Effects of CO_2-induced ocean environmental changes on marine life: implications for aquaculture

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ABSTRACT

The world's oceans are becoming warmer and acidic. The atmospheric carbon dioxide concentration has increased from 280 ppm at pre-industrial revolution to above 380 ppm today. The 4th IPCC report predicts that it will range from 540 to nearly 1,000 ppm by the end of the century. The increased CO_2 not only warms surface seawater, but also acidifies it (usually termed as ocean acidification) by diffusing across the ocean surface and forming carbonic acid. Our knowledge is still scarce as to how these ocean environmental changes will affect marine life. The early studies on the impact of ocean acidification focused on corals aiming to clarify effects of high-CO_2 seawater on their calcification processes. However, more recent studies have revealed that in fact ocean acidification, either alone or coupled with warming, could have detrimental impacts on a variety of biological processes in different taxa. We have shown that early development of marine bivalves (oysters and mussels) could be severely disrupted under elevated CO_2 conditions (ca. 2,000 ppm). When a marine shrimp was exposed to seawater equilibrated with air containing 1,000 ppm CO2 for 30 weeks, survival was only 55% as compared with 90% in the control. Gonad maturation of a sea urchin was delayed by one month under the same CO_2 conditions at ambient temperature, but when accompanied with increased temperature of 2°C above ambient, gonad maturation was not only delayed but also significantly suppressed; the number of eggs in the ovary was reduced to only 20% of the control. It has been shown that tropical animals already live near their thermal tolerant maxima, and therefore even small increases of environmental temperature could reduce their environmental fitness.

These recent findings bear significant implication in aquaculture and fisheries production, in particular, in tropical countries. This paper will summarize recent data on these topics and discuss possible adaptation measures.

Ocean environment is changing as a result of increasing concentrations of greenhouse gases in the atmosphere, with carbon dioxide (CO_2) being the most important anthropogenic greenhouse gas. The atmospheric concentration of CO_2 is projected to reach from the current level of 386 ppm to 540 to 970 ppm by the end of this century (IPCC 2007), and thereafter up to 1.900 ppm in around the year 2300 (Caldeira & Wickett 2003; see also Caldeira & Wickett 2005 for other projections). The buildup of greenhouse gases in the atmosphere has led to ocean warming and sea level rise (WBGM 2006; IPCC 2007). In the Southeast Asian region, mean surface temperature is predicted to rise 2.5°C (range, 1.5 - 3.7°C) by the end of this century (IPCC 2007).

Increasing atmospheric CO_2 is also acidifying ocean waters through formation of carbonic acid. It has been estimated that the oceans have absorbed about 50% of the anthropogenic CO_2 emitted to the atmosphere between 1800 and 1994 (Sabine et al. 2004), and roughly 30% of more recent emissions (Feely et al. 2004). It is generally held that average surface ocean pH has already declined by 0.1 units from the pre-industrial level (Orr et al. 2005), and is projected to decrease 0.3 to 0.46 by the end of this century, depending on CO_2 emission scenarios (Caldeira & Wickett 2005).

Effects of warming

Increases in water temperature stimulate metabolism of living organisms as long as warming remains within tolerable thermal ranges of a particular species. Metabolism, and therefore oxygen requirement, of most animals increases 2- to 3-fold for a 10°C increment, the value called Q10, which is expressed as:

$$Q10 = (Mo2^{T2}/Mo2^{T1})^{10/(T2-T1)}$$

where Mo2 represents oxygen requirement at temperature T1 or T2

Thus, when ambient temperature increases by 2.5°C, oxygen requirement will be enhanced by 20 to 30%. At the same time, warming decreases the solubility of O_2 (and CO_2) in water. A temperature increase by 10°C reduces O_2 concentration by approximately 20% in both freshwater and seawater given that the partial pressure of O_2 remains constant.

