

Effects of increased atmospheric CO₂ on sea urchin early development

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ABSTRACT: Increased carbon dioxide (CO₂) concentration in the atmosphere will change the balance of the components of carbonate chemistry and reduce the pH at the ocean surface. Here, we report the effects of increased CO₂ concentration on the early development of the sea urchins *Hemicentrotus pulcherrimus* and *Echinometra mathaei*. We examined the fertilization, early cleavage, and pluteus larval stage to evaluate the impact of elevated CO₂ concentration on fertilization rate, cleavage rate, developmental speed, and pluteus larval morphology. Furthermore, we compared the effects of CO₂ and HCl at the same pH in an attempt to elucidate any differences between the two. We found that fertilization rate, cleavage rate, developmental speed, and pluteus larval size all tended to decrease with increasing CO₂ concentration. Furthermore, CO₂-seawater had a more severe effect than HCl-seawater on the fertilization rate. By contrast, the effects on cleavage rate, developmental speed, and pluteus larval morphology were similar for CO₂- and HCl-seawater. Our results suggest that both decreased pH and altered carbonate chemistry affect the early development and life history of marine animals, implying that increased seawater CO₂ concentration will seriously alter marine ecosystems. The effects of CO₂ itself on marine organisms therefore requires further clarification.

KEY WORDS: CO₂ · pH · Sea urchin · Early development · Biological impact · Global change

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INTRODUCTION

Increasing atmospheric carbon dioxide (CO₂) concentration (Houghton et al. 1992, Keeling & Whorf 1994) is predicted to lead to a rise in CO₂ concentrations at the ocean surface, due to continuous gas exchange between the air and seawater (Takahashi et al. 1997). By the end of this century, the concentration of CO₂ in the air is expected to rise to 750 ppm, which is about twice the present 365 ppm, and the CO₂ concentration in seawater will attain the same level (IPCC 2001). This will in turn increase the concentration of dissolved inorganic carbon (DIC) (i.e. CO_{2(aq)}, HCO₃⁻, and CO₃²⁻), which will then lead to a decrease in pH at the ocean surface. Current models predict that the pH of surface seawater will drop from 8.0 to 7.8 by the year 2100 (Wolf-Gladrow et al. 1999). Between 2100 and 2200, the atmospheric CO₂ concentration is expected to increase to the range of 1500 to 2100 ppm resulting in a further decline in pH value (Sundquist 1993, Wigley et al. 1996).

To modulate the increasing atmospheric CO₂, some have proposed the sequestration of CO₂ captured from power plants, which would be piped into the deep ocean (Nozaki 1991, Liro et al. 1992, Ohsumi 1995, Auerbach et al. 1996). The CO₂ concentration close to the injection point is, however, expected to be as high as 20 000 ppm and the pH there less than 5.8 (Herzog et al. 1996). Furthermore, models of CO₂ disposal suggest that the volume of acidic (pH < 7.0) seawater could be hundreds of cubic kilometers (Caulfield et al. 1997a, Huesemann et al. 2002). These changes in seawater chemistry might have a serious, direct impact on marine organisms.

The effects of lowered pH have been studied experimentally in many species of marine organism using strong acids, such as hydrochloric acid (HCl) and sulfuric acid (H₂SO₄) (Calabrese & Davis 1966, Kuwatani & Nishii 1969, Rose et al. 1977, Bamber 1987, 1990, Yamada & Ikeda 1999). These studies have shown that acidification below pH 7.0 has harmful consequences (such as decreased survival rate) in many organisms.

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Otherwise, in most studies, pH fluctuations in the range of 0.5 to 1.0 unit have little influence on survival (Knutzen 1981). However, these studies are not sufficient to evaluate the environmental impact of raised atmospheric CO₂ on marine ecosystems for 3 reasons. First, studies have mostly examined survival rates at low pH; little attention has been paid to other biological processes, such as reproduction, growth rates, and morphological abnormalities, or to the effects of small changes in pH (<1.0 unit). Second, most studies have focused on the impact on adult organisms. The impact on early developmental stages should also be evaluated, since these stages are more sensitive to environmental stress. Third, increased CO₂ concentration may have other effects on marine organisms besides those caused by reducing pH. In this study, we therefore examined the effect of a small fluctuation in pH resulting from an increase in atmospheric CO₂ concentrations.

Watanabe et al. (2001) compared the survival rates of copepods raised in seawater acidified to the same degree with either CO₂ or HCl, and found that CO₂ had a greater impact. The impact of raised CO₂ concentrations in seawater has been examined mainly for coral reef organisms and phytoplankton. Elevated CO₂ concentrations and low pH are reported to reduce the calcite production rate of coccolithophores and corals (Riebesell et al. 1993, 2000, Gattuso et al. 1998). Thornton & Shirayama (2001) found that both sea urchins and snails raised in seawater containing elevated CO₂ concentrations had lower survival and growth rates after a 10 wk exposure.

