct effects of increased $CO_2$ concentration in seawater are expected to have a significant influence on marine organisms and potentially the functioning of the biological pump.

Because of the anticipated effects of increased atmospheric $CO_2$ concentration on marine and terrestrial ecosystems (Parmesan and Yohe, 2003; Root *et al*., 2003), several strategies to control the rise of anthropogenic $CO_2$ have been proposed. Among these, the direct injection of $CO_2$ into the deep ocean is receiving increasing attention (Marchetti, 1977; Liro *et al*., 1992; Ohsumi, 1995; Herzog *et al*., 1996; Broecker, 1997; Caulfield *et al*., 1997; Parson and Keith, 1998). Such direct-injection methods would lead to $CO_2$ concentrations as high as 20,000 ppm near the injection point, resulting in seawater pH less than 5.8 (Herzog *et al*., 1996). The numerical model of the $CO_2$ plume method suggests that $CO_2$-enriched seawater would diffuse into the surrounding seawater, and that the volume of acidic (pH < 7.0) seawater could be hundreds of cubic kilometers (Caulfield *et al*., 1997; Huesemann *et al*., 2002).

Elevated $CO_2$ concentration and decreased pH in seawater could seriously impact the marine organisms. Therefore, to both anticipate future impacts of elevated atmospheric $CO_2$ concentration and the effects of direct $CO_2$ injection on marine ecosystems, we need to understand the impacts of elevated $CO_2$ concentration and low pH in the seawater on marine biota.

Effects of elevated $CO_2$ concentration have been most often studied on corals and phytoplankters. The growth rate of phyoplankton has been reported as being influenced by fluctuations in DIC concentration (Riebesell *et al*., 1993; Wolf-Gladrow *et al*., 1999). In addition, the calcification rate of corals and coccolithophores decreases with increased $CO_2$ concentration (Gattuso *et al*., 1998; Riebesell *et al*., 2000). However, to understand the effects of elevated $CO_2$ on the whole marine ecosystem, and on the ocean carbon cycle, studies of organisms at higher trophic levels are also needed.

The lethal effects of low pH have been studied for several marine invertebrates, using strong acids such as hydrochloric acid (HCl) and sulfuric acid ($H_2SO_4$) (Knutzen, 1981; Adams *et al*., 1997; Yamada and Ikeda, 1999). Kuwatani and Nishii (1969) studied the sub-lethal effects of low pH due to strong acids; they detected that the growth rate of the pearl oyster decreased with lowering pH. Furthermore, in a recent study, Kikkawa *et al*. (2004) compared the acute toxicity of $CO_2$-acidified and HCl-acidified seawater for eggs and larvae of marine fish and found that seawater acidified by $CO_2$ was more toxic.

Here, we evaluate the lethal and sub-lethal effects of increased $CO_2$ on zooplankton (planktonic copepods and sea urchin embryos). From the results of increased $CO_2$ on the egg production of the planktonic copepod (*Acartia steueri*), and on the fertilization and morphology of the pluteus larvae of sea urchins (*Hemicentrotus pulcherrimus* and *Echinometra mathaei*), the impacts of increased $CO_2$ on the marine ecosystem are discussed.

## 2. Materials and Methods

### *2.1 Survival and egg production of Acartia steueri*

The copepod *Acartia steueri* was collected from Tanabe Bay, off coast of the Kii Peninsula, Honshu Island, Japan, near the Seto Marine Biological Laboratory of Kyoto University. Experiments were conducted from April to June 2003. Plankton samples were collected and brought to the laboratory within one hour. Adult females were selected from the sample under a stereo-microscope and incubated for one day to acclimate them to laboratory conditions.

