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EFFECTS OF CO_2 OCEAN SEQUESTRATION ON MARINE FISH

Atsushi Ishimatsu
Institute for East China Sea Research
Nagasaki Univ.

Masahiro Hayashi
Institute for East China Sea Research
Nagasaki Univ.

Takashi Kikkawa
Central Laboratory
Marine Ecology Research Institute

Jun Kita
Demonstration Laboratory
Marine Ecology Research Institute

ABSTRACT

Ocean sequestration of CO_2 has been proposed as a possible measure to retard the increasing rate of the atmospheric CO_2 concentration. Since some negative impacts on marine animals and ecosystems are likely to ensue, we must carefully investigate biological effects of ocean CO_2 sequestration before embarking on this mitigation practice. Considering the expected depths for CO_2 ocean sequestration (> 1,000 m), it is desirable to use deep-sea animals for the experimental assessment of CO_2 ocean sequestration. In addition, experimental protocols preferably mimic environmental conditions at the releasing site: CO_2 concentrations vary due to mixing with surrounding seawater at low temperatures (0-2 °C) and under high pressures.

This paper describes our recent experiments to elucidate the effects of high CO_2 on marine fishes. A deep-sea fish *Careproctus trachysoma* (habitat depth 400-800 m) can be captured alive and be used for in vivo CO_2 exposure experiments. 100% mortality occurred when the fish was exposed to seawater equilibrated with a gas mixture containing 3% CO_2 conditions at 2 °C within 48 h, whereas mortality was never observed when shallow-water fishes (*Mustelus manazo*, *Paralichthys olivaceus* and *Seriola quinqueradiata*) were tested under the same CO_2 conditions but at higher temperatures (17-20 °C). It is currently not clear whether this difference in mortality is due to often presumed high susceptibility of deep-sea organisms to environmental perturbations.

Subsequent experiments demonstrated that low water temperature accelerates mortality by CO_2 exposure. Thus, half lethal time decreased from 105h to only 5 h when water temperature was decreased from 26 °C to 20 °C (CO_2 8.5%, *Sillago parvisquamis*). Therefore, the high CO_2 susceptibility of *C. trachysoma* could be solely due to low water temperature.

Temporally varying CO_2 conditions resulted in markedly different mortality patterns when compare with mortality recorded under constant CO_2 conditions. Step-wise increases in ambient CO_2 resulted in much lower mortalities than under one-step increases to the same CO_2 levels. Further, a sudden drop of CO_2 from 9–10% CO_2 to air level (0.038%) killed all the surviving fish within a few minutes.

INTRODUCTION

Since oceans have an immense capacity to store CO_2 due to the vast volume and high CO_2 solubility of seawater, ocean sequestration of CO_2 in the deep sea has been proposed as a potential mitigation method against climate change, and its feasibility and potential impacts on the marine ecosystem have been discussed (Barry et al. [1]; Carman et al. [2]; Ishimatsu et al. [3]; Kita and Ohsumi [4]; Pörtner et al. [5]; Vetter and Smith [6]). Among several potential options the most practical near-term procedure for CO_2 storage in the deep sea appears to be injection at depths of 1,000 to 1,500 meters through either a pipeline from a land-based facility or a pipeline towed by a moving ship (Anderson and Newell [7]).

Whichever method may be used, environmental impacts of CO_2 ocean sequestration must be carefully investigated. In particular, deep-sea organisms are usually considered to be more susceptible to environmental perturbations than shallow-water counterparts, because deep-sea organisms have evolved in and adapted to highly stable conditions prevailing in the great depths (Seibel and Walsh [8]), although there is little experimental verification of the supposition.

Previous studies on the effect of CO_2 ocean sequestration have exclusively used shallow-water organisms (Ishimatsu et al. [3]). Hayashi et al. [9] demonstrated that mortality occurred during exposure to seawater equilibrated with a gas mixture containing 5% and 7% of CO_2 for two bony fishes and a dogfish, respectively. To our knowledge, there is no published account on experimental assessment of CO_2 mortality in deep-sea fish.