We have focused here on the impacts on early development to evaluate sub-lethal effects of increased CO₂ concentrations. We studied 2 sea urchin species, *Hemicentrotus pulcherrimus* and *Echinometra mathaei*, as sea urchins are frequently indicator species of environmental alteration (Kobayashi 1971, 1995). The effects on the early development of the sea urchins were examined by studying (1) fertilization rate, (2) cleavage rate, (3) developmental speed, and (4) pluteus morphology. Furthermore, we compared the effects of CO₂ and HCl at the same pH to examine the influence of a change in DIC concentration on the early development of marine invertebrates.

MATERIALS AND METHODS

Test animals. The sea urchins *Hemicentrotus pulcherrimus* and *Echinometra mathaei* were collected from the subtidal rocky shore near the Seto Marine Biological Laboratory, Wakayama, Japan. Due to differences in breeding season, experiments using *H. pulcherrimus* were conducted from January to March at 14°C,

whereas those using *E. mathaei* were carried out from June to October at 24°C. To select the best quality gametes, only batches that had fertilization rates exceeding 95% in control seawater were used. *H. pulcherrimus* was used in all experiments, while *E. mathaei* was used only in the fertilization bioassay.

Preparing seawater for culture. Seawater filtered through a Millipore filter was placed in six 1 l beakers in each experiment, and the CO₂ concentration of the seawater was controlled by aerating the water with air containing different concentrations of CO₂ (CO₂-seawater). The flow rates of air and CO₂ were adjusted using a flow meter (Kofloc). The control was aerated with 500 ml min⁻¹ air, which contained 365 ppm CO₂. To alter the CO₂ concentration, the water was also aerated with 0.25, 0.5, 1.0, 2.5 and 5.0 ml min⁻¹ CO₂, respectively. The CO₂ concentrations of the gas were as follows: control, +500, +1000, +2000, +5000, and +10 000 ppm. The seawater was aerated by experimental gases for more than 2 h, until the partial pressure of CO₂ concentrations (P_{CO2}) in the seawater became equilibrated with the gas (Table 1). The pH of the seawater was measured using a pH meter (Toledo) just before performing the experiment (Table 1). To prepare HCl-seawater, filtered seawater was placed in another six 1 l beakers and 1 N HCl was added to each to match the pH of the seawater in the respective CO₂ beakers.

Bioassay. To induce spawning, 0.1 M acetylcholine chloride was injected into the perivisceral cavity of individual sea urchins. Eggs were rinsed several times with filtered seawater and maintained in a 200 ml beaker at 4°C until use. Sperm were collected with a pipette and kept at 4°C as dry sperm. The dry sperm were diluted with filtered seawater just before the experiment. To determine the effect of CO₂ on the early development of sea urchins, eggs of *Hemicentrotus pulcherrimus* or *Echinometra mathaei* were placed in 6 Petri dishes (10 × 1.5 cm) filled with CO₂-seawater with CO₂ concentrations of 365 (control), +500, +1000, +2000, +5000, and +10 000 ppm. After 1 min, a drop of sperm suspension was added to each dish. Fifteen min

Table 1. The P_{CO2} and pH of seawater aerated using 6 different CO₂ concentrations before the experiment and the pH of the seawater incubated after 3 d (ANOVA p < 0.01). Means ± SD

CO ₂ condition	P _{CO2} (kPa)	<i>H. pulcherrimus</i> pH (before) pH (after)		<i>E. mathaei</i> pH (before)
Control	0.036	8.01 ± 0.10	7.97 ± 0.19	8.11 ± 0.03
+ 500	0.086	7.77 ± 0.07	7.64 ± 0.15	7.82 ± 0.09
+ 1000	0.136	7.61 ± 0.05	7.57 ± 0.10	7.71 ± 0.09
+ 2000	0.236	7.38 ± 0.04	7.33 ± 0.05	7.33 ± 0.05
+ 5000	0.536	7.03 ± 0.07	7.10 ± 0.09	7.12 ± 0.02
+ 10 000	1.036	6.83 ± 0.05	6.88 ± 0.09	6.79 ± 0.02

after insemination, about 500 embryos were transferred from each dish into three 20 ml vials and 100 embryos were transferred into a 50 ml vial.

To determine the fertilization rate, 10% buffered-formalin seawater solution was added to one of the 20 ml vials for each CO₂ concentration. The remaining 20 ml and 50 ml vials were filled with seawater at the respective CO₂ concentrations and any air spaces were removed to prevent exchange of CO₂ with the ambient air. One 20 ml vial for each CO₂ concentration was incubated for 105 min and the other for 210 min after insemination, to determine cleavage rate and developmental speed. The 50 ml vials were incubated for 3 d to determine pluteus larval morphology. After 3 d of incubation, the pH of the seawater was measured to verify that the concentration of CO₂ had not changed (Table 1), and one drop of concentrated formalin was then added to each bottle to halt development.