All test seawater, including control, was enriched with phytoplankton *Isochrysis galbana* ($4 \times 10^4$ cells $mL^{-1}$) and *Chaetoceros gracilis* ($0.5 \times 10^4$ cells $mL^{-1}$) as food of copepods. Control seawater was then bubbled with atmospheric air at a flow rate of 500 mL $min^{-1}$ until the pH of the seawater stabilized. Experimental seawater was bubbled with gas mixtures of atmospheric air at a flow rate of 500 mL $min^{-1}$ and 1.0 or 5.0 mL $min^{-1}$ $CO_2$ until the pH of the seawater stabilized. The $PCO_2$ values of supplied gas was 2,000 and 10,000 ppm higher than the control (+2,000 ppm and +10,000 ppm), respectively. The flow rates of air and $CO_2$ were adjusted using a flow meter (Kofloc RK1600R). The pH of the each seawater was measured using a pH meter (Toledo MP225) immediately before the experiments were performed (Table 1).

Vials (50 mL) were filled with the test seawater; 7 to 10 replicates were prepared for each $CO_2$ condition. The

Table 1. $CO_2$ condition (control concentration, +2,000 or +10,000 ppm; see text for detail) and pH of the test seawater measured before and after incubation of *Acartia steueri*.

| $CO_2$ | pH | |
|---|---|---|
| | before | 2 days after |
| control | 8.14 ± 0.04 | 8.17 ± 0.05 |
| +2,000 | 7.40 ± 0.08 | 7.55 ± 0.10 |
| +10,000 | 6.84 ± 0.11 | 7.02 ± 0.09 |

Means ± S.D. (standard deviation).

Table 2. $CO_2$ condition (control concentration, +2,000 or +10,000 ppm; see text for detail) and pH of the seawater measured before and after incubation of sea urchin *Hemicentrotus pulcherrimus* and *Echinometra mathaei*.

| $CO_2$ condition | *H. pulcherrimus* | | *E. mathaei* |
|---|---|---|---|
| | pH (before) | pH (after) | pH (before) |
| control | 7.99 ± 0.10 | 7.97 ± 0.16 | 8.11 ± 0.00 |
| +500 | 7.74 ± 0.02 | 7.73 ± 0.13 | 7.80 ± 0.01 |
| +1,000 | 7.59 ± 0.00 | 7.56 ± 0.11 | 7.68 ± 0.04 |
| +2,000 | 7.35 ± 0.04 | 7.33 ± 0.08 | 7.34 ± 0.02 |
| +5,000 | 7.03 ± 0.07 | 7.16 ± 0.06 | 7.13 ± 0.01 |
| +10,000 | 6.83 ± 0.01 | 6.95 ± 0.10 | 6.78 ± 0.00 |

Means ± S.D.

vials were filled carefully to eliminate all air bubbles that could enable the exchange of $CO_2$ with the seawater, and closed with screw caps. Two female copepods were placed in each vial and incubated for 8 days at 24°C under a 14:10 light/dark cycle.

The survival of the adult females and the number of both unhatched and hatched eggs were recorded every 2 days. The seawater in each vial was replaced with fresh test seawater every 2 days. The pH of the seawater was measured after 2 days of incubation to verify that the $CO_2$ concentration in the seawater was constant for this period (Table 1). Egg production was expressed as the mean egg production of all egg-laying females during each 2-day interval (eggs female$^{-1}$ 2 days$^{-1}$). Each experiment was conducted twice.

Two-way analysis of variance (ANOVA) was used to evaluate the effects of $CO_2$ concentrations on the survival and egg production rates of adult female copepods. To test for significant differences in the egg production rate among $CO_2$ concentrations (control, +2,000 ppm, +10,000 ppm), we used the Tukey-Kramer test.

### *2.2 Fertilization rate and pluteus larval morphology*

The sea urchins *H. pulcherrimus* and *E. mathaei* were collected from the subtidal rocky shore near the Seto Marine Biological Laboratory in Wakayama, Japan. Due to differences in breeding season, experiments using *H. pulcherrimus* were conducted from January to March 2002; those using *E. mathaei* were conducted from June to October 2002.

