Available online at www.sciencedirect.com
SCIENCE DIRECT®
Marine Pollution Bulletin 48 (2004) 108–110
MARINE POLLUTION BULLETIN
www.elsevier.com/locate/marpolbul

Comparison of the lethal effect of CO_2 and acidification on red sea bream (*Pagrus major*) during the early developmental stages

Takashi Kikkawa [a,b,*], Jun Kita [a,1], Atsushi Ishimatsu [b]

[a] *Central Laboratory, Marine Ecology Research Institute, 300 Iwawada, Onjuku, Chiba 299-5105, Japan*
[b] *Marine Research Institute, Nagasaki University, 1551-7 Taira, Nagasaki 851-2213, Japan*

Abstract

To compare the acute toxicity of CO_2- and HCl-acidified seawater, eggs and larvae of a marine fish, *Pagrus major*, were exposed to seawater equilibrated with CO_2-enriched gas mixtures ($CO_2 = 5\%$ or 10%, $O_2 = 20.95\%$ balanced with N_2) or seawater acidified with 1 N HCl at two pH levels (pH 6.2 ($= 5\%$ CO_2) and 5.9 ($= 10\%$ CO_2)) for 6 h (eggs) or 24 h (larvae). Mortalities of eggs were 85.8% (CO_2) and 3.6% (HCl) at pH 6.2, and 97.4% (CO_2) and 0.9% (HCl) at pH 5.9, while those of larvae were 61.2% (CO_2) and 1.6% (HCl) at pH 6.2, and 100% (CO_2) and 5.0% (HCl) at pH 5.9. Thus, previous research on the effects of acidified seawater on marine organisms, as a substitute for CO_2, has largely underestimated the toxic effects of CO_2.

Keywords: Toxicity; Hypercapnia; Acidification; Marine fish; Early developmental stage; Ocean dumping

As a possible measure to reduce the rate of increase of atmospheric CO_2, ocean sequestration of CO_2 has been proposed. The feasibility and potential impacts on the marine ecosystem of this method of CO_2 disposal are well documented (Handa and Ohsumi, 1995; Ormerod and Angel, 1996). To evaluate the lethal effects of CO_2 on marine animals, Auerbach et al. (1997) proposed mortality curves based on existing data from the literature for marine organisms with limited mobilities (e.g. fish larvae, copepods and mollusks). Their analysis was based on experiments in which organisms were exposed to seawater acidified by adding mineral acids (HCl or H_2SO_4) and not by CO_2. However, these two media may exert different physiological influences on aquatic organisms, as the membrane permeabilities of hydrogen ions and gaseous CO_2 differ (Heisler, 1986; Morris et al., 1989). In fact, Brownell (1980) reported 24 h median lethal pH (24 h-LC_{50}) of 4.5–5.1 for the larvae of three marine fish, cape rocking, *Gaidropsarus capensis*; cape sole, *Heteromycteris capensis* and white sea bream, *Diplodus sargus*, when seawater was acidified with HCl. These values are considerably lower than the 24 h-LC_{50} values (pH 6.1–6.5) we recently found for larvae and juveniles of two marine fish, red sea bream, *Pagrus major* and Japanese whiting, *Sillago japonica*, when seawater pH was reduced by elevating CO_2 pressures (Kikkawa et al., unpublished data). However, no direct comparison has been made in this respect. Therefore, this study was aimed at comparing the toxicity of seawater acidified by either CO_2 or acid to fish at two early developmental stages.

