Effects of CO₂ Ocean Sequestration on Deep-Sea Animals

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I. INTRODUCTION

Emissions of anthropogenic greenhouse gases and aerosols continue to alter the atmosphere in ways that have been unequivocally warming the climate system [1]. As potential mitigation methods, carbon capture and storage (CCS) technologies are now being actively investigated, although there are significant environmental, technical, and political uncertainties relating to these technologies [2]. The oceans have an immense capacity to store CO₂ due to their vast volume and the high solubility of CO₂ in seawater. Hence, CO₂ storage in the deep sea has been proposed as one of the CCS technologies, and its feasibility and potential impacts on the marine ecosystem have been discussed. CO₂ storage in the deep sea would create CO₂-rich plumes around an injection point of liquefied CO₂; near-field pH depression could be as high as 3.0 pH unit if liquid CO₂ is injected from a fixed pipe. Environmental impacts of such high-CO₂/low pH plumes are scarcely known. As a most fundamental parameter to assess the acute impacts of this technology, data must be urgently accumulated on the acute lethality of elevated CO₂ concentration for various biota.

Considering the expected depths for ocean CO₂ sequestration (> 1,500 m), experimental assessment of ocean CO₂ sequestration must use deep-sea animals to evaluate its biological impacts. However, this is obviously a difficult task due to practical problems of obtaining, handling, and maintaining these organisms in healthy conditions for experimental use. In addition, CO₂ conditions around releasing sites are expected to vary temporally, which should be taken into account when designing experimental protocols [3].

This paper describes our recent experimental results to elucidate the effects of high CO₂ on a deep-sea fish. A few species can be captured alive from depths of ca. 400 meters and be used for in vivo CO₂ exposure experiments. We have developed an experimental setup that allows us to expose deep-sea organisms to high pressures (up to 20 kPa) at low temperatures (1-2°C) for extended periods. This setup also allows us to monitor physiological state of exposed animals through electrocardiogram for cardiac activities and electromyogram for respiratory movements. We will discuss the latest results on the effects of CO₂ on a deep-sea fish *Careproctus trachysoma* (Liparididae) under pressures of 6-10 kPa at 2°C. Preliminary results indicate that the fish would die earlier under high pressures than when they are exposed to the same CO₂ conditions (20,000 µatm) at atmospheric pressure. Electrocardiogram has been successfully recorded from the fish, demonstrating that heart rate rapidly declined when they were exposed to 20,000 µatm CO₂ under high pressure.

The fish that has been obtained live for CO₂ experiment are all demersal species, which may have different CO₂ sensitivities from pelagic organisms. To establish a method to estimate CO₂ sensitivities of pelagic species, we are examining effects of high CO₂ exposure on some morphological (the branchial chloride cells) and biochemical (Na⁺/K⁺ ATPase activity of the gills) parameters that can be obtained from dead animals, as possible indirect indices for CO₂ tolerance of the animals.

II. MATERIALS AND METHODS

CO₂ mortality of deep-sea fish under the atmospheric pressure

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 of 10,000 20,000, 30,000 and 50,000 µatm PCO₂ 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). Oxygen consumption was determined for *C. trachysoma*, *Bothrocara hollandi*, and *Petroschmidtia toyamensis* to examine possible relationship between fish activity and CO₂ tolerance. Oxygen consumption was measured by closed respirometry.

CO₂ mortality of deep-sea fish under high pressure

Individuals of *C. trachysoma* were collected from a deep-seawater intake facility in Toyama Prefecture, Japan from the depth of 384 m. The fish were kept for several days in a concrete tank by the facility, before they were transported to Research Institute of Innovative Technology for the Earth (RITE) in Kyoto, Japan.

The high pressure experimental setup consists of a high-pressure chamber (max. tolerable pressure 50 MPa, stainless steel, 105 cm long, outer diameter 32 cm, capacity 14 L), a high-pressure pump (max. output pressure 20 MPa), a CO₂ equilibration column, a gas mixing device, and a water cooler (**Fig. 2**). Seawater was equilibrated with CO₂ gas mixtures (PCO₂ 20,000 μ atm) under the atmospheric pressure and pressurized before fed into the high-pressure chamber. The high-pressure chamber, the equilibration column were placed in a cold room at 0 °C.