To prepare the test seawater with different $CO_2$ concentrations, seawater was bubbled with gas mixtures of atmospheric air at a flow rate of 500 mL min$^{-1}$ and 0.25 (+500 ppm), 0.5 (+1,000 ppm), 1.0 (+2,000 ppm), 2.5 (+5,000 ppm) or 5.0 mL min$^{-1}$ $CO_2$ (+10,000 ppm) until the pH stabilized. The control seawater was aerated only by atmospheric air, with a flow rate of 500 mL min$^{-1}$. The pH of the seawater was measured using a pH meter before performing the experiment (Toledo MP225; Table 2).

To induce spawning, 0.1 M acetylcholine chloride was injected into the perivisceral cavity of individual sea urchins. *Hemicentrotus pulcherrimus* or *E. mathaei* eggs were placed in Petri dishes (10 × 1.5 cm) filled with each test seawater. Six replicate Petri dishes were used for each $CO_2$ concentration. One minute after the eggs were added, one drop of sperm suspension was added to each dish.

Fifteen minutes later, about 500 embryos were fixed using a 10% buffered-formalin seawater solution to determine the fertilization rate. Of these embryos, 300 were randomly selected for investigation. Fertilization was defined as the presence of a fertilization membrane. Six and three batches were used for the *H. pulcherrimus* and *E. mathaei*, respectively.

Next we transferred 100 more embryos from each dish into 50-mL vials containing the test seawater solutions. The vials were filled carefully to eliminate all air bubbles that could enable the exchange of $CO_2$ with the seawater, and closed with screw caps. These were incubated for 3 days, at which they developed into four-armed pluteus larvae. The *H. pulcherrimus* and *E. mathaei* embryos were incubated at 14°C and 24°C respectively. For *H. pulcherrimus*, the pH of the seawater was measured after three days of culture to verify that the concentration of $CO_2$ had not changed (Table 2). For *E. mathaei*, the pH was not measured after the 3 day incubation because it was already confirmed for *H. pulcherrimus* that the $CO_2$ concentration almost did not change during the incubation. One drop of concentrated formalin was added to each vial to halt development and 10 larvae from each vial were sampled randomly and mounted on glass slides. These larvae were photographed and the length from the tip of the arm to the posterior end of larval body (body length) was measured under a microscope using an ocular micrometer. For the experiment measuring the size of pluteus larvae, five and three batches were used for the *H.*

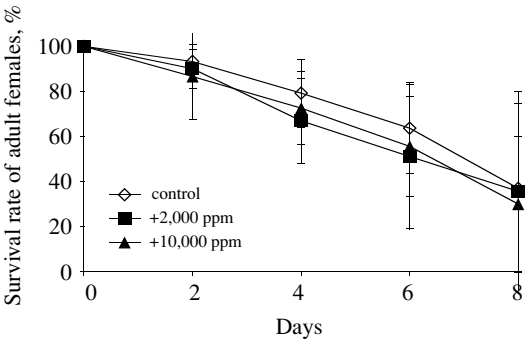


Fig. 1. Survival rate of adult female copepods (*Acartia steueri*) cultured in seawater with different $CO_2$ concentrations.

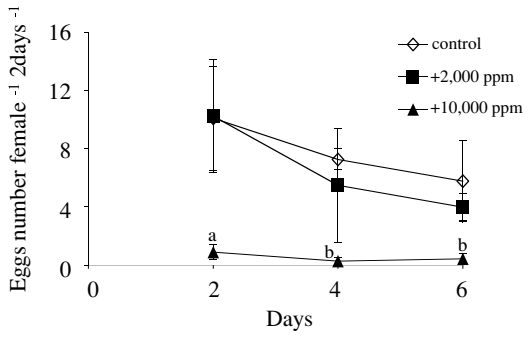


Fig. 2. Egg production rate of adult female copepods (*Acartia steueri*) cultured in seawater with different $CO_2$ concentrations. Means were significantly different compared to (a) control and +2,000 ppm and (b) control (Tukey-Kramer, $p < 0.05$).

