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Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates

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ABSTRACT: CO_2 emissions arising from the burning of fossil fuels have altered seawater chemistry far more rapidly than the Earth has previously experienced, and the rate and extent of this change are expected to affect shallow water marine organisms. The increased CO_2 diffuses from the atmosphere into ocean surface waters, resulting in increased partial pressure of CO_2, and reduced [CO_3^{2-}] and pH. The CO_2-driven ocean acidification leads to a decrease in calcium carbonate ($CaCO_3$) saturation state in the ocean surface waters and has potential impacts on calcifiers. The present study focuses on the effects of ocean acidification on early developmental and reproductive stages of calcifiers, both of which are believed to be the most vulnerable stages to environmental change within a life cycle. Laboratory experiments revealed that ocean acidification has negative impacts on the fertilization, cleavage, larva, settlement and reproductive stages of several marine calcifiers, including echinoderm, bivalve, coral and crustacean species. There appear to be significant ontogenetic impacts and species-specific differences in tolerance to the high CO_2 levels. The conclusion is that future changes in ocean acidity will potentially impact the population size and dynamics, as well as the community structure of calcifiers, and will therefore have negative impacts on marine ecosystems. Further studies are needed to evaluate the potential impacts on non-calcifiers, as well as the synergistic impacts of ocean acidification and climate change. Studies should also focus on the adaptive capability of marine organisms, which will be crucial to the ability to forecast how marine organisms and ecosystems will respond to the world's oceans as they warm and acidify.

KEY WORDS: CO_2 · Ocean acidification · Seawater chemistry · Calcifiers · Early development · Reproduction · Rapid environmental change

INTRODUCTION

Approximately one-third of the CO_2 that has entered the atmosphere over the past 100 yr has been absorbed into ocean surface waters and has resulted in the elevation of partial pressure of CO_2 (pCO_2) in seawater and reduction of seawater pH (Caldeira & Wickett 2003, Royal Society 2005, German Advisory Council on Global Change 2006, Denman et al. 2007). One biological impact of ocean acidification is its effect on calcifiers, because seawater acidification results in a decrease of [CO_3^{2-}], thereby reducing the calcium carbonate ($CaCO_3$) saturation state, which is determined by [CO_3^{2-}][Ca^{2+}] / *K*sp (*K*sp is the stoichiometric solubility of $CaCO_3$; Kleypas et al. 2006). Of the 2 major biologically secreted forms of $CaCO_3$ in modern calcifiers, aragonite is more soluble than calcite (Zeebe & Wolf-Gladrow 2001). Orr et al. (2005) reported that high-latitude surface oceans will become undersaturated with respect to aragonite by the year 2050, and lead to aragonite shell dissolution (Feely et al. 2004, Orr et al. 2005). Recent studies have shown that the calcification rate of calcifiers, such as corals, coccolithophores, foraminiferans and bivalves, decreases with increasing pCO_2, even in seawater supersaturated with respect to $CaCO_3$ (Gattuso et al. 1998, Riebesell et al. 2000, Bijma et al. 2002, Kleypas et al. 2006, Gazeau et al. 2007). Additionally, increased pCO_2 may also have complex effects on the physiology, growth and reproductive success of marine calcifiers. Indeed, recent studies have

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demonstrated that adult calcifiers exposed to hypercapnia suffer from physiological stress in addition to reduced calcification (Pörtner et al. 2004, Michaelidis et al. 2005, Miles et al. 2007, Spicer et al. 2007). To understand the effect of ocean acidification at a population level, however, it is important to focus on the most sensitive life cycle stages to environmental change. Usually these are early developmental and reproductive stages, during which environmental requirements are often more specific and acute than at other stages (Thorson 1950). Indeed mortality of marine invertebrates, including benthic calcifiers, exceeded 90% during early life stages in their natural habitat according to Gosselin & Qian (1997).

There are a number of different life cycle stages of benthic calcifiers, such as fertilization, cleavage, planktonic larva, settlement, metamorphosis, juvenile, adult and reproductive stages, which are possibly affected differently by high pCO_2 (Fig. 1). The first deposition of $CaCO_3$ is known to occur during the larval stage, as in echinoderms and bivalves, or during the settlement stage, as in corals and barnacles. Hence, these stages are highly susceptible to the potential effects of ocean acidification. Beckerman et al. (2002) suggested that environmental conditions experienced during early development can have profound effects on the subsequent performance of individuals and cohorts. Indeed, Green et al. (2004) showed that the low $CaCO_3$ saturation state may explain the exponential losses of juvenile bivalves and the low recruitment transition from the pelagic larval phase to the benthic juvenile phase. Therefore, effects of ocean acidification on larval survival rate, as well as reproduction rate, will directly influence the population abundance, distribution and community structure. To evaluate the impact of ocean acidification on calcareous organisms at a community level, the present paper focuses on the effects of high pCO_2 on early developmental stages including fertilization, cleavage, hatching, larva, settlement and reproductive stages of calcifiers.