The same procedure as described above was also used for HCl-seawater instead of CO₂-seawater.

Fertilization. To study the effect on the fertilization rate, 300 eggs were randomly sampled from each 20 ml vial, cultured for 15 min after insemination, and the numbers of fertilized eggs were counted under a microscope. Fertilization was defined as the presence of a fertilization membrane. For *H. pulcherrimus*, 6 and 4 batches were used for the CO₂- and HCl-seawater experiments, respectively. For *E. mathaei*, 3 and 5 batches were used.

Cleavage. To study the effect of CO₂ concentration on cleavage rate and developmental speed, 300 randomly sampled *Hemicentrotus pulcherrimus* eggs, that had been incubated for 105 or 210 min after insemination in the 20 ml vials, were observed under a microscope and the stage of each embryo was recorded. Six and 4 batches were used for the CO₂- and HCl-seawater experiments, respectively.

Pluteus larvae. To determine the effects of CO₂ concentration on pluteus larval morphology, 10 *Hemicentrotus pulcherrimus* larvae were sampled randomly from each 50 ml vial and mounted on glass slides. The mounted larvae were photographed and the overall length (OL), postoral arm length (PL), and body length (BL) of each larva were measured under a microscope using an ocular micrometer (Fig. 1). Six and 3 batches were used for the CO₂- and HCl-seawater experiments, respectively.

Data analysis. The means and standard deviations were computed for each experiment. Spearman's rank correlation coefficient (R_s) was calculated to compare the effects of lowering pH with CO₂ or HCl on fertilization rate, OL, PL, and BL. The Tukey-Kramer test was performed to evaluate effects of CO₂ and HCl on fertilization rate, cleavage rate and the lengths of OL, PL, and BL. Two-way repeated ANOVA was used to com-

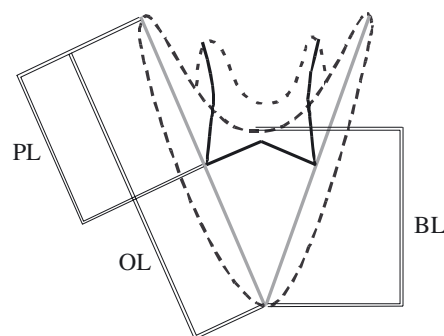


Fig. 1. *Hemicentrotus pulcherrimus*. Three morphometric measurements of the 4-armed pluteus larvae: (1) overall length (OL), (2) postoral arm length (PL), and (3) body length (BL)

pare the results of CO₂-seawater and HCl-seawater experiments. One-way analysis of variance (ANOVA) was used to compare the effects of CO₂ and HCl on the percentage of embryos at each developmental stage in the cleavage analysis.

RESULTS

Relationship between CO₂ concentration and pH

The pH of the seawater decreased with increasing CO₂ concentration (Table 1; ANOVA, $p < 0.001$). A slight difference in pH was observed between the seawater used for *Hemicentrotus pulcherrimus* and *Echinometra mathaei*, but the difference was not significant. The pH of CO₂-seawater incubated for 3 d changed within the range of 0.04 to 0.13 (Table 1). For HCl-seawater, the pH changed within the same range as CO₂-seawater during the experiment (data not shown).

Effects on fertilization

The fertilization rate of *Hemicentrotus pulcherrimus* tended to decrease with pH in both CO₂-seawater ($R_s = 0.74$, $p < 0.01$) and HCl-seawater ($R_s = 0.72$, $p < 0.01$; Fig. 2). However, the effect differed between the 2 acids. With CO₂-seawater, the fertilization rates decreased slightly compared to the control when the concentration of CO₂ exceeded +500 ppm (pH 7.8), with increasingly severe effects as the pH decreased. At +10000 ppm (pH 6.8), the fertilization rate was almost half the control value, a significant difference (Tukey-Kramer; Fig. 2). By contrast, the fertilization rate in HCl-seawater only decreased to 77% at pH 6.8. However, this was still significantly lower than in the control (Tukey-Kramer; Fig. 2). The fertilization rate was always lower in CO₂-seawater than in HCl-