Moreover, CO_2 concentration varies with time after releasing into the deep sea in a dynamic fashion dependent on the local water mixing conditions, as demonstrated by recent modeling studies (Sato and Sato [10]; Sato [11]). However, previous biological studies only employed experimental protocols in which test animals were subjected to a one-step increase in water CO_2, which may have different impacts on animals than when they were exposed to temporally changing levels of CO_2.

The purposes of the present study were (1) to test susceptibility of deep-sea fish to elevated ambient CO_2 conditions to compare results with those of the shallow-water species, (2) to study effects of water temperature on CO_2 mortality, and (3) to compare mortality of fish exposed to temporally changing conditions of CO_2 with that obtained under constant high CO_2 conditions.

Fig. 1 *Careproctus trachysoma*. The fish are caught alive from depths of 400 ± 20 m in the Japan sea where water temperature is stable at 2 °C below 200 m throughout a year. The fish can be kept in healthy conditions in captivity for over a year at atmospheric pressure as long as water temperature is kept below 0-2 °C.

MATERIALS AND METHODS

1. CO_2 MORTALITY OF A DEEP-SEA FISH

A deep-sea fish, *Careproctus trachysoma* (Fig. 1), which inhabits depths of 400-800 m, were captured live from the depths of 400 ± 20 m off the coast of Toyama Prefecture, Japan with deep-sea trawling. It takes 40-50 min to raise the net from the fishing depth to the deck. The fish were kept in a rearing tank at 2 ° C for 6-21 days before use without feeding. After acclimation to an experimental chamber, the fish were subjected to sea water equilibrated with gas mixtures containing 1, 2, 3 and 5% CO_2 in air for 72 h. The gas mixture was prepared with a gas mixing apparatus (EYELA, GMU-1, Tokyo). Average body weight (± SD) of the fish was 217 ± 40 g (N = 14). During 2% exposure, fish behavior and respiratory frequency were recorded by direct observation and video recording.

2. EFFECTS OF WATER TEMPERATURE ON FISH MORTALITY BY CO_2

A eurythermal fish, *Sillago parvisquamis* (preferred temperature range: 14.7 ～ 36.7 ℃ , final temperature preferendum: 28.5 °C, Tsuchida [12]), was used in this experiment. The fish were acclimated for ca. 1 month at 20, 22, 24 and 26 °C before use, and exposed to sea water equilibrated with a gas mixture containing 8.5% CO_2. The pH of the test water was 6.0. A total of 40 individuals were used. Average body weight and body length were 0.18 ± 0.05g and 32.6 ± 0.4mm, respectively. Fish were starved for 24 h before use.

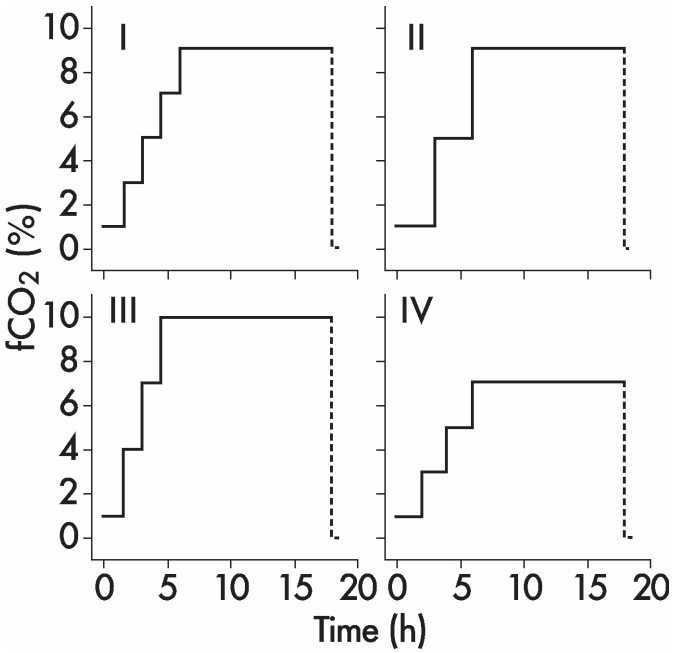

Fig. 2 Time course changes in fractional CO_2 concentration (fCO_2) of the gas used for equilibrating experimental seawater in step-wise CO_2 exposure test. Dashed line shows the final transfer of the test fish to normocapnia and subsequent 15 min exposure to normocapnic seawater.

