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Comparison of the acid-base responses to CO_2 and acidification in Japanese flounder (*Paralichthys olivaceus*)

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Abstract

To investigate whether the biological toxicity of aquatic hypercapnia is due to the direct effects of CO_2 or to the effects of acidification of seawater by CO_2, the Japanese flounder (*Paralichthys olivaceus*) was subjected to seawater equilibrated with a gas mixture of air containing 5% CO_2 (pH 6.18) or seawater acidified to the same pH with 1 N H_2SO_4. All the fish died within 48 h in the CO_2 exposure group, whereas no mortality occurred in the acid group. Acid-base parameters as well as plasma ion concentrations were severely perturbed in the CO_2 exposure group, whereas they were minimally affected in the acid group. These results clearly demonstrate that the mortality in the CO_2 group is a direct result of the elevated levels of dissolved CO_2 and not to the effects of the reduced water pH.

Keywords: Acid-base response; CO_2; pH; Toxicity; Marine fish; Ocean dumping

The atmospheric concentration of carbon dioxide (CO_2) has been rapidly rising in recent years (IPCC, 2001). To mitigate environmental impacts of the increasing concentration of atmospheric CO_2, several methods of sequestering CO_2 from the atmosphere have been proposed. Among them, ocean sequestration is receiving increasing attention, in which CO_2 captured from flue gases and other sources is released into deep ocean waters (Handa and Ohsumi, 1995; Ormerod and Angel, 1996). CO_2 ocean sequestration potentially will perturb biological processes in the ocean (Seibel and Walsh, 2002). However, the impact of this mitigation procedure on the ocean ecosystem is little understood, and therefore must be carefully investigated before embarking on this strategy. A priori, the toxic effects of CO_2 on marine organisms should be assessed through CO_2 exposure experiments, but the lack of such experimental data in the past has led to the use of the data on the toxic effects of seawater acidified by adding mineral acids (HCl or H_2SO_4) in modeling the potential consequences of ocean disposal of CO_2 (e.g., Auerbach et al., 1997).

We recently compared acute mortality of eggs and larvae of a marine teleost, red sea bream (*Pagrus major*), subjected to CO_2- and HCl- acidified seawater, and found that mortality of the CO_2 group was significantly higher than in the HCl group (Kikkawa et al., 2004). We suggested a higher membrane permeability of CO_2, as compared with that of H^+, to be responsible for the observed difference in mortality, but direct comparison of the physiological responses between acidification by CO_2 and mineral acid has not been made. Therefore, the present study was aimed at comparing the acid-base responses of the Japanese flounder (*Paralichthys olivaceus*) exposed to CO_2- and H_2SO_4-acidified seawater.

Experimental fish weighing 490 ± 40 (S.D.) g ($N = 11$) were purchased from a local hatchery (NCC Kaihatsu, Nomozaki, Nagasaki, Japan), and kept in an indoor