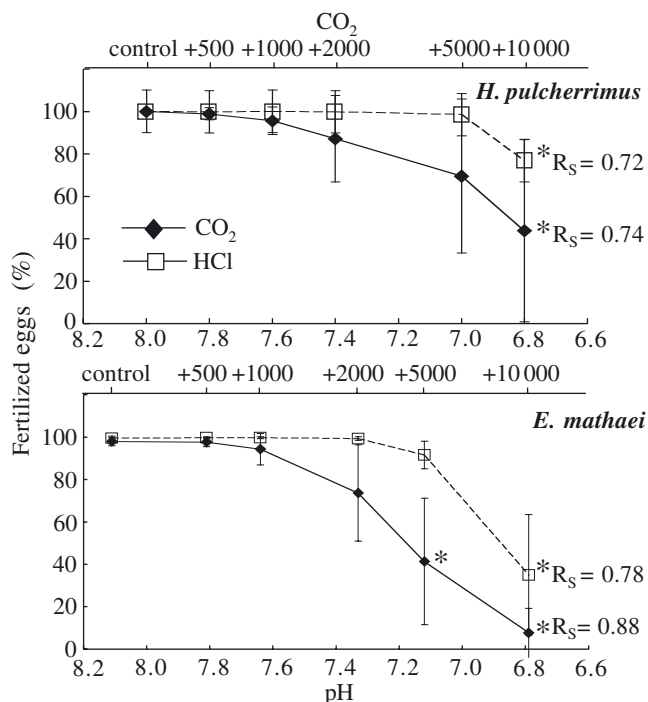


Fig. 2. *Hemicentrotus pulcherrimus* and *Echinometra mathaei*. Percentage of eggs fertilized in CO₂-seawater and HCl-seawater at different pHs. For *H. pulcherrimus*, each value is the average of 6 experiments for CO₂-seawater and 4 for HCl-seawater. For *E. mathaei*, each value is the average of 3 experiments for CO₂-seawater and 5 for HCl-seawater. Error bars = SD. R_s = Spearman's rank correlation coefficient. *Means were significantly different from control (Tukey-Kramer)

seawater at the same pH, although the differences were not significant (ANOVA).

As in *Hemicentrotus pulcherrimus*, the fertilization rates of *Echinometra mathaei* tended to decrease with pH in both CO₂-seawater ($R_s = 0.88$, $p < 0.01$, Fig. 2) and HCl-seawater ($R_s = 0.78$, $p < 0.01$; Fig. 2). The effects of pH on fertilization rate also differed between CO₂- and HCl-seawater (Fig. 2). In CO₂-seawater, the fertilization rate of *E. mathaei* tended to drop linearly with increasing CO₂ concentration and was less than 10% at +10 000 ppm (pH 6.8). The fertilization rates at +5000 (pH 7.1) and +10 000 ppm were significantly different from that of the control (Tukey-Kramer; Fig. 2). Furthermore, *E. mathaei* seemed to be affected more severely than *H. pulcherrimus*, especially at higher CO₂ concentrations (Fig. 2). In HCl-seawater, the fertilization rates of *E. mathaei* decreased only after the pH dropped below 7.3. At pH 7.1, the fertilization rate decreased slightly to 94% and at pH 6.8, it decreased drastically to 34% (Fig. 2). The fertilization rate at pH 6.8 was significantly different from that of the control (Tukey-Kramer, Fig. 2). There was also a significant

difference between the effects of CO₂- and HCl-seawater on the fertilization rate in this species (ANOVA, $p < 0.05$).

Effects on early cleavage

The percentage of fertilized *Hemicentrotus pulcherrimus* eggs (fert) that had not yet cleaved (1 cell fert⁻¹) and of embryos that had already completed their first cleavage (2 cell fert⁻¹) 105 min after insemination differed significantly with pH in both CO₂-seawater (Fig. 3A; ANOVA, $p < 0.01$) and HCl-seawater (Fig. 3B; ANOVA, $p < 0.01$). In both CO₂- and HCl-seawater, the percentage of 1 cell fert⁻¹ increased as the pH of the seawater decreased, whereas that of 2 cell fert⁻¹ decreased with decreasing pH (Fig. 3). The percentage of 1 cell fert⁻¹ was significantly higher at +10 000 ppm (pH 6.8) than in the control in CO₂-seawater (Fig. 3A; Tukey-Kramer) and at pH 7.0 and 6.8 in HCl-seawater (Fig. 3B; Tukey-Kramer). There was no significant difference in the percentage of either 1 or 2 cell fert⁻¹ between CO₂- and HCl-seawater at the same pH.

At 210 min after insemination, some *Hemicentrotus pulcherrimus* fertilized eggs reached the 8-cell stage while some remained at the 1-cell stage, and