Metabolic stimulation generally accelerates growth of fish and other animals, and is in this sense considered to be advantageous for aquaculture production. However, to meet enhanced O_2 requirement, aquatic animals must ventilate their gills and other respiratory organs with a larger volume of water per unit time, when dissolved oxygen is relatively low. This would pose an increased load to the respiratory and circulatory system of animals. Pörtner (2002, 2008) and Pörtner & Langenbuch (2005) proposed a hypothesis of oxygen-limited thermal tolerance and performance capacity to give a mechanistic explanation for thermal tolerance of animals. According to this hypothesis, the performance of an animal will be curtailed at both lower and higher than optimal temperatures, since O_2 transport capacity of ventilatory and circulatory systems will become unable to deliver sufficient oxygen to metabolizing tissues. At even more extreme temperatures, anaerobic metabolism develops to provide energy, but only for limited time periods. It is currently unclear whether the hypothesis can fully explain thermal effects on animal physiology.

We recently compared combined effects of CO_2 and temperature on embryonic development of two tropical fish species. When incubation temperature was increased from 29°C (control) to 31°C, 100% mortality occurred within 144 h after fertilization in one species (*Amphiprion clarkia*), in contrast to only modest negative effect for the other species (*A. ocellaris*). Although statistical comparison was not made due to small sample size, it appears that heart rate failed to increase with time in those individuals incubated at 31°C in *A. clarkia,* while no such trend was seen for *A. ocellaris* (unpublished data).

Effects of CO_2-induced acidification

Earlier studies on ocean acidification have centered on how and to what extent low pH seawater affects calcification of major calcifying organisms such as corals and some phytoplankton species (e.g. coccolithophores). However, there is now increasing evidence that ocean acidification in fact has multi-faceted biological impacts on both calcifying and non-calcifying organisms. These new findings have implications for aquaculture production in not only marine but also freshwater systems, whilst potential effects of CO_2-induced acidification in the freshwater realm have been virtually unexplored.

Bivalves and gastropods. Experimental evidence has revealed that CO_2-induced reductions in seawater pH suppress calcification in *Crassostrea gigas* and *Mytilus edulis* (Gazeau et al. 2007); growth of *M. edulis* (Berge et al. 2006) and *M. galloprovincialis* (Michaelidis et al. 2005); and early development of *M. galloprovincialis* (Kurihara et al. 2008a) and *C. gigas* (Kurihara et al. 2007). Kurihara et al. (2007) have shown that only 5% of *C. gigas* developed into normal veliger larvae when incubated under 2,270 µatm pCO_2 conditions* even though no obvious effect was detected up to the trochophore stage. Larval shell mineralization was completely inhibited in 45% of the CO_2 larvae in these conditions (Kurihara et al. 2007). All CO_2-exposed larvae of *M. galloprovincialis* showed morphological aberrancies under 2,000 µatm conditions (Kurihara et al. 2008a, Fig. 1).

Shirayama & Thornton (2005) exposed a marine snail *Strombus luhuanus* to pCO_2 of only 560 µatm for six months, and found a significant reduction in both shell height and wet weight

*1 µatm = 1 ppm in a gaseous phase under a barometric pressure of 1 atm but not in an aqueous phase; in an aqueous phase, concentration of a gas is the product of partial pressure and solubility of the gas