3. EFFECT OF TEMPORALLY CHANGING CO_2 CONDITIONS ON FISH MORTALITY

Parental fish of *Sillago japonica* were reared at 25°C under 15L:9D light conditions. We used three clutches obtained from these fish by natural spawning. Test fishes were 46–70 days old, 34.0 ± 0.4 mm in total length and 0.29 ± 0.01 g in wet body weight (mean ± SE). Fish were starved for 5 h before use.

One-step exposure

The exposure system was the same as that reported by Kikkawa et al. [13]. Twenty vessels, each containing one fish, were simultaneously immersed in hypercapnic seawater in an experimental tank. Exposure duration was 18 h. Vessels containing a dead individual were immediately removed from the tank. The same treatment was adapted to the control group.

Step-wise exposure

We used four different patterns of step-wise increases in seawater CO_2 levels to study their impacts on fish survival (Fig. 2). Two tanks were used for exposure of test fish to step-wise increases in CO_2 level. Prior to an experiment, seawater in one tank was equilibrated with a gas mixture of 1% CO_2, and seawater in the other tank was bubbled with a gas mixture of a higher CO_2. Twenty fish in containers were submerged in the seawater in the 1% CO_2 tank for a pre-scheduled period, and then transferred to the second tank of a higher CO_2. During the second exposure, CO_2 of the gas mixture for the first tank was increased to the third level of CO_2. This procedure was repeated to produce step-wise increasing patterns of CO_2 given in Fig. 2.

RESULTS AND DISCUSSION

1. CO_2 MORTALITY OF A DEEP-SEA FISH

Under captivity at 2 °C, *C. trachysoma* did not accept food. Undigested food items were found in the alimentary canal of the fish by post mortem dissection after CO_2 exposure experiment, suggesting slow metabolism at this low temperature. On the basis of these observations, we presumed that different lengths of starvation did not affect physiological responses to high CO_2 conditions of the fish.

Table 1 shows cumulative mortality of *C. trachysoma* during exposure to seawater equilibrated with gas mixtures containing 1-5% CO_2 in air. At 3% CO_2 conditions, all fish died within 48 h. This is in sharp contrast to our previous results on shallow-water species (*Mustelus manazo*, *Paralichthys olivaceus* and *Seriola quinqueradiata*) in that no mortality occurred by the end of 72 h exposure. In these fishes, mortality occurred only at 5 and 7% for the two bony fish (*P. olivaceus* and *S. quinqueradiata*) and the dogfish (*M. manazo*), respectively (Hayashi et al. [9]). Even at 2% CO_2 conditions, one fish died within 72 h, and the surviving individuals lost equilibrium and rested on their side on the bottom of experimental chambers. Therefore, 2% CO_2 conditions could also have fatal effects if exposure prolonged.

Respiratory frequency decreased from 31.6 ± 5.2 breaths min^{-1} during control conditions to 18.7 ± 5.0 at 3 h and then gradually increased to 27.0 (N = 2) at 72 h.

Shallow-water fish increase ventilatory volume mainly through increases in respiratory amplitude with moderate increases in frequency (Ishimatsu and Kita [14]). The observed transient decrease in respiratory frequency in *C. trachysoma* is in contrast to these earlier observations.