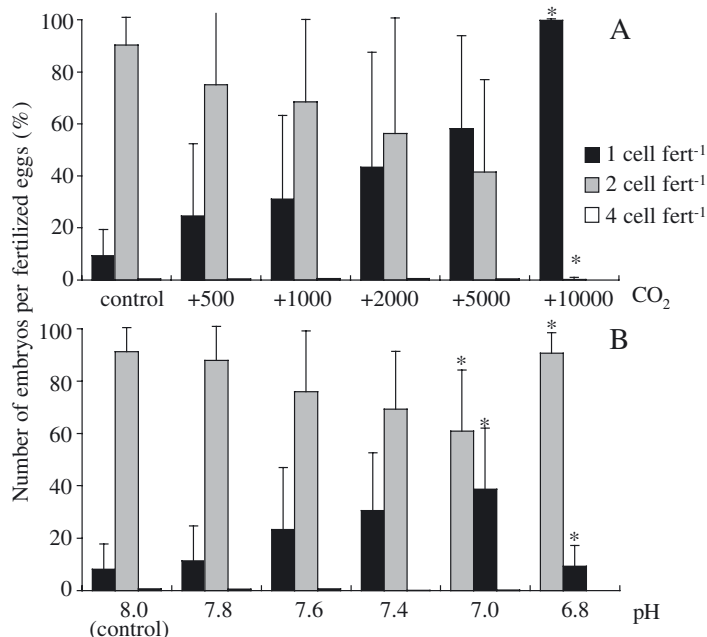


Fig. 3. *Hemicentrotus pulcherrimus*. Percentage of 1-, 2-, and 4-cell stage embryos in fertilized eggs cultured for 105 min after insemination in (A) CO₂-seawater and (B) HCl-seawater at different pHs. Each value is the average of 6 experiments for CO₂-seawater and 4 for HCl-seawater. Error bars = SD. *Means were significantly different from control (Tukey-Kramer).

abnormal embryos were observed in both CO₂-seawater (Fig. 4A) and HCl-seawater (Fig. 4B). The percentage of earlier-stage embryos, such as 1-cell or 2-cell stage embryos, tended to increase as the pH decreased in both CO₂-seawater (Fig. 4A; ANOVA, $p < 0.01$) and HCl-seawater (Fig. 4B; ANOVA, $p < 0.01$). The percentage of 1- and 2-cell stage embryos was significantly higher at pH 6.8 (CO₂ = +10000 ppm) than in the control in both CO₂- and HCl-seawater (Fig. 4; Tukey-Kramer).

The percentage of later-stage embryos, such as 4- and 8-cell stage embryos, differed significantly with pH in both CO₂-seawater (Fig. 4A; ANOVA, $p < 0.05$) and HCl-seawater (Fig. 4B; ANOVA, $p < 0.05$). Although the average percentage of 8 cell fert⁻¹ tended to decrease with increasing pH, it did not differ significantly from the control at all pHs in both CO₂- and HCl-seawater (Fig. 4A,B; Tukey-Kramer). There were no significant differences between the percentages of embryos at each stage in CO₂-seawater and HCl-seawater for all pHs.

Effects on pluteus larvae

All 3 parameters measured (Fig. 1), overall length (OL), post-oral arm length (PL), and body length (BL),

tended to decrease with pH in both CO₂-seawater (OL: $R_S = 0.82$, $p < 0.01$; PL: $R_S = 0.75$, $p < 0.01$; BL: $R_S = 0.83$, $p < 0.01$) and HCl-seawater (OL: $R_S = 0.92$, $p < 0.01$; PL: $R_S = 0.85$, $p < 0.01$; BL: $R_S = 0.94$, $p < 0.01$; Fig. 5). In CO₂-seawater, all OL, PL, and BL were significantly different at all pH values from those in the control (Tukey-Kramer, Fig. 5). In HCl-seawater, OL and BL were significantly shorter at pH 7.6, 7.4 and 7.0 than in the control, whereas PL differed significantly only at pH 7.4 and 7.0 (Tukey-Kramer, Fig. 5). The data for pH 6.8 (CO₂ = +10 000 ppm) are not shown because in this treatment virtually no embryos developed to the pluteus stage. There were no significant differences between the effects of CO₂- and HCl-seawater on OL, PL, and BL under these conditions (ANOVA, Fig. 5).

The larvae grown at pH 7.8 (CO₂ = +500 ppm) were smaller (Fig. 6B,H) than the controls (Fig. 6A,G), although of similar shape. In contrast, larvae grown in seawater more acidic than pH 7.6 (CO₂ > +1000 ppm), were smaller and tended to be trapeziform rather than of the normal triangular form (Fig. 6C,D,E,I,J & K). Larvae grown at pH 6.8 (CO₂ = +10 000 ppm) were extremely abnormal, lacking arms and we were unable to find any spicules (Fig. 6F,L).