Fish were anesthetized in 2-phenoxyethanol solution (0.5 mL L⁻¹) for 5-10 min before surgery. The fish were then equipped with stainless steel electrode to record electrocardiogram (ECG) and respiratory movements from electromyogram (EMG) of the gill muscle. Before placing the fish in the chamber, the fish were housed in a mesh chamber to restrict free swimming.

After acclimatizing the fish in the chamber under the atmospheric pressure for 24 h, the pressure of the chamber was raised to 10 MPa at a rate of 1 MPa h⁻¹, and left for another 24 h before hypercapnic exposure. Then, the equilibration gas was switched from air (CO₂ 380 μ atm) to CO₂-enriched air (20,000 μ atm). During pressurization and the CO₂ exposure period of up to 72 h, seawater temperature, salinity and pH was monitored. Both ECG and EMG signals were recorded continuously using a data-acquisition system (Keyence) at a sampling rate of 1000 Hz. The respiratory movement was also confirmed by visual inspection.

Fig. 1 *Careproctus trachysoma*. The fish are caught alive from depths of ca. 400 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.

III. RESULTS

CO₂ mortality of deep-sea fish under the atmospheric pressure

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 CO₂ 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 CO₂ conditions of the fish.

Table 1 shows cumulative mortality of *C. trachysoma* during exposure to seawater equilibrated with gas mixtures containing 10,000-50,000 μ atm PCO₂ in air. At 30,000 μ atm PCO₂ 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 shallow-water fishes, mortality occurred only at 50,000 and 70,000 μ atm for the two bony fish (*P. olivaceus* and *S. quinqueradiata*) and the dogfish (*M. manazo*), respectively [4]. Even at 20,000 μ atm CO₂ conditions, one *C. trachysoma* died within 72 h, and the surviving individuals lost equilibrium and rested on their side on the bottom of experimental chambers. Therefore, 20,000 μ atm CO₂ conditions could also have fatal effects on *C. trachysoma* 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

Fig. 2 Left: The high pressure chamber used for CO₂-exposure experiment of the deep-sea fish under pressure. **Right:** The chamber was placed in a cold room and 2 °C seawater was recirculated through the chamber using a high pressure pump (not shown). The seawater was equilibrated with CO₂ gas mixtures under the atmospheric pressure.

increases in respiratory amplitude with moderate increases in frequency [5]. The observed transient decrease in respiratory frequency in *C. trachysoma* is in contrast to these earlier observations.

Oxygen consumption of *C. trachysoma*, *Bothrocara hollandi*, and *Petroschmidtia toyamensis* were 0.471, 0.470, and 0.449 mmol h⁻¹ g⁻¹, respectively. Of the three species, *C. trachysoma* was the most susceptible to high CO₂ than the other two species, dismissing our hypothesis of the relationship between oxygen consumption and CO₂ susceptibility.

Table 1 Cumulative mortality of *Careproctus trachysoma* during exposure to high CO₂ conditions under the atmospheric pressure

PCO ₂	N	Time (hours)						
		0	1	3	8	24	48	72
10,000	4	0	0	0	0	0	0	0
20,000	6	0	0	0	0	0	0	17
30,000	4	0	0	0	0	25	100	
50,000	4	0	0	0	25	100		

CO₂ mortality of deep-sea fish under high-pressure

In the year 2006, CO₂ exposure was conducted under 10 MPa conditions. Control fish survived for 72 h under 10 MPa, normocapnic conditions (PCO₂ 380 µatm, **Fig. 3**). The heart rate and respiratory frequency were 12.2 and 14.0 beats min⁻¹, respectively at 0 h and remained stable throughout the exposure period. In sharp contrast, the fish exposed to 20,000 µatm PCO₂ under 10 MPa was confirmed to be dead at 13 h. The heart rate decreased from 13.9 beats min⁻¹ at 0 h to 9.4 beats min⁻¹ at 3 h, 5.8 beats min⁻¹ at 8 h and was not detected at 13 h. Respiratory frequency also decreased from 15.1 min⁻¹ at 0 h, 12.8 min⁻¹ at 3h, 8.7 min⁻¹ at 8 h and was undetectable at 13 h.

