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Preliminary Study of the Effects of CO_2 on the Survival and Gowth of Olive Flounder (*Paralichthys olivaceus*) Juveniles

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As a result of human industrial development, carbon dioxide (CO_2) is currently accumulating in the atmosphere and dissolving into the oceans. Sequestration into the deep sea has been proposed as a possible solution to this increasing atmospheric CO_2, although the impact of such a program on marine ecosystems is unknown. We examined the effects of increased CO_2 levels on the growth of the olive flounder, *Paralichthys olivaceus*. Juvenile olive flounder 40 days post hatching were exposed to two levels of CO_2 (3.60-7.55 and 4.05-11.46 kPa) in running seawater for 26 days. During the exposure period, the pH and CO_2 levels of the water were measured, and the numbers of dead individuals were counted in each aquarium. Following the exposure period, the total lengths (mm) and body weights (mg) of the juvenile fish were measured. Both CO_2 treatments significantly increased fish mortality compared to controls (19.87±4.53% vs. 7.14% and 75.96±1.36% vs. 7.14% for high and low doses, respectively). After the high CO_2 treatment, total length (14.98±6.58 mm vs. 19.52±1.83 mm) and body weight (28.92±13.85 mg vs. 67.35±18.32 mg) of the exposed flounder were reduced compared to the control fish; however, no significant differences in these values were observed after the low CO_2 dose. These results suggested that CO_2 exposure inhibits growth in the juvenile stage and that CO_2-enriched seawater is toxic in the early life stages of olive flounder.

Key words: Carbon dioxide, Olive flounder, Growth, Mortality

Introduction

In recent years, the concentration of anthropogenic carbon dioxide (CO_2) in the atmosphere has been rising rapidly (IPCC, 2001). It is estimated that more than 50% of anthropogenic CO_2 is absorbed by the oceans (Sabine et al., 2004). Increased CO_2 levels in the oceans could decrease pH; indeed, a 0.3–0.5 unit decrease in pH is expected over the next century (Feely et al., 2004). As a result, the potential effects of ocean acidification on marine ecosystems have come increasingly into focus in recent years (Brower et al., 2004; Gazeau et al., 2007; Michaelidis et al., 2007; Iglesias-Rodriguez et al., 2008).

In addition, ocean sequestration has been proposed as a possible way to reduce increasing anthropogenic CO_2, (Cole et al., 1995; Ormerod and Angel, 1996). However, this extra CO_2 may negatively impact marine ecosystems including marine organisms (Adams et al., 1997; Auerbach et al., 1997). Most previous studies focused on the effects of CO_2 on shell-structured organisms such as phytoplankton, shellfish and crabs; however very few studies have examined the impacts of increased marine CO_2 on fish (Kikkawa et al., 2003; Lee et al., 2003; Ishimatsu et al., 2004, 2008). Fish are important and essential components of marine ecosystems, and they constitute the main protein sources for people in many countries. Successful recruitment to fishery stocks is dependent on the growth and survival of early life stages (eggs, larvae, and juveniles). Ocean acidification appears to affect the recruitment dynamics of fishes, and it is expected that the impact of CO_2 on these stages could be more crucial than that on the adult stage.

Although very few studies have examined the effects of CO_2 on fish, most studies have focused on adults or freshwater species (Fivelstad et al., 1999; Ingermann et al., 2002; Fivelstad et al., 2007; Kaufman et al., 2007). We investigated the effects of

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increased CO_2 levels on the juvenile stages of the olive flounder, a marine species.

Materials and Methods

Experimental fish and rearing conditions

To investigate the effects of CO_2 on juvenile olive flounder, we used a CO_2 exposure tank (80 L, high CO_2 group), a natural seawater tank (80 L, control group), and a mixing tank (80 L, low CO_2 group; Fig. 1). Carbon dioxide of 99.9% purity was added to the water from a pressurized gas bottle at a flow rate of 300 ml/min. The control group received only natural seawater with no CO_2 added (21 mL/min); the high CO_2 group only received carbon dioxide enriched water (21 mL/min); and the low CO_2 group received a 1:1 mixture of natural seawater and CO_2-enriched water (21 mL/min). Water flow was kept continuous using a siphon, and more than 30 L of water was exchanged per day. Juvenile olive flounder (40 days post-hatching) were reared in 30 L tanks (30 fish/tank) at 20°C under a photoperiod of 14L:10D. The fish were fed an artificial diet three times per day. All experiments were conducted in duplicate.