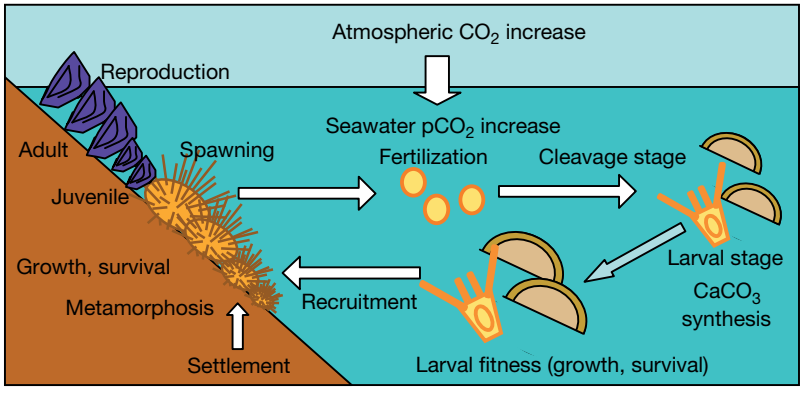

Fig. 1. Different life-cycle stages of benthic calcifiers, including reproduction, fertilization, planktonic larva, settlement, metamorphosis, juvenile and benthic adult stages, that are potentially affected in different manners by ocean acidification

EFFECTS ON FERTILIZATION, CLEAVAGE AND HATCHING STAGE

The fertilization rate of sea urchins decreased with increasing pCO_2 concentration (360 to 10 360 µatm, pH 8.1 to 6.8) in eggs of both *Hemicentrotus pulcherrimus* (Fig. 2; $r_s = 0.74$, $p < 0.001$) and *Echinometra mathaei* (Fig. 2; $r_s = 0.88$, $p < 0.001$; Kurihara & Shirayama 2004a,b). However, the impact of increasing pCO_2 on fertilization differed between females, as revealed by the large SDs (Fig. 2), possibly reflecting a degree of genetic variation for CO_2 tolerance within populations. Additionally, in contrast with the linear decrease of fertilization rate in high pCO_2 seawater, the fertilization rate decreased at pH levels only <7.0 when seawater was acidified with HCl (Fig. 2; Kurihara & Shirayama 2004a,b). Effects of low pH using mineral acids on sperm motility have been well studied for sea urchins. Christen et al. (1983) demonstrated that sperm motility was suppressed at pH < 7.0. Polyspermic fertilization was also reported in *Anthocidaris crassispina* sea urchin eggs fertilized at pH 7.0 (Kobayashi 1971). Recently, Havenhand et al. (2008) found that sperm swimming speed and percent sperm motility of the sea urchin *Heliocidaris erythrogramma* exposed to 1000 µatm pCO_2 (pH 7.7) seawater decreased compared to controls. These results suggest again that high pCO_2 may affect egg fertilization more strongly than mineral acids. One of the reasons for this difference is likely to be the diffusion capability of CO_2 and protons. Ion transport is an energy (ATP)-consuming process (Heisler 1993), whereas molecular CO_2 directly diffuses across the biological cell membrane far faster than protons (Gutknecht et al. 1977), and hence CO_2 can readily enter into eggs or sperm and decrease the intracellular pH. Since the intracellular pH of sea urchin eggs is known to rise after insemination (Lopo & Vacquier 1977) and trigger the initiation of embryonic development (Johnson et al. 1976), in addition to the impact on sperm motility, the low intracellular egg pH may prevent fertilization and subsequent development.

The fertilization rates of marine bivalves, the oyster *Crassostrea gigas* and the mussel *Mytilus galloprovincialis* were unaffected in 2000 µatm