DISCUSSION

Effects of elevated CO₂ on the early development of sea urchin embryos

In this study, we have assessed the effects of high CO₂ concentrations on the early development of sea urchin embryos. All of the parameters studied, i.e. fertilization rates, cleavage rates, and size of pluteus larvae, tended to decrease with increased CO₂ concentration. We also compared the effects of CO₂ and HCl at the same pH to evaluate any differences between the two. The 2 compounds had different effects on fertilization rate, whereas their effects on cleavage rate, developmental speed, and pluteus larval morphology were the same. It was especially noteworthy that, in contrast to CO₂-seawater, there seemed to be a threshold in HCl-seawater where the fertilization rate only began to decrease when the pH was <7.1–7.0 (Fig. 2). Christen et al. (1983) pointed out that the low pH (7.0) caused by strong acids prevents sperm motility. In our experiment, however, we observed many sperm swimming around eggs that failed to be fertilized in both CO₂- and HCl-seawater (data not shown). In another species of sea urchin, *Anthocidaris crassipina*, polyspermy

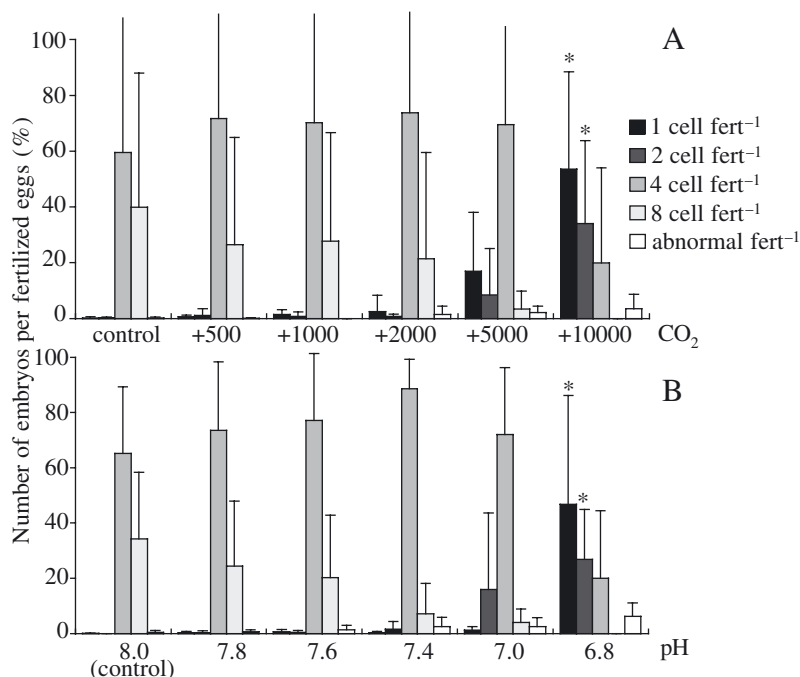


Fig. 4. *Hemicentrotus pulcherrimus*. Percentage of 1-, 2-, 4-, and 8-cell stage embryos in fertilized eggs cultured for 210 min after insemination in (A) CO₂-seawater and (B) HCl-seawater at different pHs. Each value is the average of 6 experiments for CO₂-seawater and 4 for HCl-seawater. Error bars = SD. * Means were significantly different from control (Tukey-Kramer)

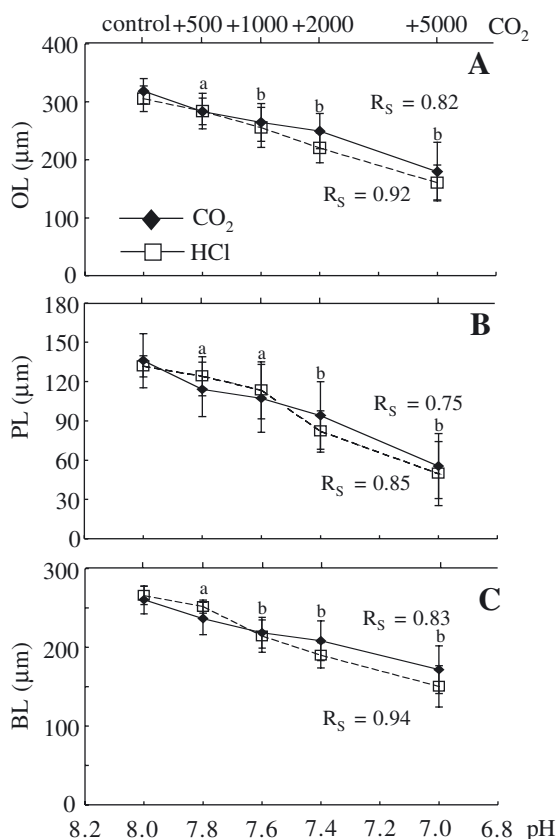


Fig. 5. *Hemicentrotus pulcherrimus*. Three morphometric measurements for 4-armed pluteus larvae cultured for 3 d at different pHs in CO₂-seawater and HCl-seawater. (A) OL; (B) PL; (C) BL. Each value is the average of 5 experiments for CO₂-seawater and 3 for HCl-seawater. Error bars = SD. R_s = Spearman's rank correlation coefficient. Means were significantly different from the control (Tukey-Kramer) (a) only CO₂-seawater and (b) both CO₂- and HCl-seawater

was induced when eggs were fertilized at pH 7.0 (Kobayashi 1973), although we could not find any such eggs. Gregg & Metz (1976) indicated that low pH (6.5) caused by strong acids inhibited the acrosomal reaction of sea urchin sperm, which could explain the lower fertilization rates observed in our study. Since the fertilization rate decreased at a much higher pH in CO₂-seawater, however, the increased CO₂ concentration itself may have an inhibitory effect. Elevated CO₂ concentration leads to an increase in total inorganic carbon ($TC = CO_{2(aq)} + HCO_3^- + CO_3^{2-}$) in seawater, and such changes in CO₂ chemistry may affect the fertilization mechanism, leading to reduced fertilization.