*pulcherrimus* and *E. mathaei*, respectively.

Spearman's rank correlation coefficient ($r_S$) was calculated to compare the effect of increased $CO_2$ concentration on the fertilization rate and the pluteus larvae body length. Tukey-Kramer a *posteriori* comparisons were performed to evaluate the effects of $CO_2$ on the fertilization rate and on the size of pluteus larvae, as compared to controls.

## 3. Results

### *3.1 Survival and egg production of Acartia steueri*

The survival rates of adult females decreased with time in all test seawater treatments (ANOVA, $df = 4$, $F = 22.84$, $p < 0.01$; Fig. 1). No differences in survival rates with $CO_2$ concentration were observed throughout the experiment (ANOVA, $df = 2$, $F = 0.15$, $p = 0.85$; Tukey-Kramer; Fig. 1). However, significant differences in egg production rates were detected at different $CO_2$ concentrations (ANOVA, $df = 2$, $F = 10.58$, $p < 0.05$; Fig. 2).

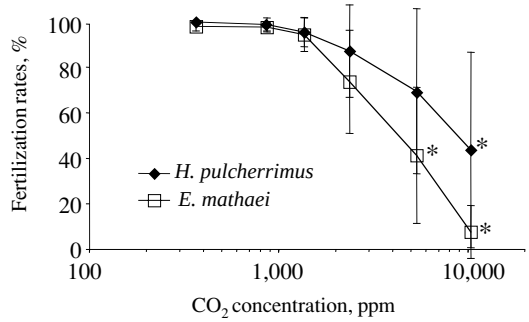


Fig. 3. Fertilization rate of *Hemicentrotus pulcherrimus* and *Echinometra mathaei* in seawater with different $CO_2$ concentrations. Each value is the average of six and three batches for *H. pulcherrimus* and for *E. mathaei* respectively. Error bars = S.D. $r_S$ = Spearman's rank correlation coefficient. *: Significant difference from control (Tukey-Kramer, $p < 0.05$).

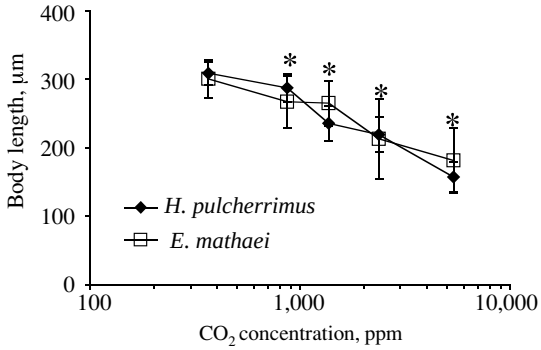


Fig. 4. Body length of four-armed pluteus larvae of *Hemicentrotus pulcherrimus* and *Echinometra mathaei* cultured for 3 days in seawater with different $CO_2$ concentrations. Each value is the average of five and three batches for *H. pulcherrimus* and *E. mathaei*, respectively. Error bars = S.D. *: Significant difference from control (Tukey-Kramer, $p < 0.05$). Data for larvae cultured in +10,000 ppm $CO_2$ are not shown because almost no embryos developed to this stage.

The egg production rate of copepods cultured for 4 and 6 days in seawater with +2,000 ppm $CO_2$ was slightly lower than copepods cultured in control seawater, although the differences were not significant (Tukey-Kramer; Fig. 2). The copepods cultured in seawater with +10,000 ppm $CO_2$ produced almost no eggs during the experiments; their egg production rate differed significantly from both the control and +2,000 ppm seawater treatments (Tukey-Kramer, $p < 0.05$; Fig. 2). When the copepods cultured in seawater with +10,000 ppm $CO_2$ were transferred to control seawater, they resumed normal egg production (data not shown).