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fiberglass tank (capacity 1000 l) with filtered, recirculating well-aerated seawater at 20 °C, without feeding for less than 3 weeks. For blood sampling, the flounder were cannulated as described in Hayashi et al. (2004). Fish were then allowed to recover for about 48 h in Plexiglass chambers supplied with continuous flow of well-aerated seawater at a rate of 3 l min^{-1}. The experimental setup consisted of a fish chamber, a water reservoir and a gas equilibration column. Total water volume of the setup was ca. 40 l. Fresh seawater was supplied into the experimental setup continuously at a rate of 0.3 l min^{-1}. Two control blood samples were taken, 1 h apart, before exposures began. Subsequently, fish were subjected to one of the following acidic (pH 6.18) conditions: (1) CO_2-acidified seawater: Water in the experimental setup was equilibrated with a gas mixture (95% air plus 5% CO_2) prepared with a gas mixing flowmeter (GF-3/MP, Cameron Instruments, TX, USA) at a gas flow rate of 6 l min^{-1}. (2) H_2SO_4-acidified seawater: Water in the experimental setup was switched to seawater that had been acidified by adding 1 N H_2SO_4. To remove excess CO_2 produced by acidification, the seawater was vigorously bubbled with air for at least two days before use. During the H_2SO_4 exposure, seawater pH was continuously monitored, and adjusted to 6.18 with a pH controller (NPH-660, Nissin, Tokyo, Japan) and peristaltic pumps adding either 0.1 N H_2SO_4 or 0.1 N NaOH as required. Acidified stock seawater was continuously added into the experimental setup at the rate of 0.3 l min^{-1}. Blood samples were taken at 0.5, 1, 3, 8, 24, 48 and 72 h of each exposure. Hematocrit (Hct), arterial pH (pHa) and partial pressure of CO_2 (Pa_{CO_2}), and plasma ion concentrations ($[HCO_3^-]p$, $[Cl^-]p$, $[Na^+]p$ and $[K^+]p$) were measured as described in Hayashi et al., 2004). Seawater pH was measured at every sampling time. It decreased from normocapnic values of 8.16 ± 0.01 (S.D.) and 8.20 ± 0.06 to 6.17 ± 0.03 (CO_2) and 6.18 ± 0.00 (H_2SO_4) within 1 h and 3 h, respectively.

Fish subjected to CO_2-acidified seawater started to die 8 h after the onset of exposure, and all fish died within 48 h. In contrast, no fish died until the end of 72 h exposure to H_2SO_4-acidified seawater. Figs. 1 and 2 summarize the changes of pHa, Pa_{CO_2}, plasma ion concentrations and Hct during the CO_2- and H_2SO_4-acidified seawater exposures. The pHa of the CO_2-exposed fish fell significantly from the pre-exposure value of 7.66 ± 0.02 (S.D.) to 6.87 ± 0.07 within 0.5 h, but was subsequently restored. In contrast, H_2SO_4 exposure caused a more gradual but steady decrease in pHa. Pa_{CO_2} increased from 0.24 ± 0.03 (S.D.) kPa (pre-exposure) to 7.09 ± 1.00 in 1 h in the CO_2 group. The H_2SO_4 exposure resulted in a much smaller, but significant increase in Pa_{CO_2}. The CO_2 exposure resulted in significant increases in $[HCO_3^-]p$, $[Na^+]p$ and $[K^+]p$. $[Cl^-]p$ decreased, but not significantly (statistical comparison was not applied due to mortality of some of the experimental specimens). The H_2SO_4 exposure caused hardly any significant changes in the plasma ion concentrations. Hct rose significantly during the CO_2 exposure but not during the H_2SO_4 exposure.