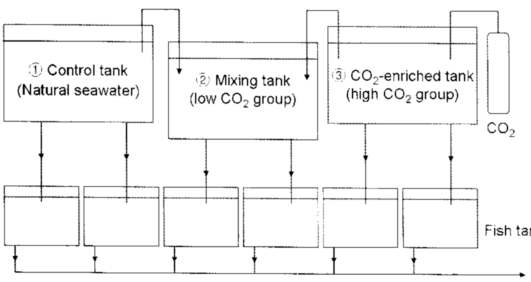

Fig. 1. Schematic diagram of CO_2-exposure experiment. Control groups were natural seawater flow (①), high CO_2 groups were CO_2-airated seawater flow (③), low CO_2 groups were mixing seawater flow with both of ① and ③ (②). Arrow heads indicate water flows.

The CO_2 concentrations were determined by the single acid addition method described by Parsons et al. (1984). Solubility values from Randall (1970) were applied to convert the concentration of carbon dioxide in mg/L to partial pressure in mmHg and then converted to kPa. pH was measured using a pH meter (Orion). pH and pCO_2 were measured every 2 days. Dead individuals were counted to evaluate the mortality over the 26 day exposure period. After 26 days, the remaining fish were measured for total length (mm) and body weight (mg).

Statistics

All mortality, length, and weight data are expressed as mean and standard error of the mean (SEM). SPSS 11.0 for Windows was used for the Kruskal-Wallis test with a Bonferroni adjustment. A value of $P<0.05$ was considered statistically significant.

Results

The pH values reflected the CO_2 levels. During the exposure period, the water pH ranged from 7.66 to 8.34 in the control group, 7.12 to 7.88 in the low CO_2 group and 6.46 to 7.23 in the high CO_2 group (Fig. 2). The ranges of water pCO_2 (kPa) remained constant (0.10-0.13) in the control group and ranged from 3.60 to 7.55 in the low CO_2 group and from 4.05 to 11.46 in the high CO_2 group.

During the 26-day exposure period, 5 and 19 individuals died from the low and high CO_2 groups, respectively, whereas only two individuals died from the control group (Fig. 3). The mortalities for both the low and high CO_2 groups were significantly higher than those for the control group (19.87±4.53% vs. 7.14% and 75.96±1.36 % vs. 7.14%, $P<0.05$).

After the 26 day exposure period, the increases in total length (14.98±6.58 mm vs. 19.52±1.83 mm) and body weight (28.92±13.85 mg vs. 67.35±18.32 mg) were significantly lower in the high CO_2 group than the control group ($P<0.05$).

Discussion

Our study demonstrated that CO_2-enriched water is toxic and that it increased the mortality of juvenile olive flounder. Recent studies with young olive flounder have reported median lethal concentrations of CO_2 of 4.96 kPa in 24 h and 4.61 kPa in 72 h for marine fish species (Grottum and Sigholt, 1996; Kikkawa et al., 2003). However, these studies examined the effects of CO_2 using constant levels or relatively low levels over short exposure periods. In the present study, we investigated the effects of high and continuous CO_2 levels on juvenile stages. Using the CO_2 sequestration model, Sato and Sato (2002) reported that when CO_2 was released into the deep sea at a depth of 2,000 m, CO_2 levels greater than 30 kPa of pCO_2 would occur. These extremely high levels of CO_2 could induce high fish mortalities. The effects of CO_2 levels (4.05-11.46 kPa in the high CO_2 group) seen in the present study provide fundamental data for further investigations using realistic natural conditions.

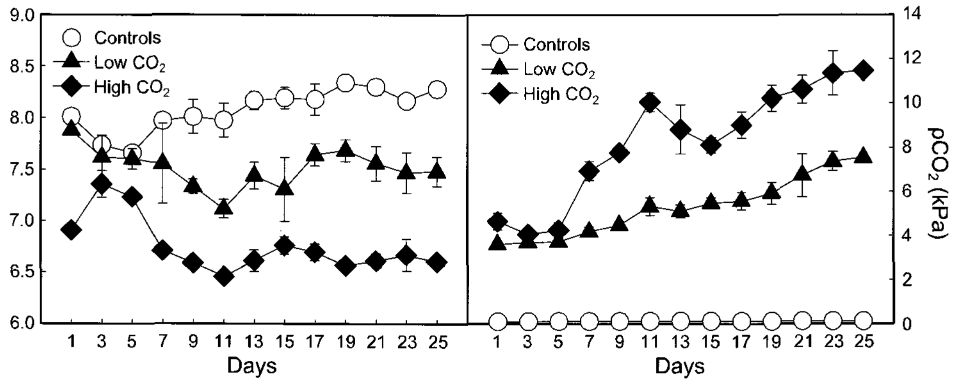

Fig. 2. Water pH (left) and CO_2 levels (right) measured during exposure period. Values are mean ± SEM of duplicate.