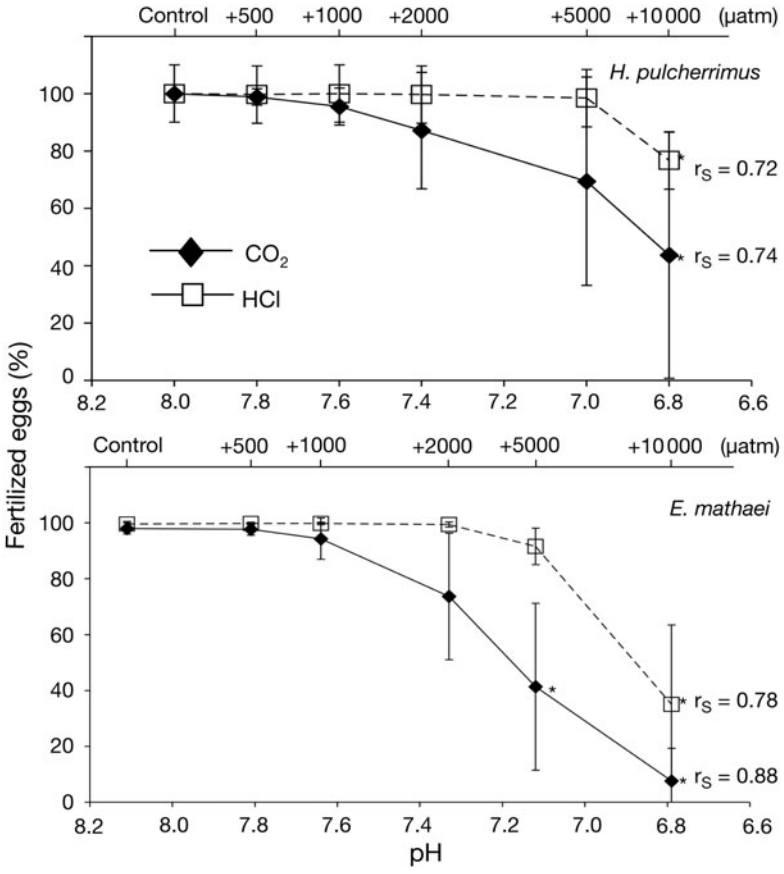

Fig. 2. *Hemicentrotus pulcherrimus* and *Echinometra mathaei*. Fertilization rate of eggs fertilized under 6 different pH conditions. Seawater was acidified with CO_2 or HCl; 6 and 3 batches were used for *H. pulcherrimus* and for *E. mathaei*, respectively. Error bar: SD; r_s: Spearman's rank correlation coefficient; *: significant difference compared to control (Tukey-Kramer, $p < 0.05$)

pCO_2 (pH 7.4) seawater (Kurihara et al. 2007, Kurihara et al. unpubl. data), whereas Desrosiers et al. (1996) reported that polyspermic fertilization in the giant scallop *Placopecten magellanicus* increased at seawater pH < 7.5. Additionally, during the scallop embryonic stage, the time to complete the first cleavage was shortest at pH 8.2 and increased with decreasing pH. Similarly, the cleavage speed of sea urchin embryos *Hemicentrotus pulcherrimus* and *Echinometra mathaei* slowed with decreasing pH (Kurihara & Shirayama 2004a,b). When embryos of the sea urchin *Sphaerechinus granularis* were reared in seawater acidified with HCl or H_2SO_4, mitotic abnormalities were induced at pH < 6.5 (Pagano et al. 1985a,b, Cipollaro et al. 1986). Incubating zygotes in seawater acidified by mineral acids reduces protein synthesis (Grainger et al. 1979). Such impacts on protein synthesis and mitotic activity probably decrease growth and cleavage rates.

Both hatching and nauplius survival decrease with increasing pCO_2 in the copepods *Acartia erythraea*, even though negative impacts were significant only at pCO_2 levels higher than those projected to occur in the future ocean (Kurihara et al. 2004a,b). Similarly, Mayor et al. (2007) also demonstrated a decrease of hatching success in the copepod *Calanus finmarchicus* only at 8000 µatm pCO_2 (pH 6.9). When *A. tsuensis* eggs were reared under 2000 µatm pCO_2 (pH 7.3) until they developed into adults, survival, growth and morphology were unaffected at all stages (Kurihara & Ishimatsu 2008). Additionally, the hatching rate was unaffected during ensuing generations (0 to 2 generations).

EFFECTS ON LARVAL DEVELOPMENT

The larval development of several calcifiers is affected by elevations of seawater pCO_2. When *Hemicentrotus pulcherrimus* and *Echinometra mathaei* embryos were reared under 6 different CO_2 concentrations until they developed to the pluteus larval stage, larval and arm sizes were significantly smaller with increasing pCO_2 and their morphology, principally the larval skeletogenesis, tended to be abnormal (Fig. 3a to f; Kurihara & Shirayama 2004a,b). Similarly, the larval shells of *Crassostrea gigas* and *Mytilus galloprovincialis* were strongly affected by high pCO_2 conditions (Fig. 3g to k). When oyster eggs were reared under 1000 µatm pCO_2 (pH 7.8), though CO_2-treated larvae were completely shelled, they showed malformations such as convex hinges (Fig. 3h), which are typical criteria to identify abnormal development of veliger larvae in embryotoxicology bioassays (His et al. 1997). When oyster eggs were reared under 2000 µatm pCO_2 (pH 7.4), >70% of the CO_2-treated larvae were either completely non-shelled, or only partially shelled (Fig. 3i), and only 4% of CO_2-treated embryos developed into normal 'D-shaped' veliger larvae by 48 h after fertilization, in contrast to about 70% successful development in control embryos (Fig. 3g; Kurihara et al. 2007). A negative impact of 2000 µatm pCO_2 (pH 7.4) was also observed in *M. galloprovincialis* larvae. Though all CO_2-treated mussel larvae were completely shelled in contrast with oyster larvae, larval size was about 20% smaller than that of larvae from the control conditions and showed morphological abnormalities such as convex hinges, protrusion of mantle and malformed shells (Fig. 3i,k; Kurihara et al. in press).