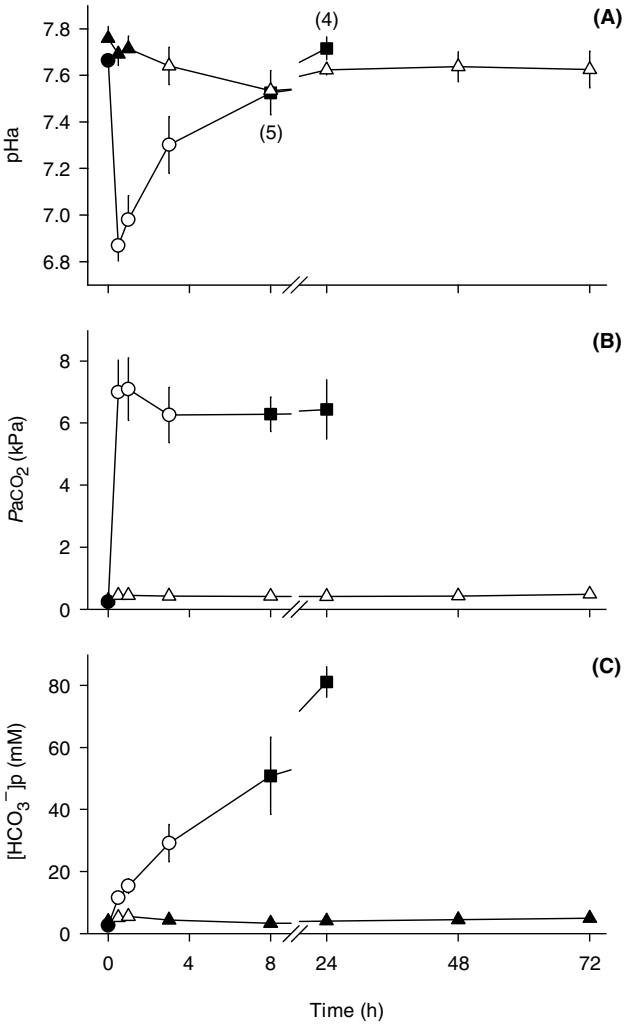

Fig. 1. Arterial pH (pHa; **A**), partial pressure of CO_2 (Pa_{CO_2}; **B**) and concentration of plasma HCO_3^- ($[HCO_3^-]p$; **C**) of the Japanese flounder during CO_2- (●: $N = 6$) and H_2SO_4-acidified (▲: $N = 5$) seawater exposure (mean ± S.D.). Open symbols indicate significant differences from 0-h values ($p < 0.05$; one-way ANOVA, Dunnett's test). N decreased due to mortality in the CO_2-acidified seawater exposure (■), to which no statistical comparison was applied (the numbers in parentheses indicate numbers of surviving fish).

Even though the fish were exposed to the same seawater pH, mortality occurred only during the CO_2 exposure, and no fish died during the H_2SO_4 exposure. Similar results were obtained by Kikkawa et al. (2004), in which mortalities of eggs and larvae of red sea bream (*Pagrus major*) were significantly higher in the CO_2 treated groups (eggs; >85%, larvae; >60%) than in the acid treated groups (eggs; <5%, larvae; <5%), tested at two levels of seawater pH lowered by either elevating the seawater CO_2 pressure or the addition of HCl.