Table 1 Cumulative mortality of *Careproctus trachysoma* during exposure to high CO_2 conditions

		TIME (HOURS)						
%CO_2	*N*	0	1	3	8	24	48	72
1	4	0	0	0	0	0	0	0
2	6	0	0	0	0	0	0	17
3	4	0	0	0	0	25	100	
5	4	0	0	0	25	100		

Experimental temperature 2°C.

The deep-sea environment is characterized by low temperature and high pressure. Our results on *C. trachysoma* were obtained at atmospheric pressure and might not therefore accurately reflect *in situ* CO_2 susceptibility of the species. We have recently conducted a preliminary CO_2 exposure experiment on *C. tracysoma* using a high pressure chamber (max. pressure 50 MPa) at 5 °C, and successfully recorded electrocardiograms from fish exposed to seawater equilibrated with 1% CO2 at a hydrostatic pressure of 10 MPa for 3 h. Fish survived the exposure and showed a gradual decrease in heart rate from 25 beats min^{-1} (normocapnia at 10 MPa) to 20 after 3 h at 1% CO_2. Difficulty in obtaining sufficient number of *C. trachysoma* has precluded further experimentation.

2. EFFECTS OF WATER TEMPERATURE ON FISH MORTALITY BY CO_2

Figure 3 demonstrates the effect of water temperature on CO_2 mortality in a bony fish (*Sillago parvisquamis*). Lowering water temperature shortened survival. Thus, at 26 °C half lethal time (time at which 50% mortality occurs) was calculated to be 105 h, as compared with only 5.0 h at 20 °C. It is unclear why the fish died earlier at lower temperatures. The observed higher CO_2 susceptibility of *C. trachysoma* might be due to the lower water temperature than those used for shallow-water species.

3. EFFECT OF TEMPORALLY CHANGING CO_2 CONDITIONS ON FISH MORTALITY

Mortalities obtained by one-step (Fig. 4) and step-wise CO_2 exposures (Fig. 5) were markedly different. Among one-step exposures, no mortality occurred in 1% CO_2 exposure until 18 h. Mortality plateaued around 0.2 in 3% CO_2. Far higher mortalities of 0.8 resulted from 5% CO_2 exposures within 1.5 h, and nearly all fish died within 15 min by both 7 and 9% CO_2 exposures. In contrast, step-wise elevation of CO_2 resulted in far lower mortalities in comparable CO_2 conditions.

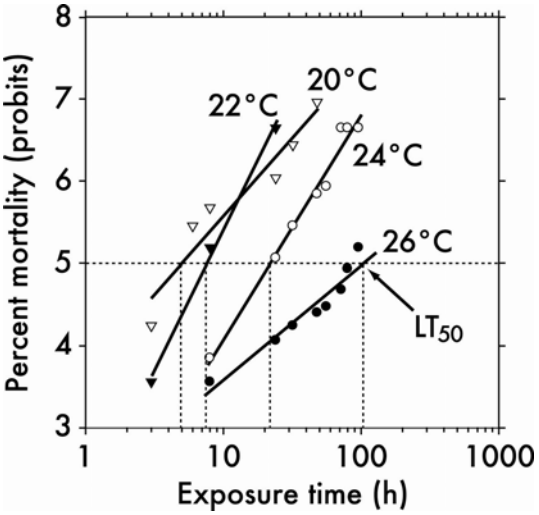

Fig. 3 Effect of water temperature on mortality of *S. parvisquamis* exposed to high CO_2 conditions (8.5% CO_2 in air).