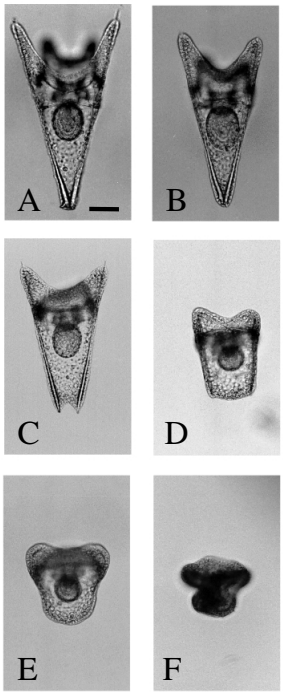


Fig. 5. Morphology of four-armed *Hemicentrotus pulcherrimus* pluteus larvae cultured for 3 days in seawater with different $CO_2$ concentrations. A: control; B: +500 ppm; C: +1,000 ppm; D: +2,000 ppm; E: +5,000 ppm; and F: +10,000 ppm. Scale bar = 50 $\mu$m.

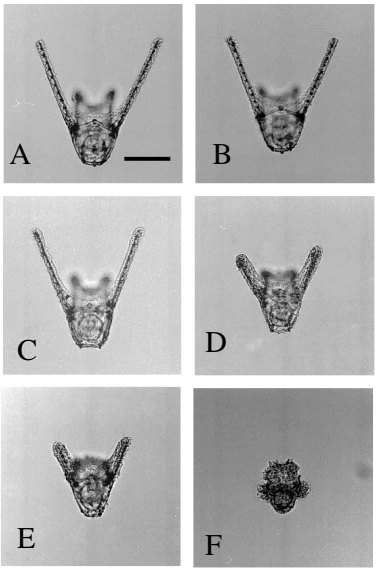


Fig. 6. Morphology of four-armed *Echinometra mathaei* pluteus larvae cultured for 3 days in seawater with different $CO_2$ concentrations. A: control; B: +500 ppm; C: +1,000 ppm; D: +2,000 ppm; E: +5,000 ppm; and F: +10,000 ppm. Scale bar = 50 $\mu$m.

### *3.2 Fertilization rate and pluteus larval morphology*

Fertilization rates decreased with increasing $CO_2$ concentration in both *H. pulcherrimus* ($r_S = -0.69$, $p < 0.01$) and *E. mathaei* ($r_S = -0.87$, $p < 0.01$; Fig. 3). Fertilization rates decreased slightly when the $CO_2$ concentration exceeded +500 ppm (pH 7.8), and this tendency became more pronounced at higher $CO_2$ concentrations. At +10,000 ppm (pH 6.8), the fertilization rate of *H. pulcherrimus* and *E. mathaei* decreased to 44 and 7.6%, respectively (Fig. 3). For *H. pulcherrimus*, the fertilization rate of eggs inseminated under seawater with +10,000 ppm $CO_2$ was significantly lower than that of control (Tukey-Kramer, $p < 0.05$; Fig. 3). The same result was also observed for *E. mathaei* in seawater with + 5,000 and +10,000 ppm $CO_2$ (Tukey-Kramer, $p < 0.05$; Fig. 3).

The body length of the pluteus larvae tended to decrease with increasing $CO_2$ concentration in both *H. pulcherrimus* ($r_S = -0.76$, $p < 0.01$) and *E. mathaei* ($r_S = -0.66$, $p < 0.01$; Fig. 4). For both sea urchin species, larval body length was significantly smaller in test seawater of all $CO_2$ concentrations than it was in control seawater (Tukey-Kamer, $p < 0.05$; Fig. 4). Data for the pluteus larvae cultured in +10,000 ppm $CO_2$ (pH 6.8) are not shown in Fig. 4 because almost no embryos developed to this stage in either sea urchin species.