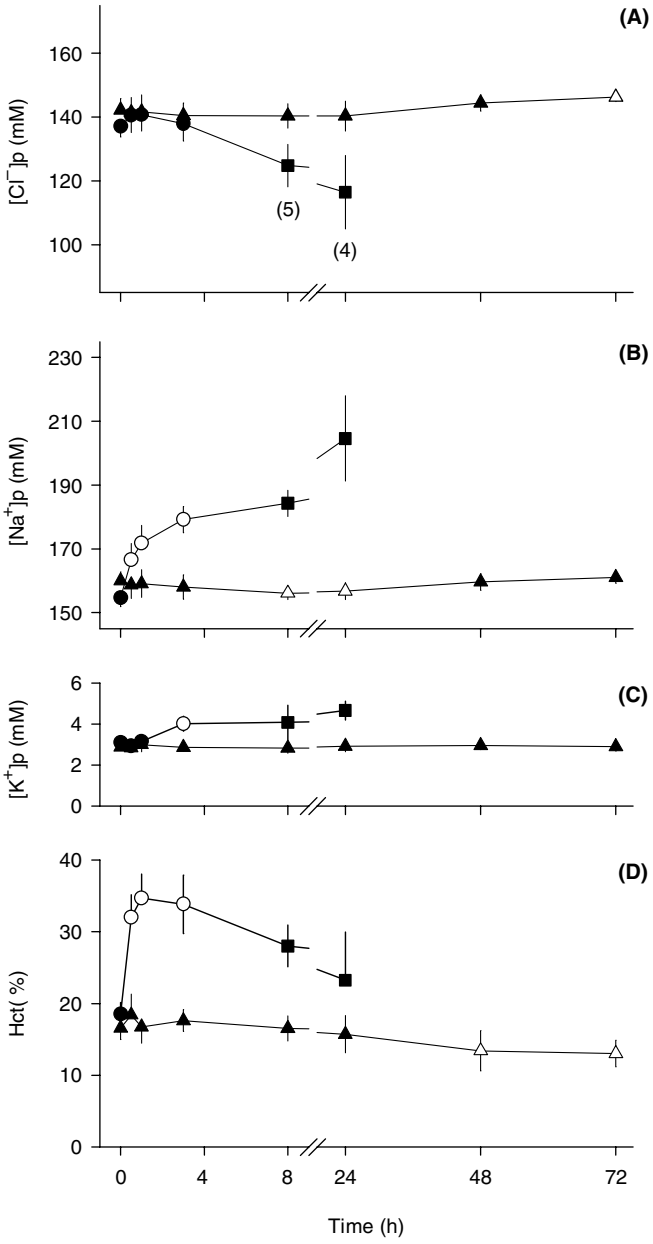

Fig. 2. Concentrations of plasma Cl^- ($[Cl^-]p$; **A**), Na^+ ($[Na^+]p$; **B**) and K^+ ($[K^+]p$; **C**) and hematocrit (Hct; **D**) of the Japanese flounder during CO_2- (●: $N = 6$) and H_2SO_4-acidified (▲: $N = 5$) seawater exposure (mean ± S.D.). Open symbols indicate significant differences from 0-h values ($p < 0.05$; one-way ANOVA, Dunnett's test). N decreased due to mortality in the CO_2-acidified seawater exposure (■), to which no statistical comparison was applied (the numbers in parentheses indicate numbers of surviving fish).

In this study, the elevation of ambient CO_2 rapidly disturbed the acid-base and ionic status of the plasma. Since the biomembrane permeability of gaseous CO_2 is much higher compared with that of H^+, CO_2 diffuses immediately into the body fluid according to the P_{CO_2} gradient (Heisler, 1986; Morris et al., 1989). Blood P_{CO_2} of water-breathing animals is an order of magnitude lower than in air-breathing animals (Pa_{CO_2} of fish ranging 0.1–0.5 kPa as compared with 5.3 kPa in humans, Heisler, 1986), so that even a small increase in water P_{CO_2} would reverse the normal CO_2 gradient, resulting in a rise in blood P_{CO_2}. Increased blood P_{CO_2} shifts the CO_2–H_2CO_3 equilibrium to cause acidification of the blood. The resultant acidosis is usually compensated by the uptake of bicarbonate ions from or the extrusion of protons into the surrounding water as long as the magnitude of the CO_2 rise remains sublethal (Hayashi et al., 2004).

Although pHa was restored to the pre-exposure level within 24 h, this does not mean that the effects of CO_2 were eliminated, since changes in ionic the status were an unavoidable consequence of pHa compensation. In

fact, the flounder in the CO_2 group started dying after pHa had been completely restored, suggesting that the mortality was not directly due to acidosis. Lee et al. (2003) determined the cardiac responses of the marine fish, yellowtail (*Seriola quinqueradiata*), to a lethal level of aquatic hypercapnia, and pointed out that cardiac failure probably played an important role in inducing death during hypercapnia.

The more gradual fall of pHa in the acid group attests to the above-mentioned lower membrane permeability of H^+ as compared with CO_2 (McDonald and Wood, 1981; Ultsch et al., 1981). Freshwater fish generally suffer from the loss of inorganic plasma ions, in addition to the decrease of pHa, in severely acidic (ca. pH 4.5) environments (Milligan and Wood, 1982), but no ion loss was observed when rainbow trout (*Oncorhynchus mykiss*) was exposed to water pH of 6.0 (Giles et al., 1984). It is therefore probably not surprising that the flounder in the H_2SO_4 group showed hardly any significant changes in acid-base parameters and plasma ion levels, even though marine fish face ionic and osmotic gradients that are opposite from what freshwater fish experience.

In spite of the same pH level used in this study, mortality and all measured physiological parameters were largely affected by CO_2-acidified seawater whereas they were hardly changed by H_2SO_4-acidified seawater exposure. These results support our hypothesis that the physiological effects of aquatic hypercapnia are caused by CO_2 itself and not by an accompanying reduction of water pH.

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