It is currently unclear why mortality differed widely between the two exposure protocols. Environmental hypercapnia is immediately transmitted to the arterial blood resulting in the rise of plasma partial pressure of CO_2 and a correlated decrease in plasma and intracellular pH. However, the pH soon recovers after initiation of hypercapnia by elevation of the bicarbonate concentration (Heisler [15]). Hayashi et al. [9] doubted that the drop of blood pH was the direct cause of death in *P. olivaceus* exposed to environmental hypercapnia (5% CO_2) because the blood pH had recovered to pre-exposure levels before the fish died. Lee et al. [16] tentatively concluded that cardiac failure is the major physiological disorder leading to death of fish subjected to hypercapnic conditions. From the results of this experiment, we suggest that the low-level CO_2 exposures may have activated physiological buffering mechanisms of the hypercapnic fish, which might have contributed to better fish survival at the higher CO_2 conditions. Interestingly, the sudden drop of water CO_2 level rapidly killed the fish. The sudden drop of CO_2 level to normocapnia would result in respiratory alkalosis (higher than normal pH due to the slower rate of return to equilibrium conditions of body fluid HCO_3^- concentrations that had been

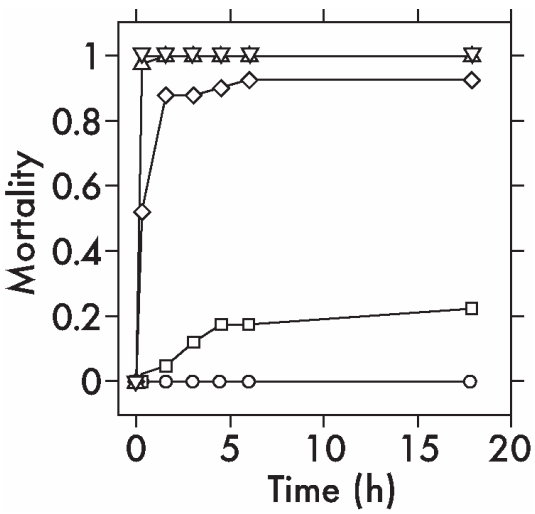

Fig. 4 Time courses of fish mortality under one-step CO_2 exposures to five CO_2 levels. Circles: 1% CO_2, squares: 3% CO_2, diamonds: 5% CO_2, triangles: 7% CO_2 and inverted triangles: 9% CO_2. Results based on two clutches.

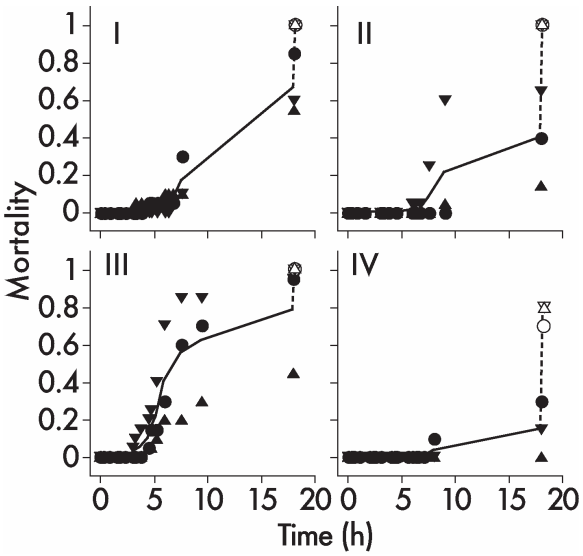

Fig. 5 Time courses of fish mortality in the four cases (Case I to IV; see Fig. 2) of step-wise CO_2 exposures. Different symbols show results from different clutches. Lines represent average. Open symbols show the mortalities at 15 min of normocapnic exposure following hypercapnia and dashed lines show the change of the average mortality during the last 15 min of the normocapnic exposure.

accumulated for pH compensation during hypercapnia (Cameron [17]), which might act as the trigger of the death on sudden exposure to normocapnia.