Eggs of red sea bream (*Pagrus major*) were obtained after natural spawning on May 20–23, 2001 and incubated in 100 l tanks supplied with aerated, sand-filtered seawater at 20 °C at the Marine Ecology Research Institute. Exposure tests were conducted upon two developmental stages; embryos at the stage where auditory vesicles were forming (21 h after fertilization) and preflexion larva (10–12 days after hatching). The mean egg diameter and total length of larvae (±SD) were 0.87 ± 0.013 and 5.74 ± 0.506 mm, respectively. Test seawater was prepared as follows: (1) CO_2 group: seawater was bubbled with a gas mixture of 5% or 10% CO_2 and 20.95% O_2 balanced with N_2 prepared by a gas mixing flow meter (GF-3/MP, Cameron Instrument Company, Texas, USA) at a flow rate of 300 ml min^{-1}

* Corresponding author. Address: Central Laboratory, Marine Ecology Research Institute, 300 Iwawada, Onjuku, Chiba 299-5105, Japan. Tel.: +81-470-68-7512; fax: +81-470-68-5115.
E-mail address: kikkawa@kaiseiken.or.jp (T. Kikkawa).
[1] Present address: Research Institute of Innovative Technology for the Earth, 9-2 Kizugawa-dai, Kizu-Cho, Soraku-Gun, Kyoto 619-0292, Japan.

0025-326X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0025-326X(03)00367-9

Table 1
Seawater carbonic systems of CO_2 and acid groups

Parameters	Condition A		Condition B	
	CO_2 (5%)	Acid	CO_2 (10%)	Acid
pH	6.16	6.19	5.86	5.87
$[H^+]$ (mmol l^{-1})	6.92×10^{-7}	6.46×10^{-7}	1.38×10^{-6}	1.35×10^{-6}
Pco_2 (kPa)	4.95	0.037	9.90	0.037
$[CO_2]$ (mmol kg^{-1})[a]	1.58	1.17×10^{-2}	3.16	1.17×10^{-2}
$[HCO_3^-]$ (mmol kg^{-1})	2.16	1.71×10^{-2}	2.16	0.82×10^{-2}
$[CO_3^{2-}]$ (mmol kg^{-1})	2.05×10^{-3}	1.74×10^{-5}	1.03×10^{-3}	4.00×10^{-6}

pK_1 (6.026) and pK_2 (9.181) from Mehrbach et al. (1973). CO_2 solubility (0.03241 mol kg^{-1} atm^{-1}) from Weiss (1974).
[a] Includes negligible concentration of H_2CO_3 (Heisler, 1986). Assuming atmospheric pressure of 101.3 kPa.

for 2 h. Equilibrating seawater with 5% and 10% CO_2 reduced seawater pH to 6.2 and 5.9, respectively. (2) Acid group: seawater was acidified by adding 1 N HCl, and vigorously aerated for about 24 h before use to eliminate excess CO_2 produced by acidification. Seawater pH of the acid group was acidified as per those in the CO_2 group (Table 1), and was readjusted to desired levels immediately before experimentation. Salinity, total CO_2 concentration, pH and alkalinity of the original seawater were 34.46 (PSU), 1.75 mM, 8.1 and 2.29 meq l^{-1}, respectively. Eggs were placed in five cylindrical polycarbonate vessels (capacity 85 ml, 39–51 eggs per vessel) with open ends covered with a fine mesh net, and larvae were placed in three polycarbonate bottles (capacity 1000 ml, 16–28 larvae per bottle) with round net-covered windows on the side wall. Eggs and larvae were then submerged in test seawater in a temperature-controlled (20 °C) PVC tank for 6 h (eggs) or 24 h (larvae). At the end of the exposure, eggs were transferred to 300 ml beakers filled with natural seawater, and incubated overnight. Numbers of normally hatched larvae, dead eggs, malformed eggs, dead larvae, and malformed larvae were counted one day after hatching. Only normally hatched individuals were regarded as "survived", and mortality was calculated accordingly. Larval mortality was counted immediately after the exposure. A larva was judged to be dead if the heart was not beating.

Mortalities were significantly higher in the CO_2 groups than in the acid groups irrespective of developmental stage (Welch's *t*-test was used for data at pH 6.2; embryo $p < 0.0001$, larva $p < 0.05$. Student's *t*-test was used for data at pH 5.9; both groups $p < 0.0001$, Fig. 1). CO_2 is clearly far more toxic than acid when seawater pH is reduced to the same pH, and therefore, the use of acid toxicity results for evaluating CO_2 toxicity greatly underestimates impacts of the gas. It is obvious that CO_2 must be used to correctly assess the environmental impacts of CO_2 ocean sequestration on marine organisms.