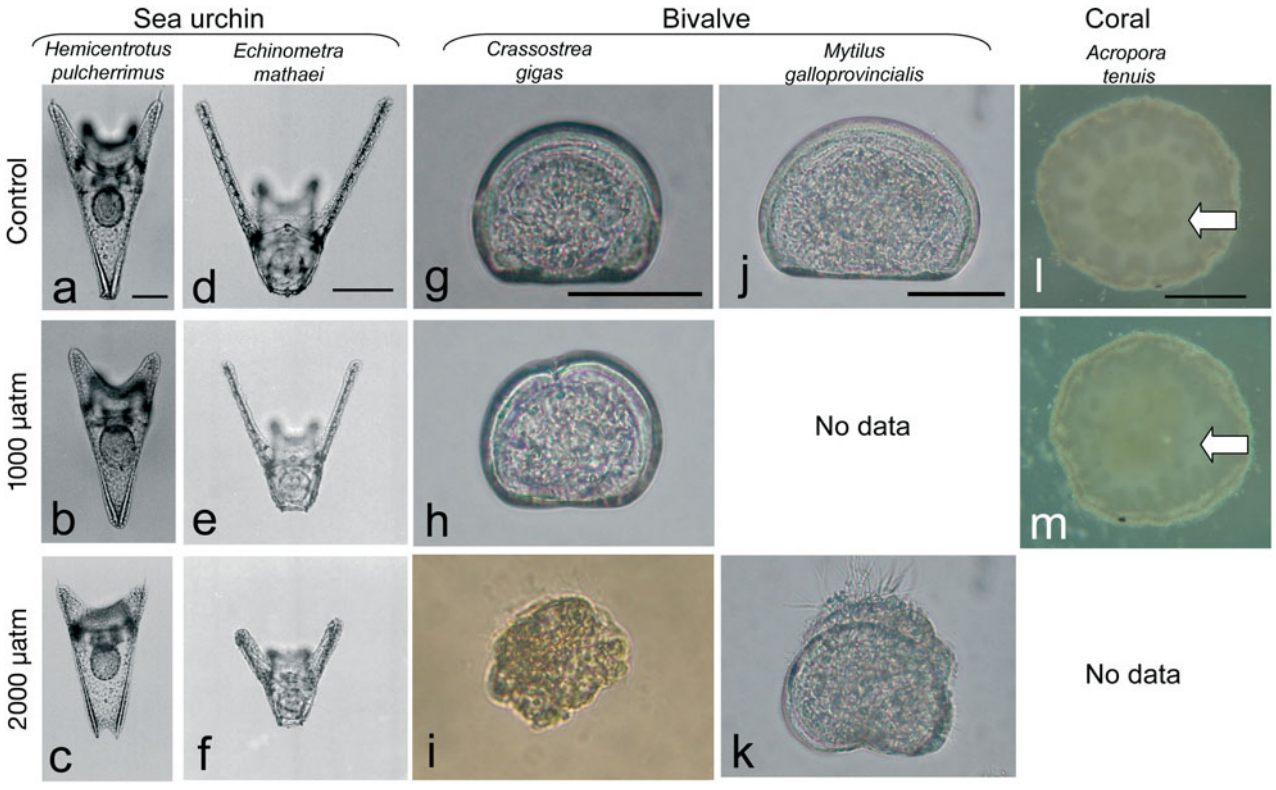

Fig. 3. Larval or polyp morphology of sea urchins *Hemicentrotus pulcherrimus* (a to c) and *Echinometra mathaei* (d to f), bivalves *Crassostrea gigas* (g to i) and *Mytilus galloprovincialis* (j,k), and the coral *Acropora tenuis* (l,m) incubated in the control (a,d,g,j,l), 1000 µatm pCO_2 (b,e,h,m) and 2000 µatm pCO_2, (c,f,i,k). Scale bars = 50 µm (a to j), 500 µm (l,m); the bars in (a,d,g,j,l) apply to the panels of the whole column

All these results suggest that high pCO_2 affected larval skeleton and shell synthesis. To evaluate the mechanism of this effect, I have recently examined the effect of high CO_2 (1000 and 2000 µatm pCO_2 / pH 7.7 and 7.45) on the expression of the gene related to spicule elongation (SM50) (Peled-Kamar et al. 2002), and of the gene that regulates the direction of crystal growth (SM30) in embryos of the sea urchin *Hemicentrotus pulcherrimus*. No effect was observed on the expression of these genes, even though spicule size and morphology of larvae were affected (Kurihara et al. unpubl. data). Further experiments evaluating effects on other proteins such as msp130, known to be related to Ca^{2+} transportation (Farach-Carson et al. 1989), will help clarify effects on calcification.

Encounter and clearance rates of food particles depend on larval body size, and, therefore, smaller larvae are more prone to starvation (Anger 1987, Strathmann 1987, Hart & Strathmann 1995). Simkiss & Wilbur (1989) pointed out that the $CaCO_3$ structures have vital functions for calcified larvae, such as defense against predation, as well as roles in feeding, buoyancy control and pH regulation. Predation is generally considered to be the most important cause of larval mortality (Morgan 1995). Research to date on ocean acidification strongly suggests that it will lead to a reduction in fitness and survivorship of sea urchin and bivalve larvae due to both size reduction and disruption of $CaCO_3$ skeletogenesis.

EFFECTS ON LARVAL SETTLEMENT

Mortality and shell dissolution rates of the bivalve *Mercenaria mercenaria* juveniles were significantly higher in $CaCO_3$-undersaturated conditions at the sediment–seawater interface than in supersaturated conditions (Green et al. 2004). They also demonstrated that the mortality rates were higher for small size classes (0.2 and 0.3 mm) than for larger individuals (1.0 and 2.0 mm). To examine the effect of ocean acidification on the settlement and the subsequent growth of coral polyps, eggs of the coral *Acropora tenuis* were reared under control and 1000 µatm pCO_2 (pH 7.6) conditions for 2 wk. In contrast with sea urchin and bivalve larvae, coral was unaffected by high pCO_2 until the larval stage. An impact of CO_2, however, was observed after settlement, while they developed into the polyp stage. The morphology of the CO_2-treated polyp endoskeleton was disturbed and malformed

compared to the radial pattern of control polyps (Fig. 3l,m). When hatched embryos of the marine shrimp *Palaemon pacificus* were cultured until settlement stage under 2000 µatm pCO_2 seawater (pH 7.6), no significant effect was observed on planktonic larval stages; however, CO_2-treated metamorphosing and settling juveniles were significantly smaller than in the control (2-way repeated-measures ANOVA; Fig. 4). Relatively small perturbations in initial populations of settling marine bivalves have been shown to induce large alterations in adult populations (Gosselin & Qian 1997, Hunt & Scheibling 1997). Hence, the impact of ocean acidification on settlement stages may well have profound ecological implications for their populations.