The present results cast some doubt about the applicability of the concept for modeling the lethal effects of CO_2 under varying conditions by integrating data obtained under constant-state conditions, at least for fish (Auerbach et al. [18]). The method apparently is unable to predict the delay of incipient death by step-wise increase of water CO_2 level and the death by sudden drop of water CO_2 level, both of which are shown in the present study on fish. However, a recent study (Sato et al. [19]) demonstrated that mortality of the copepod species (*Metamphiascopsis hirsutus*) under step-wise CO_2 would be predictable from the results of a one-step CO_2 exposure experiment. Therefore, the prediction model of Auerbach et al. [18] would be applicable for predicting the mortality for copepods subjected to changing CO_2 levels, but not for fish. No data are available on the responses of other marine animals to changing CO_2 levels in water under laboratory conditions. In the field, Vetter and Smith [6] observed effect of 60 min exposure to CO_2-rich plume (plume pH ca. 6.0) on the deep-sea amphipod *Eurythenes* of. *obesus* at a hydrothermal vent off Hawaii. The exposure resulted in quick immobilization of the animals, but they subsequently resumed active swimming activity when removed from the plume.

CONCLUSIONS

Aquatic animals are in general more susceptible to increases in ambient CO_2 levels than terrestrial animals, because of their lower body fluid PCO_2 (Ishimatsu et al. [20]). Thus, even small elevations in water CO_2 may adversely affect physiological functions of these animals. Even though effects of CO_2 may largely be attributable to disturbance of body fluid pH of exposed animals, high CO_2 have stronger impacts on them than seawater acidification due to a higher permeability of CO_2 than H^+ added to water (Kikkawa et al. [21]). Therefore, investigations assessing CO_2 sequestration must use CO_2-enriched sea water rather than adding nonvolatile acids to the medium.

At the low temperatures prevailing at the great depths where CO_2 ocean sequestration is considered, CO_2 would have severe effects on marine animals as suggested above. Additionally, most deep-sea fish are taxonomically distinct from shallow-water species, and they are supposedly less tolerant to environmental perturbations. The present data on *C. trachysoma*, support this contention: fish died at a lower CO_2 level than found for shallow-water species, although this needs to be verified using more species. In particular, we need to gain knowledge about CO_2 susceptibility of pelagic deep-sea fish, since the ocean CO_2 sequestration by a moving ship method would release CO_2 into mid-layers of the ocean and thereby would affect pelagic organisms. *C. trachysoma* is a demersal fish, living near or at the sea floor. However, we have so far succeeded in obtaining only demersal species in live conditions from the depths, and pelagic species have been only obtained moribund or dead.

In situ deep-sea CO_2 exposure experiments such as one conducted by Barry et al. [1] and Vetter and Smith [6] are certainly another highly valuable and informative approach to the issue. With this approach, deep-sea fish and other fauna can be exposed to hypercapnia under *in situ* conditions of low temperature and high pressure, even though it requires specialized equipments and a submersible.

We must certainly understand not only acute effects of CO_2 on deep-sea fish but also long-term impacts on them to evaluate environmental consequence of CO_2 ocean sequestration, although this is a more difficult task to fulfill. Sébert et al. [22] have developed an experimental system more than 10 years ago that allowed long-term experiments under high pressures for aquatic animals, and reported the oxygen consumption of *Anguilla anguilla* over a period of 31 days. Using such systems for deep-sea fish experiments is certainly a possibility for assessing the effects of chronic CO_2 under high pressures, although measurable physiological parameters in these systems are limited. Again, *in situ* deep-sea CO_2 exposure (Barry et al. [1]) may be a useful method for evaluating long-term CO_2 effects.

Finally, fish is only one constituent of marine ecosystems, which are composed of myriads of organisms, mutually dependent and influenced in complex manners. Organisms belonging to different phyla may well have different degrees of tolerance to CO_2, and highly developed animals, such as fish, generally show higher tolerance to environmental perturbations than less developed organisms, although the latter play vital roles in marine ecosystems, and are essential to fish as food organisms. Thus, even if fish tolerate elevated ambient CO_2 caused by CO_2 ocean sequestration, they may eventually be affected through reductions of feed population. Moreover, organisms generally show lower tolerance to environmental perturbations at early developmental stages such that eggs, juveniles and larvae than adults of the same species (Kikkawa et al. [13]). These, and many other aspects, must be thoroughly investigated to evaluate environmental impacts of CO_2 ocean sequestration.

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