The morphology of pluteus larvae was also affected by the $CO_2$ concentration. Malformed skeletogenesis was observed in both *H. pulcherrimus* (Fig. 5) and *E. mathaei* (Fig. 6). *Hemicentrotus pulcherrimus* larvae cultured in seawater that contained $CO_2$ at concentrations greater than +1,000 ppm (pH < 7.6) were smaller in size and more trapeziform, as compared to their normal triangular form (Figs. 5C–F). The larvae of both sea urchins that were cultured at +10,000 ppm $CO_2$ (pH 6.8) were extremely abnormal: they lacked arms, spicules, stomodaeum and intestines (Figs. 5F and 6F).

## 4. Discussion

### *4.1 Effects of increased $CO_2$ on marine organisms*

Our results show that the planktonic copepods and sea urchin embryos were affected by $CO_2$ in a concentration-dependent manner. Sub-lethal effects on reproduction such as the reduced egg production rate of *A. steueri* and fertilization rate or abnormal morphology of sea urchin larvae were observed at $CO_2$ concentrations that did not have lethal effects.

The lethal effects of low pH, produced using strong acids, have been studied in several planktonic copepods (Rose *et al*., 1977; Yamada and Ikeda, 1999). These stud-

ies have shown that only acidification to pH less than 6.5 has harmful consequences, such as a decreased survival rate. Knutzen (1981) reviewed the effects of low pH on several marine organisms and reported that most were markedly affected when pH was below 7.0. Consistent with these observations, the female copepods examined in this study were not lethally affected when reared in seawater at $CO_2$ concentration ≤ +10,000 ppm (pH > 6.8) for 8 days. Therefore, $CO_2$ concentrations that cause pH to be lowered by ≥1.0, in relation to normal seawater (~pH 8.0), may not be acutely toxic to marine zooplankton. However, Yamada and Ikeda (1999) demonstrated that the tolerable pH range for marine plankton decreases with increasing exposure time. Thus, longer exposure times in our study could have revealed toxic effects of elevated $CO_2$ concentrations on adult copepods.

In contrast, in this study, sub-lethal effects were observed for both adult copepods and sea urchin embryos at $CO_2$ concentrations ≤ +10,000 ppm (pH > 6.8). By evaluating the impact of $CO_2$ on sea urchin embryos, it was shown that the fertilization rate of the eggs, the morphology and skeletogenesis of pluteus larvae were affected by increased $CO_2$ concentrations. Because the skeleton of the sea urchin is made of calcium carbonate, elevated $CO_2$ concentrations may reduce calcification rates, as has been observed in corals (Gattuso *et al*., 1998) and coccolithophores (Riebesell *et al*., 2000). Bamber (1987) studied the effects of acidic seawater on clams and showed that shell dissolution occurred at pH < 7.55, and feeding and growth rate were inhibited at pH < 7.0. In addition, Grice *et al*. (1973) observed that the hatching rate of eggs of the marine copepod *Temora longicornis* was affected at pH levels that did not affect the survival rate of adult copepods. We recently studied the effect of increased $CO_2$ on the hatching rate and nauplius larval mortality of the copepod *Acartia erythraea*. It was shown that the hatching rate decreased significantly ($p < 0.05$) at +10,000 ppm $CO_2$ seawater (pH = 6.8) and the larval mortality increased significantly ($p < 0.05$) at +5,000 ppm $CO_2$ seawater (pH = 7.0) (Kurihara *et al*., 2004). Therefore, it is thought that marine organisms were sub-lethally affected even at $CO_2$ concentrations that did not induce lethal effects. Even though adult organisms may not be lethally affected, the population will be affected if the egg production rate decreases, eggs are not fertilized, and larvae do not develop normally (Legendre and Rivkin, 2002). These effects may result in drastic changes to the whole ecosystem over time.