EFFECTS ON REPRODUCTION

While effects of hypercapnia on fish reproduction have been studied to some extent (Ishimatsu et al. 2005), less is known for invertebrates. Some recent studies suggest that ocean acidification exerts negative impacts on invertebrate reproduction. Siikavuopio et al. (2007) reported that gonad growth was reduced by 67% when the green sea urchin *Strongylocentrotus droebachiensis* was exposed to high pCO_2 (pH 6.98) for 56 d. When the sea urchin *Hemicentrotus pulcherrimus* was reared under 1000 µatm pCO_2 (pH 7.8) for 10 mo, gonad development was delayed, and the spawning period was shortened to almost half that of the control (Kurihara et al. unpubl. data). The marine shrimp *Palaemon pacificus* cultured under 1000 µatm pCO_2 (pH 7.9) seawater for 30 wk showed reduced reproduction compared to the control (Kurihara et al. 2008). On the other hand, egg production of all copepods studied (e.g. *Acropora steueri*, *A. erythraea* and *A. tsuensis*) was not affected when reared under the high pCO_2 projected to occur in the future ocean (>2000 µatm pCO_2; Kurihara et al. 2004a,b, Kurihara & Ishimatsu 2008). Consequently, although some organisms appear less sensitive to elevated pCO_2, ocean acidification would directly affect the population size of several calcifiers.

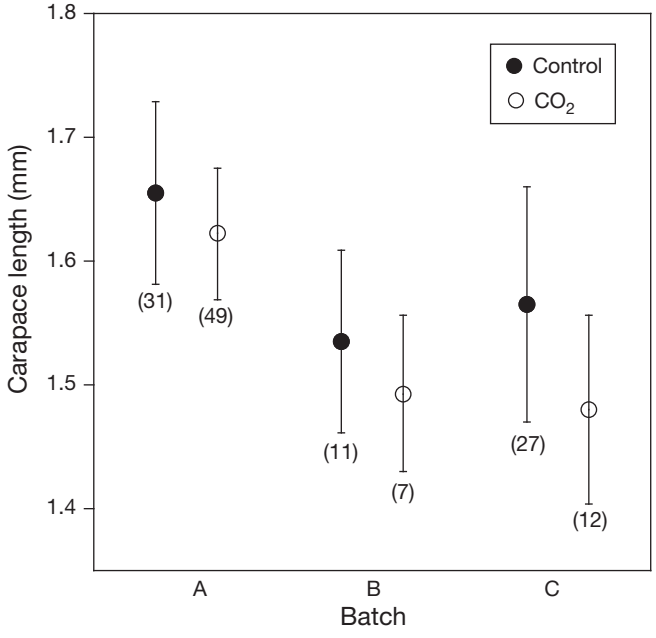

Fig. 4. *Palaemon pacificus.* Carapace length of a just settled marine shrimp juvenile reared under control and 2000 µatm pCO_2. Three different batches (A to C) were used for the experiment. The size of shrimp in CO_2 seawater was significantly smaller than that of control (2-way repeated-measures ANOVA). Number of shrimp shown in parentheses. Error bars: SD

ONTOGENIC IMPACTS OF HIGH CO_2

Table 1 lists the effects of low pH condition (by addition of CO_2 or mineral acids) on the early developmental stages of marine calcifiers and their adult stages. The data indicate that ocean acidification has negative impacts on both larval and adult stages of corals, mollusks, echinoderms and crustaceans. Although data are limited for direct comparison of CO_2 tolerance between larval and adult stages, larvae appear to be more sensitive than adults. For example, whereas calcification of oyster adults reared under 2000 µatm pCO_2 (pH 7.4) decreased by about 50%, approximately half of the oyster larvae completely lacked a shell when cultured under the same pCO_2 concentration (Table 1; Gazeau et al. 2007, Kurihara et al. 2007). Although adult oyster shells are mainly composed of calcite (Stenzel 1964), oyster larval shell is completely formed of aragonite. Since the solubility of aragonite is higher than that of calcite, the $CaCO_3$ shells of bivalve larvae are probably affected more severely than those of adults. Additionally, although the growth and size of the adult sea urchin *Hemicentrotus pulcherrimus* was not affected when cultured for 10 mo under 1000 µatm pCO_2 (pH 7.8), the larval size of *H. pulcherrimus* was significantly reduced compared to the control when reared under 860 µatm pCO_2 (pH 7.8) for 3 d. Larvae of bivalves such as *Crassostrea gigas* and *Mercenaria merceneria* and also sea urchins such as *Paracentrotus lividus* and *Strongylocentrotus purpuratus* are known to initially deposit amorphous calcium carbonate (ACC), with a solubility 30 times larger than that of aragonite (Breãeviç & Nielsen 1989, Weiss et al. 2002, Addadi et al. 2003, Politi et al. 2004). For larval shells of bivalves, the ACC transformed into aragonite, and then to calcite in adult oysters, or into a mixture of aragonite and calcite in adult mussels (Hubbard et al.