When fertilized *Hemicentrotus pulcherrimus* eggs were kept in CO₂-seawater for 105 and 210 min, the percentage of embryos at later stages tended to decrease with increasing CO₂ concentration (Figs. 4 & 5). Furthermore, some of the fertilized eggs kept at CO₂ concentrations higher than +5000 ppm for

210 min did not cleave at all (Fig. 5). These results reveal 2 effects of CO₂ on the development of sea urchin embryos: a dose-dependent developmental delay and a decline in the cleavage rate at CO₂ levels above +5000 ppm (i.e. a decrease in the number of zygotes capable of cleaving at concentrations higher than +5000 ppm CO₂). In chick embryos, the rates of thymidine-³H incorporation into DNA and cell multiplication both show a positive correlation with pH (Rubin 1971). If the same relationships exist for sea urchins, development could be delayed in acidic CO₂- and HCl-seawater. The reduction in intracellular pH after fertilization, caused by incubating zygotes in strong acids, leads to a reduction of protein synthesis (Grainger et al. 1979). When *Sphaerechinus granularis* sea urchin embryos were reared in seawater acidified with HCl or H₂SO₄, mitotic abnormalities were induced at pH <6.5 (Pagano et al. 1985 a,b, Cipollaro et al. 1986). Such impacts on protein synthesis and on mitotic activity are thought to lead to decreased growth and cleavage rates.

When embryos were cultured for 3 d in CO₂- or HCl-seawater, the morphology and size of the pluteus larvae were both affected in a pH-dependent manner (Figs. 5 & 6). Embryos cultured at +10 000 ppm (pH 6.8) completely lacked a skeleton (Fig. 6F,L). The larval skeleton of the sea urchin is composed of calcium carbonate (Okazaki & Inoue 1976); therefore, we postulate that these decreases in the length of pluteus larvae result from decreased calcium carbonate assimilation efficiency due to the low pH, as has been observed in phytoplankton (Riebesell et al. 1993, 2000) and coral (Gattuso et al. 1998). The size decrease may also be the result of retarded development. Furthermore, low pH is also thought to induce morphological abnormalities. Pagano et al. (1985a) used HCl to study the effects of low pH on embryos of the sea urchin *Paracentrotus lividus*, and demonstrated that larval morphology is critically affected at pH 7.5.

Ecological impacts of increased CO₂ concentration in the seawater

Our results reveal that increased CO₂ concentrations in seawater affect various stages in the early life history of sea urchins. A delay in the developmental rate increases mortality of embryos during the planktonic period due to predation (Vance 1973a,b, Christiansen & Fenchel 1979, Emlet et al. 1986). The typical triangular form and long arms of the pluteus larvae are important for swimming and feeding (Strathmann 1971, Emlet 1983). The clearance rate of food in suspension increases with increasing length of the ciliated band (Strathmann 1971, Hart 1991), which is known to