The effects of increased $CO_2$ are supposed to be highly related with the effects of low pH (Heisler, 1993). Low pH is known to change the activity of enzymes (Hochachka and Somero, 2002) and inhibit protein synthesis (Morgan *et al*., 2001). Elevated $CO_2$ concentration probably affects reproduction and development by altering the enzyme activity or inhibiting protein synthesis. These stresses may also affect the physiology and lead to sub-lethal effects such as retarded growth and reduced metabolic activity. However, recent studies have shown that the effects of seawater acidified by strong acid and $CO_2$ are different (Watanabe *et al*., 2001; Kikkawa *et al*., 2004). We also observed that $CO_2$ seawater affects the fertilization rate at higher pH values than strong acid (Kurihara and Shirayama, 2004). Therefore, it has been suggested that the effects of $CO_2$ are not only due to the decrease of pH but there is another mechanism that also affects marine organisms.

### *4.2 Effects of increased atmospheric $CO_2$ concentration on the shallow-water ecosystem*

The present results suggest that in addition to the effects of global warming, increased $CO_2$ concentration in seawater, due to the rise of atmospheric $CO_2$ concentration, would directly affect marine organisms. Moreover, it is thought that synergistic effects of both increased $CO_2$ concentration and temperature will impact on marine organisms. Indeed, Morgan *et al*. (2001) showed that an increase of 2°C increases the effect of low pH on freshwater fishes. In our study, *E. mathaei*, which was cultured at a higher temperature than *H. pulcherrimus*, was more affected by increased $CO_2$ (Fig. 3). Synergistic effects of increased $CO_2$ concentration (or reduced pH) and elevated temperatures require further evaluation.

As the atmospheric $CO_2$ concentration continue to increase, it is predicted that marine organisms will be impacted by both the elevation of temperature and $CO_2$ concentration in the seawater. Because, these marine organisms play an important role in the biological pump, effects on them may decrease the carbon flux to the deep sea. In addition, the present results suggest that organisms utilizing calcium carbonate in their bodies (such as coralline algae, corals, mollusks, foraminiferans, and echinoderms) will be principally affected by increased $CO_2$ concentration. Because the calcifying activities of marine biota mediate the transfer of $CO_2$ into the deep-ocean (Volk and Hoffert, 1985), effects on these marine biota may also affect the biological pump. These organisms are important members of coastal marine ecosystems, which are the most productive and diverse of all marine ecosystems (Levinton, 1995). Several echinoderms are keystone species in rocky tidal shores (Paine, 1966, 1974; Sebens, 1985, 1986; Benedetti-Cecchi, 2000). Mobile deposit-feeding clams and sediment-dwelling sea urchins are critical species for bioturbation of sediments (Rhoads and Young, 1970; Tsuchiya and Kurihara, 1981; Austen *et al*., 1998). Therefore, impacts on these animals could have serious consequences for the health and biodiversity of marine ecosystems.

### 4.3 *Effects of direct $CO_2$ injection on the deep-water ecosystem*

Our results also have implications that are relevant to deep-ocean $CO_2$ sequestration strategies. In this study, the effects of increased $CO_2$ concentration were observed using shallow-water species; the effects on deep-sea species are still unknown. However, deep-sea organisms are considered to be more sensitive to environmental change than are shallow-water species, because the deep-sea environment is very stable (Shirayama, 1997).

The life history of deep-sea organisms is characterized by slow growth, long life span, and a low reproductive rate, all of which are influenced by a low metabolic rate (Nybakken, 2001). Therefore, effects on reproduction and early stage embryo morphology, such as those described here, are likely to have even greater consequences for deep-sea species. In addition, deep-sea organisms have slow colonization rates (Nybakken, 2001). Therefore, impacts on deep-sea communities from injected $CO_2$ could persist for longer periods than impacts on neritic systems.

In conclusion, we suggest that both the elevation of atmospheric $CO_2$ concentration and direct injection of $CO_2$ into the deep sea would affect marine organisms, which might, in turn, slow the biological pump and ultimately change the oceanic carbon cycle.


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