Table 1. Effects of low pH condition (by addition of CO_2 or mineral acids) on the early developmental stages and adults of marine calcifiers. Organisms that are impacted at CO_2 concentrations expected to occur in the future ocean (380~2000 µatm pCO_2 / pH 8.2~7.3) are given in **bold**. However, most of the studies evaluating effects of acidification on bivalves used mineral acids and not CO_2. ppmv: parts per million by volume

Taxon	CO_2 (ppmv) or Acid	pH	Exposure period	Effect	Source
Acropora tenuis	CO_2 1000	7.6	14 d	Reduced growth of polyp size	Present study
Crassostrea gigas **(larva)**	CO_2 2000	7.4	48 h	Inhibition of shell synthesis, reduced larval size	Kurihara et al. (2007)
	CO_2 1000	7.8	48 h	Shell malformation	Kurihara et al. (unpubl. data)
C. gigas **(adult)**	CO_2 698–2774	8.07–7.55	2 h	Decreased calcification rate	Gazeau et al. (2007)
	H_2SO_4	6.0–7.5	30 d	pH < 7.0, reduced feeding, growth, size, shell weight	Bamber (1990)
Mytilus galloprovincialis **(larva)**	CO_2 2000	7.4	6 d	Shell malformation, reduced larval size	Kurihara et al. (in press)
M. galloprovincialis **(adult)**	CO_2 5000	7.3	3 mo	Reduced growth, metabolism rate	Michaelidis et al. (2005)
Mytilus edulis (young, adult)	H_2SO_4	6.0–8.0	30 d	pH < 7.0, growth, feeding depression, shell dissolution	Bamber (1990)
M. edulis (adult)	CO_2 421–2351	8.13–7.46	2 h	Decreased calcification rate	Gazeau et al. (2007)
Crassostrea virginica (larva)	HCl	6.0–9.25	12 d	pH < 6.25, increased mortality rate; x pH < 6.75, decreased growth rate	Calabrese & Davis (1966)
Mercenaria mercenaria (larva)	HCl	6.0–9.25	10 d	pH < 6.25, increased mortality rate; x pH < 6.75, decreased growth rate	Calabrese & Davis (1966)
M. mercenaria (juvenile)	CO_2 50 000	7.1	21 d	Shell dissolution	Green et al. (2004)
Venerpis decussata **(adult)**	H_2SO_4	3.5–8.2	8–30 d	pH < 7.5, shell dissolution; pH < 7.0, feeding inhibition; pH < 6.1, 50% mortality	Bamber (1987)
V. decussata **(juvenile, 3–4 mm)**				pH < 6.4, 50% mortality	Bamber (1987)
Placopecten magellanicus **(egg)**	Unknown	7.0–9.0	5 h	pH < 7.5, polysperm, slow cleavage speed	Desrosiers et al. (1996)
Acartia steueri (adult)	CO_2 2000–10 000	7.4–6.8	8 d	pH < 6.8, decreased egg production	Kurihara et al. (2004a,b)
Acartia erythraea (egg, larva)	CO_2 2000–10 000	7.4–6.8	2 d	Increased nauplius mortality, hatching rate	Kurihara et al. (2004a,b)
A. erythraea (adult)	CO_2 5000–10 000	7.0–6.8	8 d	pH < 7.0, decreased egg production	Kurihara et al. (2004a,b)
Acartia tsuensis (egg, larva)	CO_2 2000	7.4	9 d	No effect	Kurihara et al. (2008)
A. tsuensis (adult)	CO_2 2000	7.4	27 d	No effect	Kurihara et al. (2008)
Calanus finmarchicus (egg)	CO_2 8000	6.95	72 h	Decreased hatching success	Mayor et al. (2007)
C. finmarchicus (adult)	CO_2 8000	6.95	5 d	Decreased egg production	Mayor et al. (2007)
Palaemon pacificus **(egg, juvenile)**	CO_2 2000	7.6	23~36 d	Decreased settling size	Present study
P. pacificus **(adult)**	CO_2 1000, 2000	7.9, 7.6	30,15 wk	Decreased survival, growth, egg production	Kurihara et al. (2008)
Antarctic krill (egg, larva)	CO_2 1000, 2000	7.7, 7.4	26 d	Decreased hatching success	Kurihara et al. (unpubl. data)
Hemicentrotus pulcherrimus **(egg, larva)**	CO_2 860–10 360	7.8–6.8	3 d	pH < 7.8, skeletal malformation, reduced larval size, fertilization decrease with increasing CO_2	Kurihara & Shirayama (2004a,b)
H. pulcherrimus **(adult)**	CO_2 560	7.9	6 mo	Decreased survival, growth rate	Shirayama & Thornton (2005)
	CO_2 1000	7.8	8 m	Decreased reproduction, no effects on survival	Kurihara et al. (unpubl. data)
Echinometra mathaei **(egg, larva)**	CO_2 860–10 360	7.8–6.8	3 d	pH < 7.8, skeletal malformation, reduced larval size	Kurihara & Shirayama (2004a,b)
E. mathaei **(adult)**				Fertilization decrease with increasing CO_2	Kurihara & Shirayama (2004a,b)
Paracentrotus lividus **(egg, larva)**	HCl	6.5–8.5	48 h	pH < 7.5, skeletal malformation; pH < 7.7, morphological abnormality; Mitotic abnormality with decreasing pH	Pagano et al. (1985a,b)
Sphaerechinus granularis (egg, larva)	HCl, H_2SO_4	5.5–8.0	5 h	pH < 6.5, total metaphase blockage	Cipollaro et al. (1986)