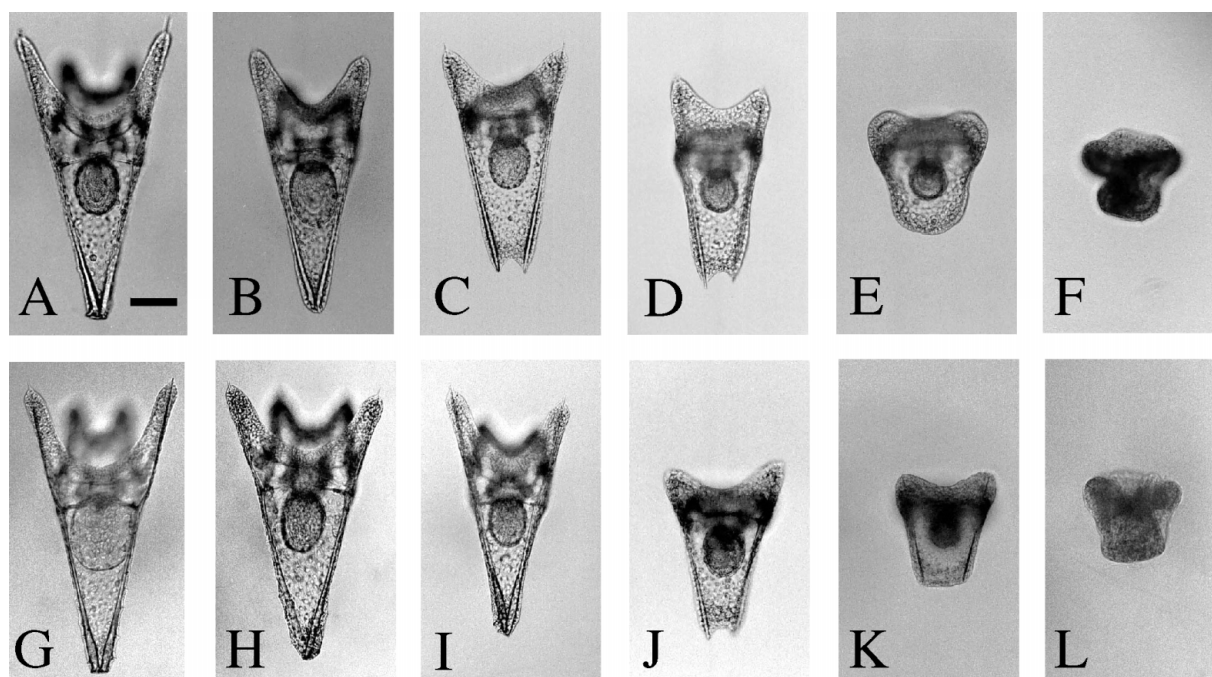


Fig. 6. *Hemicentrotus pulcherrimus*. Morphology of 4-armed pluteus larvae cultured for 3 d in (A–F) CO₂-seawater and (G–L) HCl-seawater. (A & G) control (pH 8.0); (B & H) +500 ppm (pH 7.8); (C & I) +1000 ppm (pH 7.6); (D & J) +2000 ppm (pH 7.4); (E & K) +5000 ppm (pH 7.0); (F & L) +10 000 ppm (pH 6.8). Scale bar = 50 μ m

increase allometrically with respect to overall length, arm length, and body length (Fenaux et al. 1988, McEdward 1984). Therefore, abnormal morphology and shortening of OL, PL and BL may reduce swimming and feeding capability and, in turn, survival. The skeleton is presumed to serve supportive or protective functions (Emlet 1982, Pennington & Emlet 1986); therefore, changes in skeletogenesis may also reduce the survival rate. All the effects of raised CO₂ concentration observed in this study would have a negative impact on the survival of sea urchin embryos in their early life history.

Previous studies of the impact of acidification on marine organisms focused on the survival rate under conditions prepared using strong acids, such as HCl or H₂SO₄ (Calabrese & Davis 1966, Kuwatani & Nishii 1969, Rose et al. 1977, Bamber 1987, 1990, Yamada & Ikeda 1999). As a result, a fluctuation in pH of less than 1.0 was thought to have only a minor impact (Knutzen 1981). However, we have shown that the effect of CO₂-seawater is stronger than that of HCl-seawater at the same pH. Watanabe et al. (2001) compared the survival rates of copepods raised in seawater acidified to the same degree using CO₂ or HCl, and also found that CO₂ had a greater impact. Furthermore, even a small change in pH affected the early development of sea urchins, and the effect tended to become more severe as the CO₂ concentration increased. Some aspects of

the mechanisms affecting early development are common among marine invertebrates. Therefore, increasing atmospheric CO₂ concentration may affect the early stages of many marine invertebrates in the same way that it affects sea urchins. This is especially serious for species with a high mortality rate in the early stages of development (Legendre & Rivkin 2002). It is also speculated that animals that use calcium carbonate in their bodies such as corals, mollusks, echinoderms, and foraminiferans will be highly affected. We have evaluated only the direct effects of CO₂ and low pH in this study. Low pH is, however, known to increase the impacts of certain inorganic pollutants (Pagano et al. 1986) which may further exacerbate these effects.

Recently, a large number of experimental studies evaluating the environmental impact of the oceanic disposal of CO₂ have been carried out (Auerbach et al. 1997, Haugan 1997, Omori et al. 1998). Most of these studies considered how lowered pH would affect the percentage mortality of adult organisms (Herzog et al. 1996, Adams et al. 1997, Caulfield et al. 1997b, Takeuchi et al. 1997, Yamada & Ikeda 1999). We have shown that acidic (pH <7.0) seawater drastically affects the early development of sea urchins, resulting in a reduced fertilization rate, blocked cleavage capacity and a complete absence of skeleton in pluteus larvae.

In conclusion, to anticipate the impact of increased CO₂ concentrations on marine ecosystems accurately, comprehensive information on the effects of CO₂ fluctuations on the entire life cycles of various marine organisms must be gathered.

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