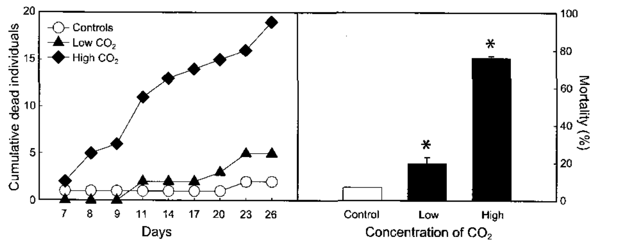

Fig. 3. Cumulative dead individuals (left) during exposure period and mortality (right). Values of mortality were mean ± SE of duplicates. Asterisks indicate significant differences from controls (P< 0.05).

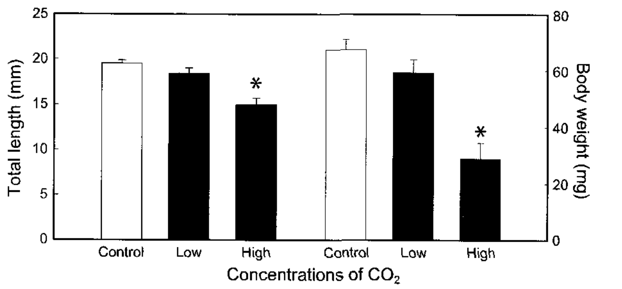

Fig. 4. Effects of CO_2 on growth of juvenile olive flounder; total length (left) and body weight (right). Values are mean ± SE of duplicate. Asterisks indicate significant differences from controls (P<0.05).

The causes of the high mortality rates induced by elevated CO_2 levels are not yet well understood. Hayashi et al. (2004) reported that decreased blood pH caused by increased CO_2 was not a direct cause of mortality in olive flounder. Recently, Lee et al. (2003) reported that depressed cardiac contractility leads to death in yellowtail, *Seriola quinqueradiata*, followed by hypercapnia. This suggests that decreesed oxygen supply is an important cause of death from CO_2 exposure.

Exposure to high CO_2 levels decreased the growth of juvenile olive flounder. Throughout the exposure period, fish in the high CO_2 group exhibited significantly reduced feeding behavior and slower swimming than did those in the control group. It is not clear whether the increased mortality was because the fish simply did not feed or whether the increased CO_2 was directly toxic. Previous studies of freshwater species showed that decreased growth caused by increased CO_2 is dependent on the fish species as well as on the size or developmental stage (Fivelstad et al., 1999, 2003; Hossfeld et al., 2008). Ishimatsu et al. (2008) reported that no published data are available on the growth of fish larvae or juveniles exposed to CO_2. Our study demonstrated that increased CO_2 levels inhibited the growth of juvenile olive flounder.

In conclusion, increased CO_2 levels are toxic and inhibit the growth of juvenile olive flounder. Our study may provide fundamental data on the effects of increased CO_2 on shallow-water fish species. Future research should be conducted examining possible CO_2 sequestration scenarios and effects on deep-sea species using realistic temperatures and CO_2 levels.

Acknowledgments

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References

Adams EE, Caulfield JA, Herzog HJ and Auerbach DI. 1997. Impacts of reduced pH from ocean CO_2 disposal: sensitivity of zooplankton mortality to model parameters. Waste Management 17, 375-380.

Auerbach DI, Caulfield JA, Adams EE and Herzog HJ. 1997. Impacts of ocean CO_2 disposal on marine life: A toxicological assessment intergrating constant-concentration laboratory assay data with variable-concentration field exposure. Environ Model Assess 2, 333-343.

Brouwer M, Larkin P, Brown-Peterson N, King C, Manning S and Denslow N. 2004. Effects of hypoxia on gene and protein expression in the blue crab, *Callinectes sapidus*. Mar Environ Res 58, 787-792.

Cole KH, Stegen GR and Spencer D. 1995. The capacity of the deep oceans to absorb carbon dioxide. In: Direct ocean disposal of carbon dioxide. Handa N and Ohsumi T, eds. Terra Sceintific Publishing Company Tokyo, Japan., 143-152.

Feely RA, Sabine CL, Lee K, Berelson W, Kleypas J, Fabry VJ and Millero FJ. 2004. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. Science 305, 362-366.