Table 1 lists the calculated concentrations of CO_2-derived molecular species in the test seawater. All CO_2-derived molecular species were ≈130-fold (5%) and 260-fold (10%) higher in the CO_2 group than in the acid group, with the H^+ concentration nearly identical for each pH level. Compared with HCO_3^- and CO_3^{2-} ions, uncharged CO_2 molecules are readily permeable to biological membranes (Vandenberg et al., 1994). Once CO_2 diffuses into intracellular compartments, it rapidly reacts with water to form carbonic acid in the presence of a ubiquitous enzyme, carbonic anhydrase, and the acid immediately dissociates into H^+ and HCO_3^- ions. The resulting intracellular acidosis will affect a number of physiological processes (Roos and Boron, 1981), to the extent that it may be lethal to marine organisms. In addition to these acidic toxicities, CO_2 itself may be toxic to animal cells (Max, 1991).

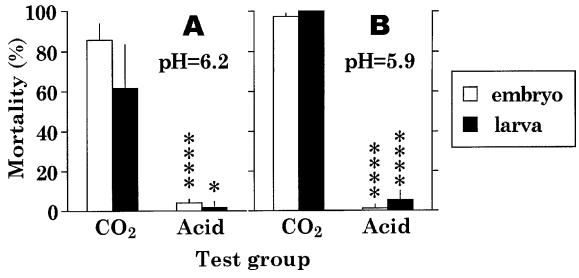

Fig. 1. Comparison of lethal effect between CO_2 and acid by mean mortalities of embryo ($n = 5$) and larva ($n = 3$) of red sea bream at two pH levels (Condition A: pH 6.2, Condition B: pH 5.9 in Table 1). Exposure periods for embryo and larva were 6 and 24 h, respectively. Asterisks show significant difference between test groups (A: Welch's *t*-test, B: Student's *t*-test, *: $p < 0.05$, ****: $p < 0.0001$).

Acknowledgements

This study was supported by the New Energy and Industrial Technology Development Organization (NEDO) and the Research Institute of Innovative Technology for the Earth (RITE).

References

Auerbach, D.I., Caulfield, J.A., Adams, E.E., Herzog, H.J., 1997. Impacts of ocean CO_2 disposal on marine life: I. A toxicological assessment integrating constant-concentration laboratory assay

data with variable-concentration field exposure. Environ. Model. Assess. 2, 333–343.

Brownell, C.L., 1980. Water quality requirements for first-feeding in marine fish larvae. II. pH, oxygen, and carbon dioxide. J. Exp. Mar. Biol. Ecol. 44, 285–298.

Handa, N., Ohsumi, T., 1995. Direct Ocean Disposal of Carbon dioxide. Terra Scientific Publishing Company, Tokyo.

Heisler, N., 1986. Acid-base Regulation in Animals. Elsevier, Amsterdam.

Max, B., 1991. This and that: the neurotoxicity of carbon dioxide. Trends Pharmacol. Sci. 12, 408–411.

Mehrbach, C., Culberson, C.H., Hawley, J.E., Pytkowicz, R.M., 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol. Oceanogr. 18, 897–907.

Morris, R., Taylor, E.W., Brown, D.J.A., Brown, J.A., 1989. Acid Toxicity and Aquatic Animals. In: Society for Experimental Biology Seminar Series 34. Cambridge University Press, Cambridge.

Ormerod, B., Angel, M., 1996. Ocean storage of carbon dioxide. Workshop 2—Environmental Impact. IEA Greenhouse and Gas R&D Programme, Cheltenham.

Roos, A., Boron, W.F., 1981. Intracellular pH. Physiol. Rev. 61, 296–434.

Vandenberg, J.I., Metcalfe, J.C., Grace, A.A., 1994. Intracellular pH recovery during respiratory acidosis in perfused hearts. Am. J. Physiol. 266, C489–C497.

Weiss, R.F., 1974. Carbon dioxide in water and seawater: the solubility of a non-ideal gas. Mar. Chem. 2, 203–215.