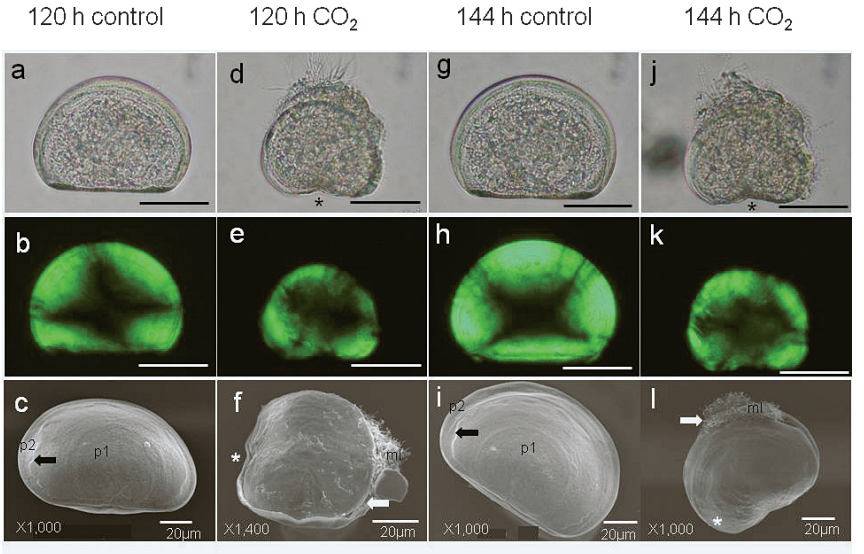

Figure 1. Morphology of *Mytilus galloprovincialis* larvae incubated for 120 h and 144 h control or in CO_2-enriched (pCO_2 2000 µatm) seawater; same individual larva in each column (Kurihara et al. 2008a)
Abbreviations: p1, prodissoconch I; p2, prodissoconch II; ml, mantle. Scale bars in upper 2 rows: 50 µm. (*) Convex hinges; (black arrows) Prodissoconch I/II boundary found only in control larvae; (white arrows) Gap between left and right shells

from 8 to 14 weeks of exposure. The average seawater pH difference was reported to be only 0.03 in their experiment (control 7.936-7.945, 560 µatm 7.897-7.902). Michaelidis et al. (2005) also demonstrated reduced oxygen consumption but increased ammonia excretion, indicating a lower metabolic rate and the net degradation of protein in *M. galloprovincialis* under their experimental conditions. Green et al. (2004) found that newly settled juveniles of the hard-shell clam *Mercenaria mercenaria* revealed substantial shell dissolution and increased mortality when they were introduced to surface sediments that were undersaturated with respect to aragonite (Warag ≈ 0.3), a level that is typical of nearshore, organic-rich surficial sediments. Within two weeks of settlement, the $CaCO_3$ shells were completely dissolved, leaving only the organic matrix of the shell. Other physiological responses elicited by acidified seawater in bivalves include a potential suppression of phagocytosis in *M. edulis* (Bibby et al. 2008), inhibition of predator-induced shell thickening in *Littorina littorea* (Bibby et al. 2007), and changes in embryonic movement and lower heart rate during development in *L. obtusata* (Ellis et al. 2009).

Sea urchins. Sea urchins are harvested in high quantity and have high commercial value. They are often the dominant grazer in many communities (Lawrence 2007) and can act as a keystone species. Shirayama & Thornton (2005) reported significant reductions in weight gain in *Hemicentrotus pulcherrimus* and *Echinometra mathaei* exposed to only 560 µatm CO_2 for six months. As in the case of *S. luhuanus* (see above), the CO_2 effect on weight became significant only after 14 weeks of exposure. Kurihara & Shirayama (2004) examined effects of CO_2 on early development of the same two sea urchin species, and found that fertilization, cleavage and pluteus morphology were all negatively affected by increasing ambient pCO_2, even though the effects on the first two parameters were significant only at very high pCO_2 levels (> 5,000 µatm). In contrast, morphological impacts were already significant at 860 µatm, which became intensified with further elevations of pCO_2. More recently, Havenhand et al. (2008) reported a small but significant drop in sperm swimming speed and percent motility in the Australian sea urchin *Heliocidaris erythrogramma* incubated at pCO_2 of 1,000 µatm (seawater pH 7.7). This led to a 20% decrease of eggs that developed into cleaving embryos, and a 26% drop of those that developed into swimming larvae. In sharp contrast, Byrne et al. (2009) found no effect of elevated seawater CO_2 on fertilization or early development (percentage of normal cleaving embryos and normal gastrulae) in the same species incubated down to the seawater pH of 7.6. The reason for this difference is not clear: both studies used *H. erythrogramma* collected at or near Sydney, collected gametes by KCl injection, acidified experimental seawater by bubbling CO_2, and conducted at almost same temperature (19.5°C and 20°C) and salinity (36 ppt). Despite these unresolved uncertainties of experimental results, the data of Byrne et al. (2009) explicitly illustrated the importance of investigating synergistic impacts of environmental changes projected for the future ocean: even though early development was unaffected by the range of pCO2 they used, increasing temperature dramatically suppressed cleavage and gastrulation of the embryos.