In the year 2007, CO₂ exposure was conducted under 6 MPa conditions, since control fish did not survive 10 MPa exposure. The reason was not clear but possibly due to seasonal changes in the physiological state of the experimental fish. When two fish were exposed to 20,000 µatm PCO₂, both died within 48 h. Similar but less pronounced decline of respiratory frequency was visually observed as compared with the observation in 2006. Other two fish were exposed to 10,000 µatm PCO₂ under 10 MPa. The fish survived 72 h exposure, but were judged to be stressed, showing occasional abrupt swimming behavior and loss of posture. Respiratory frequency decreased from 22 min⁻¹ to 14-16 min⁻¹ in one fish (no data obtained from the other). Because we used intact fish in 2007, no heart rate data could be obtained. No mortality was recorded in control fish (N = 3) under normocapnic, 6 MPa conditions. Seawater oxygen saturation during CO₂ exposure remained above 80% throughout.

IV. DISCUSSION

Aquatic animals are in general more susceptible to increases in ambient CO₂ levels than terrestrial animals, because of their lower body fluid PCO₂ [6]. Thus, even small elevations in water CO₂ may adversely affect physiological functions of these animals. Even though effects of CO₂ may largely be attributable to disturbance of body fluid pH of exposed animals, high CO₂ have stronger impacts on them than seawater acidification due to a higher permeability of CO₂ than H⁺ added to water [7]. Therefore, investigations assessing CO₂ sequestration must use CO₂-enriched sea water rather than adding nonvolatile acids to the medium.

At the low temperatures prevailing at the great depths where CO₂ ocean sequestration is considered, CO₂ would have severe effects on marine animals. We exposed a eurythermal fish *Paralichthys olivaceus* to 30,000 µatm PCO₂ at 5 and 20 °C, and found higher mortality at the lower temperature. Thus low temperature itself could have negative effects on CO₂ tolerance of fish. 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 CO₂ level than found for shallow-water species, although this needs to be verified using more species. In particular, we need to gain knowledge about CO₂ susceptibility of pelagic deep-sea fish, since the ocean CO₂ sequestration by a moving ship method

Fig. 3 Changes in heart rate and respiratory frequency of control (open circle) and experimental fish (solid circle) recorded under 10 MPa conditions.

would release CO₂ 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.

The present results suggested negative impact of high pressure on CO₂ tolerance of a deep-sea fish. Under the atmospheric conditions, only 1 out of 6 fish died in 72 h at 20,000 μ atm conditions, whereas the fish exposed to the same PCO₂ under 10 MPa died in only 13 h in 2006, and within 48 h in 2007. However, the results must be viewed with caution because of the following points. First, the number of fish used was low as stated above. Second, the fish were collected from the depth of ca. 400 m, reared under the atmospheric pressure for the total of a week or longer, pressurized in 12 h to 10 MPa before exposed to high CO₂. Although all control fish survived, the fish still might have been under some stress before being exposed to high CO₂.

In situ deep-sea CO₂ exposure experiments [8,9] 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.

The CO₂ susceptibility of deep-sea fishes may be estimated indirectly by using some morphological characteristics that correlate with CO₂ susceptibility. Our recent results suggest that chloride cell activity is stimulated by hypercapnia (unpublished [10]). Further, exceptionally CO₂-tolerant larval fish had a high density of chloride cells (unpublished [11]). Thus, if interspecific correlation between chloride cell morphometry and CO₂-susceptibility is established, then it may be possible to estimate the CO₂ susceptibility of deep-sea fishes without having live materials for experimental evaluation.

Finally, it should be noted that animals would be subjected to fluctuating PCO₂ when CO₂ droplets are released from the lower end of a pipe used for CO₂ ocean sequestration [3]. Our recent study indicated that mortality differed considerably when fish is exposed to unsteady levels of environmental CO₂ conditions, as compared with data obtained using steady CO₂ protocols [12]. This should also be included in experimental protocols to assess the effects of CO₂ under realistic conditions of CO₂ ocean sequestration.

We must certainly understand not only acute effects of CO₂ on deep-sea fish but also long-term impacts on them to evaluate environmental consequence of CO₂ ocean sequestration, although this is a more difficult task to fulfill. Reference [13] 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 CO₂ under high pressures, although measurable physiological parameters in these systems are limited. Again, *in situ* deep-sea CO₂ exposure [8,9] may be a useful method for evaluating long-term CO₂ effects.

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