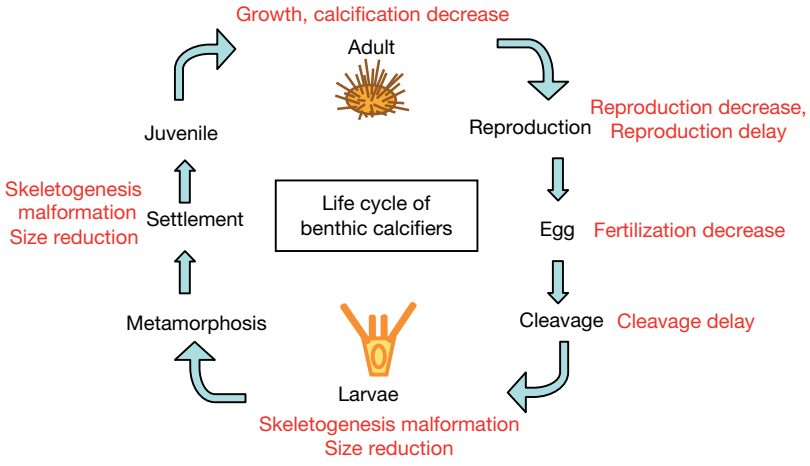

Fig. 5. Summary of CO_2 effects at different life cycle stages of benthic calcifiers under CO_2 concentrations that are expected to occur in the future ocean (380~2000 µatm pCO_2 / pH 8.2~7.3). Although the magnitude of CO_2 tolerance may differ between species and life stages, effects of high CO_2 are proposed for several different life stages, including reproduction, egg, cleavage, larva, settlement and adult stages

1981). Similarly, ACC in sea urchin larvae transformed into high magnesium calcite (Mg-calcite, >4 mol% Mg^{2+} substituting for Ca^{2+}) over a period of hours to days (Addadi et al. 2003, Politi et al. 2004). A recent study predicts that the stoichiometric solubility of Mg-calcite can exceed that of aragonite (Morse et al. 2006). Studies evaluating whether or not other calcifiers also use ACC as a transient precursor phase in their larval stages are very limited (Addadi et al. 2003). However, since research shows that both mollusks and echinoderms, on 2 separate phylogenetic branches, initially precipitate ACC before less soluble forms during later life stages, it is highly probable that this strategy is widespread among marine calcifiers. Further studies evaluating the ontogenic impacts of high pCO_2 concentration on calcifiers are anticipated.

CONCLUSIONS AND PERSPECTIVES

As discussed above, CO_2 is expected to impact the life cycles of benthic calcifiers in different ways under increasing levels (380~2000 µatm pCO_2/ pH 8.2~7.3). The effects of high pCO_2 in seawater are anticipated to occur in several different life stages, including egg, cleavage, larva, settlement, juvenile and adult stages, which are consequently likely to impact the distribution and abundance of benthic calcifiers (Fig. 5). Impacts on fertilization and reproduction can directly affect population size, and decreased calcification at larval and settlement stages is considered to affect their fitness and increase mortality. Cumulative effects across different life stages may lead to species extinctions.

CO_2 tolerance seems to differ between life stages (e.g. larva and adult). Additionally, the vulnerable stages can also differ between species. For example, although the larval stage of sea urchins and bivalves seemed to be most vulnerable to high pCO_2, the settlement stage was the most severely affected in corals and marine shrimps. This can be partially explained by the fact that most echinoderms and mollusks start shell and skeleton synthesis at their larval stage, whereas corals start at the settlement stage. The present study also demonstrates that there are significant differences in the tolerance within and between different species (Table 1). Although most calcifiers were affected at pCO_2 values >1000 µatm (pH 7.9~7.7), copepods appear less sensitive to elevated pCO_2 conditions. The fertilization rate of *Echinometra mathaei* was observed to be more affected than that of *Hemicentrotus pulcherrimus* at the same pCO_2 level (Fig. 2). Therefore, it is possible that the community structure of calcifiers will change in the future ocean. Additionally, the impact of ocean acidification may also differ between organisms that live at different latitudes. Adding studies of Antarctic and Arctic species will be important given that the saturation states of aragonite and calcite decrease faster at high versus low latitudes (Orr et al. 2005).