Fivelstad S, Olsen AB, Kluften H, Ski H and Stefansson S. 1999. Effects of carbon dioxide on Atlantic salmon (*Salmo salar* L.) smolts, at constant pH in bicarbonate rich freshwater. Aquaculture 178, 171-187

Fivelstad S, Olsen AB, Asgard T, Baeverfjord G, Rasmussen T, Vindheim T and Stefansson S. 2003. Long-term sublethal effects of carbon dioxide on Atlantic salmon smolts (*Salmo salar* L.): ion regulation, haematology, element composition, nephrocalcinosis and growth parameters. Aquaculture 215, 301-319.

Fivelstad S, Waagbø R, Stefansson S, Olsen AB. 2007. Impacts of elevated water carbon dioxide partial pressure at two temperatures on Atlantic salmon (*Salmo salar* L.) parr growth and haematology. Aquaculture. 269. 241-249.

Gazeau F, Quiblier C, Jansen JM, Gattuso JP, Middelburg JJ and Heip CHR. 2007. Impact of elevated CO_2 on shellfish calcification. Geophys Res Lett 34, 5.

Grottum JA and Sigholt T. 1996. Acute toxicity of carbon dioxide on European seabass (Dicentrachus labrax): Mortality and effects on plasma ions. Comp Biochem Physiol 115, 323-327.

Hayashi M, Kita J and Ishimatsu A. 2004. Acid-base responses to lethal aquatic hypercapnia in three marine fish. Mar Biol 144, 153-160.

Iglesias-Rodriguez MD, Halloran PR, Rickaby REM, Hall IR, Colmenero-Hidalgo E, Gittins JR, Green DRH, Tyrrell T, Gibbs SJ, von Dassow P, Rehm E, Virginia Armbrust E and Boessenkooland KP. 2008. Phytoplankton calcification in a high-CO_2 world. Science 320, 336-340.

IPCC. 2001. Climate change 2001: Synthesis Report. A Contribution of Working Groups I, II and III to the Third Assessment Report of the Intergovernmental Panel on Climate Change. University Press, Cambridge.

Ingermann RL, Holcomb M, Robinson ML and Cloud JG. 2002. Carbon dioxide and pH affect sperm motility of white sturgeon (*Acipenser transmontanus*). J Exp Biol 205, 2885-2890.

Ishimatsu A, Kikkawa T, Hayashi M, Lee KS and Kita J. 2004. Effects of CO_2 on marine fish: Larvae and adults. J Oceanogr 60, 731-741.

Ishimatsu A, Hayashi M and Kikkawa T. 2008. Fishes in high-CO_2, acidified oceans. Mar Ecol Prog Ser 373, 295-302.

Kikkawa T, Ishimatsu A and Kita J. 2003. Acute CO_2 tolerance during the early developmental stages of four marine teleost. Environ Toxicol 18, 375-382.

Kaufman RC, Houck AG. Cech Jr. JJ. 2007. Effects of temperature and carbon dioxide on green sturgeon blood-oxygen equilibria. Environ Biol Fish 79, 201-210.

Lee KS, Kita J and Ishimatsu A. 2003. Effects of lethal levels of environmental hypercapnia on cardiovascular and blood-gas status in yellowtail, *Seriola quinqueradiata.* Zool Sci 20, 417-422.

Michaelidis B, Spring A and Pörtner HO. 2007. Effects of long-term acclimation to environmental hypercapnia on extracellular acid-base status and metabolic capacity in Mediterranean fish *Sparus aurata*. Mar Biol 150, 1417-1429.

Ormerod B and Angel M. 1996. Ocean storage of carbon dioxide. Workshop 2-Environmental Impact. IEA Greenhouse and Gas R&D Programme, Cheltenham, UK. 131.

Parsons TR, Maita Y and Lalli CM. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon, Oxford, U.K., 141-149.

Randall DJ. 1970. Gas exchange in fish. In: Hoar WS and Randall DJ,_eds. Fish Physiology IV. Academic Press, New York, U.S.A., 253-286.

Sabine CL, Feely RA, Gruber N, Key RM, Lee K, Bullister JL, Wanninkhof R, Wong CS, Wallace DWR, Tilbrook B, Millero FJ, Peng TH, Kozyr A, Ono T and Rios AF. 2004. The oceanic sink for anthropogenic CO_2. Science 305, 367-371.

Sato T and Sato K. 2002. Numerical prediction of the dilution process and its biological impacts in CO_2 ocean sequestration. Mar Sci Technol 6, 169-180.

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