Clark et al. (2009) compared responses of pluteus larvae of one tropical (*Tripneustes gratilla*), two temperate (*Pseudechinus huttoni* and *Evechinus chloroticus*), and one polar (*Sterechinus neumayeri*) sea urchin to exposure to pCO_2 of 1,200 to 1,400 µatm. They found that the polar sea urchin is the most tolerant in terms of calcification, skeletal structure, and survival. These authors pointed out that the species may have greater capacity to acclimate to lowered seawater pH since it has evolved in an environment with historically higher levels of CO_2 and a greater apparent solubility product for calcite.

Crustacean decapods. Arnold et al. (2009) tested effects of 1,200 µatm pCO_2 exposure on larval development of the European lobster, *Homarus gammarus*. Again, survival, zoeal progression and carapace length were not

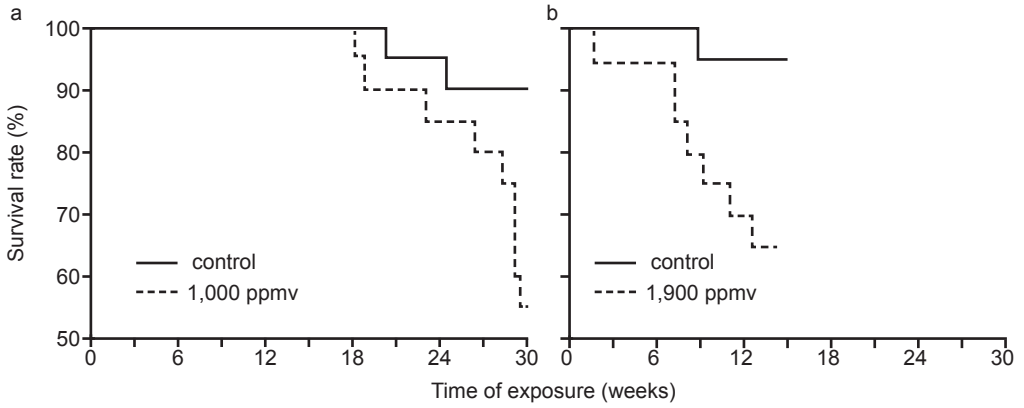

Figure 2. Effects of long-term CO2 exposure on the survival of the marine shrimp *Palaemon pacificus.* Survival was significantly suppressed (Log-rank test, $p < 0.05$) when shrimps were individually reared under a 1,000 ppm and b 1,900 ppm CO2 conditions for 30 and 15 weeks, respectively (Kurihara et al. 2008b)

significantly affected by the CO_2 exposure (Zoea I through IV, 28 days). However, dry mass of carapace and its content of calcium and magnesium were both depressed at Zoea IV stage. Thus, exposure to this level of CO_2, if prolonged, could have detrimental impacts on crustaceans. In fact, we reported that when the shrimp *Palaemon pacificus* was exposed to pCO_2 of 1,000 µatm and 1900 µmatm for 30 weeks and 15 weeks respectively, mortality set in after 7 and 18 weeks of exposure with the final survival of 55% and 65% (Kurihara et al. 2008b, Fig. 2).

Growth was suppressed in 1,900 µatm but not in 1,000 µatm. However, a closer inspection of data revealed that body length of females in the 1,000 µatm pCO_2 group tended to be smaller than in controls. Possibly related to this suppressed growth, the percentage of females that bore eggs was smaller in the 1,000 µatm pCO_2 shrimps, though this needs to be reinvestigated due to their small sample size. Characteristically, the length of the second antennae were much shorter in 1,000 µatm shrimps as determined at the end of 30 week exposure (54% of body length in the 1,000 µatm vs. 165% in the control; no measurement was conducted for the 1,900 µatm experiment).