Most calcifiers, such as corals, echinoderms, bivalves and crustaceans, play important roles in coastal ecosystems as keystone species, bioturbators and ecosystem engineers (Suchanek 1985, Gutiérrez et al. 2003). They are also socio-economically important as food sources and for industries such as tourism. On a global scale, $CaCO_3$ plays a role in regulating the oceanic carbon cycle (Feely et al. 2004). For example, marine mollusks are estimated to produce about 50 to 1000 g $CaCO_3$ m^{-2} yr^{-1} (Beukema 1982, Gutiérrez et al. 2003). For coral reef, the rate of calcification is approximately 10 kg $CaCO_3$ m^{-2} yr^{-1} (Chave et al. 1975). Given the importance of marine calcifiers to these processes, influences on their population size and composition will potentially cause negative impacts to coastal ecosystems, which, consequently, may even affect the whole oceanic ecosystem.

In contrast with marine calcifiers, effects of ocean acidification on non-calcifiers are poorly described. The present study reveals that elevated atmospheric CO_2 not only affects calcification, but also several other biological processes, such as fertilization, reproduction and physiology. There is a critical need for information on the effect of ocean acidification on non-calcifiers.

Additionally, in order to accurately assess the ecological impact of atmospheric CO_2, studies evaluating the synergetic impacts of ocean acidification and global warming on the early life and reproductive stages should be emphasized due to the vulnerability of these stages to environmental change. Impacts of global warming on the early life and reproductive stages have been studied to some extent. Foster (1971) mentions that larvae generally require a narrower temperature range for development compared to adults. O'Connor et al. (2007) demonstrated that temperature affects larval dispersal distance, with the implication that a warming ocean may influence population connectivity and structure. Svensson et al. (2005) demonstrated that unpredictable spring temperatures could lead to the mismatching of larval release with spring phytoplankton blooming, and reduce their recruitment. Thus, the interactive effect of CO_2 and temperature on early development and reproductive stages is a high priority for future studies.

Finally, a better understanding of the mechanisms behind CO_2 impacts on organisms and processes of biological adaptation and evolution is very important for any attempt to accurately forecast how marine organisms and the ecosystem will respond to ocean acidification. Most of the data gathered on the effects of ocean acidification (e.g. Table 1) highlight the impact of high pCO_2 (low $[CO_3^{2-}]$ and $CaCO_3$ saturation state) on both internal and external $CaCO_3$ skeletogenesis, even in seawater supersaturated with $CaCO_3$. Nevertheless, the mechanism behind this phenomenon is still obscure, because several studies have suggested that the major source of dissolved inorganic carbon for calcification is HCO_3^- derived from the surrounding seawater or converted by metabolic CO_2 rather than CO_3^{2-} (Tanaka et al. 1986, Furla et al. 2000, McConnaughey & Gillikin 2008). This may be partially explained by the indirect effect of decreased metabolic rate due to high pCO_2, since the respiration rate of several marine animals is observed to decrease under high pCO_2 (Langenbuch & Pörtner 2004, Michaelidis et al. 2005). Another possible explanation is that the extracellular fluid (where calcification takes place) of calcifiers becomes undersaturated for $CaCO_3$ even in $CaCO_3$ supersaturated seawater. The extracellular pH of most marine organisms is generally lower than that in the surrounding seawater (e.g. bivalve mantle hemolymph, pH 7.4~7.6), whereas $[Ca^{2+}]$ is similar to that of seawater (9 to 10 mM; Omori et al. 1988). When invertebrate calcifiers, such as bivalves and sea urchins, are exposed to high pCO_2 conditions, the hemolymph pH shows a permanent reduction (Michaelidis et al. 2005, Miles et al. 2007), suggesting that extracellular pH can become undersaturated even with a slight increase in seawater pCO_2.

On the basis of future climate scenarios, it is predicted that 15 to 37% of species and taxa will become extinct by 2050 (Thomas et al. 2004). However, it remains to be determined whether marine organisms will be able to adapt to a rapidly changing ocean environment. Recent research has revealed that organisms could evolve within decades in response to strong pressures, which Stockwell et al. (2003) termed 'contemporary evolution'. However, the capacity of marine organisms to adapt to increased seawater pCO_2 is unclear. Collins & Bell (2004) have performed the only study to examine the possible adaptation to an increased CO_2 concentration by an organism, the green alga *Chlamydomonas reinhardtii.* However, the relatively long generation length of marine calcifiers, such as echinoderms, bivalves and corals, which is an important factor for the evolutionary potential of a species, makes 'rapid evolution' of most calcifiers unlikely in response to the changes in the ocean environment (Berteaux et al. 2004).

Meanwhile, recent palaeontological studies have demonstrated that during the Paleocene-Eocene thermal maximum (PETM), when atmospheric CO_2 increased at the rate of 0.2 GtC yr^{-1} within <10 000 yr, catastrophic extinctions of 35 to 50% of benthic foraminiferan species occurred (Thomas 1998, Gibbs et al. 2006). It is also worth mentioning that the present anthropogenic rate of CO_2 emission is 8 GtC yr^{-1}, which is 16 times the rate during the PETM interval (Gibbs et al. 2006). Though further information is urgently needed on genetic variation, genetic response and adaptation of marine organisms in a high CO_2 world, the present data suggest that deleterious impacts on marine calcifier populations are very likely to occur in the future ocean.

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