Fish. Information on CO_2 effects on fish is very limited in the field of ocean acidification research, but is relatively rich in aquaculture science (Ishimatsu et al. 2008). The major difference between the two study fields relates to relevant CO_2 levels; the CO_2 levels usually employed in ocean acidification research range up to 2,000 µatm, which is the highest projected atmospheric pCO_2 in the future, whereas much higher CO_2 levels (>5,000 µatm) have been frequently used in aquaculture studies since these levels may prevail in aquaculture tanks with high stock densities. The main finding from these papers is that fish are generally more tolerant to CO_2 than invertebrates. Mortality was reported only at > 30,000 to 50,000 µatm in acute exposure experiments (duration less than 3 days), and only a few percent mortality was seen even when Atlantic salmon smolts were exposed for 60 days to 2,600 µatm pCO_2 (Ishimatsu et al. 2008).

A few examples of fish papers in ocean acidification research include Munday et al. (2009a,b,c) and Melzner et al. (2009). Munday et al. (2009a) found that embryonic development of the clownfish *Amphiprion percula* was unaffected by exposure up to 1,030 µatm, and thus apparently more tolerant than in invertebrates (Kurihara, 2008). Moreover, the larvae from some but not all parents were

longer and heavier in acidified conditions than in controls. Critical swimming speed did not differ between the control and larvae reared under high CO_2 conditions. Munday et al. (2009b) examined effects of CO_2 on olfactory performance in *A. percula*, and found that the ability of olfactory discrimination is disrupted by exposure to pCO_2 of 1,000 µatm (pH 7.8). Effect of increased seawater pCO_2 on swimming was tested by Munday et al. (2009c) and Melzner et al. (2009). Aerobic scope (difference between oxygen consumption rate at maximum swimming speed and resting) was significantly depressed by exposure to pCO_2 of 1,000 µatm at 29-32°C in one coral reef fish *Ostorhincus cyanosoma*, but not in the other species, *O. doederleini* (Munday et al. 2009c). Resting oxygen uptake remained unaffected or increased by the CO_2 exposure, unlike most findings for invertebrates. In contrast, maximum oxygen consumption either decreased or remained stable in high CO_2 conditions, leading to the observed decline of aerobic scope. There was no interaction of temperature and high CO_2 on aerobic scope. Melzner et al. (2009) acclimated Atlantic cod *Gadus morhua* to 3,000 µatm for 4 months and to 5,800 µatm for 12 months, both at 5°C. Resting and active metabolic rates, critical swimming speed and aerobic scope were all unaffected by the long-term rearing under the elevated CO_2 conditions. Gill Na+/K+ ATPase activity and protein showed significantly higher values for the fish reared in 5,800 µatm but not in 3,000 µatm.

Ambient high CO_2 levels could compromise fish growth at least under levels relevant to aquaculture. It may be inferred that fish growth is reduced due to the possible additional energetic costs imposed by elevated pCO_2, when overall oxygen consumption remains unchanged. Increments of body weight were in general unaffected by exposure to pCO_2 of up to 15,000 µatm irrespective of salinity (Fivelstad et al. 1998, 1999, 2003; Foss et al. 2003; Hosfeld et al. 2008). The condition factor ([100 × body weight] / [body length]3) tended to decrease at high pCO_2, but the threshold for this effect appears to depend on species, fish size and salinity. Growth was invariably reduced at pCO_2 >26,000 µatm. Fivelstad et al. (2007) demonstrated that negative CO_2 effect on fish growth was more pronounced at a low temperature when exposed to the same pCO_2 (16,000 µatm). Feeding may be suppressed at a very high pCO_2 (55,000 µatm, Cecchini et al. 2001; 26,500 µatm, Foss et al. 2003). To our knowledge, no paper has been published on growth from fish eggs or larvae under pCO_2 of < 2,000 µatm.

Combined effects of warming and acidification

It must be realized that ocean warming occurs in concert with acidification. Thus, to precisely understand how future ocean environmental changes will affect marine organisms, it is necessary to conduct experiments manipulating both temperature and pCO_2. We have recently examined combined effects of warming and CO_2 on gonad development of the sea urchin *Hemicentrotus pulcherrimus* reared in three conditions for 9 months (unpublished). In this experiment, sea urchins were allocated to three conditions, i.e., control group (pCO_2 380 µatm, ambient temperature), high CO_2 group (pCO_2 1,000 µatm at ambient temperature) and high CO_2 + temperature group (pCO_2 1,000 µatm at 2°C above ambient temperature). Our preliminary analysis demonstrated that high CO_2 delayed ovary maturation such that the number of eggs per cross section peaked one month later but attained a comparable level as in control, however, when the sea urchin was kept under high CO_2 and high temperature conditions, the egg number not only peaked one month later but also was dramatically suppressed (only 20% of the control level). These results clearly indicate that under more realistic conditions mimicking both future increases in seawater temperature and acidification, CO_2 could have far stronger negative impacts than when effects of CO_2 was tested alone, emphasizing the need for investigating synergistic effects of both temperature and CO_2 to better understand animal's responses to changing ocean conditions.

Implications for aquaculture

Even though climate warming in tropics is projected to be relatively small compared with high-latitude regions (IPCC 2007), tropical animals may incur disproportionally large impacts by increasing temperature in their habitats. It has been pointed out that tropical animals have narrower thermal tolerance ranges than higher-latitude animals, and also live in temperature ranges closer to their physiological optima (Tewksbury et al. 2008; Somero 2010). Therefore, even small increases in ambient temperature could bring tropical animals to critical conditions, the most well known example of which is probably coral bleaching caused by a few week's thermal anomalies of only 1-2°C (Hoegh-Guldberg 2009). Thus, aquaculture production in the Southeast Asia may be significantly affected by warming in the area (mean 2.5°C, range 1.5 to 3.7°C). Even though experimental evidence is still very limited, fish production might be reduced through negative impacts of temperature rises on early development. Acidification may also pose another significant threat to aquaculture (and catch fisheries) of bivalves and gastropods, and maybe prawns and shrimps. When these two environmental alterations occur in concert, negative impact might be far greater than what one might expect from experimental results on each factor. Science in this field is still not mature enough to answer important questions on the possible impacts of future climate change on aquaculture production.

Possible adaptation measures

Inspite of the above uncertainties, some adaptation measures could and need to be proposed on the basis of the present knowledge to sustain aquaculture production in the age of climate change. Because animals in early developmental stages seem particularly vulnerable to temperature rises and acidification, it is most essential to raise embryos and larvae under favorable conditions for their development. To reduce impacts of acidification, excess CO_2 in air supply could be absorbed into an alkaline solution before being delivered to rearing tanks. Some alkalizing agents such as soda lime could be directly added to rearing water, but their biological toxicity must be carefully tested before application. If possible, larval rearing is better conducted under favorable temperature conditions. Once larvae have grown up to a stage that is more tolerant to environmental stress, then they could probably be transferred to usual aquaculture grounds. This might be applicable to bivalves such as clams, mussels and oysters, and crustacean species. Selection of some strains that are more robust to warming and acidification should certainly be another important adaptation measure. In addition, some animals, including fish, show thermal plasticity and can adapt to higher than usual temperature (Franklin & Seebacher 2009). These observations suggest the possibility of successfully establishing thermal-tolerant aquaculture animals through breeding selection.

Some freshwater animals, particularly those inhabiting stagnant lakes and ponds, are probably less affected by high CO_2 conditions, because these water bodies are prone to show high CO_2 levels (Hughes 1976). Global warming likely reduces dissolved oxygen concentrations in these water bodies, but a large number of fish inhabiting these habitats has the capacity of breathing air, making them suitable to aquaculture in hypoxic waters. In fact, many air-breathing fish species are cultured in the Southeast Asian countries, such as A*nabas, Boleophthalmus, Channa, Clarias, Monopterus,* and *Sccobranchus*. Increasing use of these species in aquaculture production may also be considered another adaptation measure to climate change.

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