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Note

Swimming inhibition by elevated pCO_2 in ephyrae of the scyphozoan jellyfish, *Aurelia*

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Abstract: Ephyrae of the scyphozoan jellyfish, *Aurelia*, were exposed to hypercapnic seawater (pCO_2 5,000 to 50,000 μatm) for 96 h, to study the impacts of potential CO_2 seepage from a geological storage site beneath the ocean floor. Geological CO_2 storage has been proposed as a mitigation measure against global warming but ecological consequences in the case of seepage are largely unknown. No mortality occurred within the pCO_2 range used in the present study. Swimming arm pulsation was significantly depressed in animals exposed to 5,000 μatm pCO_2 compared to control animals, and immediately ceased in animals exposed to $\geq$30,000 μatm. When returned to normocapnic seawater (pCO_2 380 μatm) after 96 h exposure to 50,000 μatm pCO_2, some ephyrae showed strong arm inversion. These results indicate that even though *Aurelia* is able to survive short-term exposure to pCO_2 of up to 50,000 μatm, the strong inhibition of swimming activities under these conditions would reduce the environmental fitness of affected animals.

Key words: *Aurelia*, bioassay, carbon dioxide, CO_2 geological storage, ephyra, swimming pulsation

As a mitigation measure against global warming, geological storage aims to inject CO_2 into underground formations with an overlying caprock layer of low CO_2 permeability, and thereby separate the injected CO_2 from the atmosphere (IPCC 2005). Some countries including Japan are investigating the possibility of injecting CO_2 into geological formations beneath the ocean floor, but this requires careful evaluation of the ecological consequences in the case of potential CO_2 seepage into the marine environment and ecosystems before committing to its implementation (Anderson & Newell 2004).

A few studies have reported acute lethal CO_2 concentrations for fish, crustaceans and cephalopods to contribute to risk assessment of CO_2 ocean storage, which aims to directly inject CO_2 into the ocean depths (Kita & Ohsumi 2004, Kikkawa et al. 2008). To our knowledge, however, nothing is known about the impacts of high CO_2 conditions on gelatinous zooplankton, despite the fact that gelatinous zooplankton including cnidarians, ctenophores and chaetognaths comprise a predominant group in the marine fauna (Boero et al. 2008). Thus, we investigated acute CO_2 impacts on the mortality and swimming activity of ephyrae of the cnidarian scyphozoan, *Aurelia* sp. as a first step to evaluate acute CO_2 toxic effects on gelatinous zooplankton. The selection of the *Aurelia* ephyrae was based on the following three reasons. First, this genus is widely distributed in the coastal waters of the world (Dawson & Martin 2001, Dawson 2003, Palomares & Pauly 2009). Second, ephyrae of a uniform size can be easily obtained in the laboratory, which is a great advantage to conducting toxicity tests. Third, organisms in early developmental stages are in general more susceptible to environmental perturbations than adults (McKim 1995). The polyps of *Aurelia* have been confirmed to occur to a depth of at least 100 m (Miyake et al. 2004) so that *Aurelia* ephyrae are likely to be distributed down to the same depth. We focused on the effect of CO_2 on swimming activity, because the effects on behavior of high CO_2 concentrations are largely unknown for marine animals. Such data should be of essential importance in predicting the ecological consequences of CO_2 storage.

Ephyrae used in the present experiments were detached from polyps growing on the walls of a glass aquarium [120×45×50 (height) cm] with running seawater supply at the Central Laboratory of the Marine Ecology Research Institute. These polyps were derived from several adults collected on the Kamogawa coast, Chiba, Japan, probably signifying that they belong to *Aurelia* sp. 1 (sensu Dawson and Jacobs 2001). The polyps were fed *Artemia* nauplii and fish eggs under natural water temperature and light conditions. The ephyrae were sampled with a pipette on April 19, 2005 (Trial A, exposure trials to CO_2 partial pressures (pCO_2) of 10,000 and 30,000 μatm, see below) and April 25 (Trial B, 5,000 and 50,000 μatm trials). Water temperature was 15.8°C and 16.4°C on April 19

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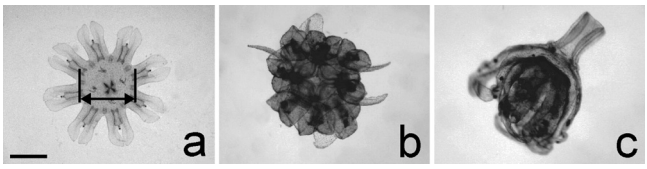

Fig. 1. Photographs of *Aurelia* ephyrae. **a**: Frontal view of a normal individual (normocapnia). The double-headed arrow indicates the bell diameter that was determined prior to the experiment. Scale: 1 mm. **b**: Frontal view of an ephyra that shows typical convolution of the ephyral arms. Photos taken at 96 h of exposure to 30,000 or 50,000 μatm pCO_2. **c**: Lateral view of an ephyra showing inversion and convolution of the ephyral arms, which occurred 24 h after returning to normocapnic seawater following 96-h exposure to 50,000 μatm pCO_2.

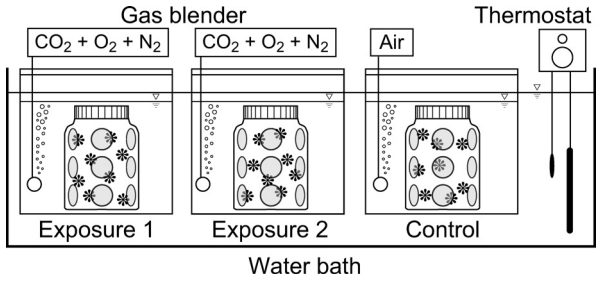

Fig. 2. Schematic of the apparatus used for the CO_2 exposure for jellyfish ephyrae. Animal containers have mesh windows on the wall.

and 25, respectively, when the sampling was made. Mean bell diameter of 10 individuals was 1.5±0.1 (SD) mm (see Fig. 1a). The CO_2 exposure trials were started in the afternoon of the respective day of sampling, and lasted for 96 h. The experimental setup was identical with the one previously reported for fish (Kikkawa et al. 2003) (Fig. 2). Briefly, three polyvinyl chloride (PVC) exposure tanks (capacity 14 L, one control and two CO_2 exposure tanks) were placed in a temperature-controlled water bath (100 L). The PVC tanks for the CO_2 group were filled with 11 L of seawater equilibrated with gas mixtures of CO_2 (0.5 to 5%), and O_2 (20.95%) balanced with N_2, which gave pCO_2 of 5,000, 10,000, 30,000 and 50,000 μatm. The gas mixtures were supplied using a gas blender (GB-3CS, Kojima Instruments Inc., Japan). The tank water was bubbled throughout the trials with either the gas mixtures or ambient air (pCO_2 380 μatm) at a flow rate of 400 mL min^{-1}. A polycarbonate bottle (1 L) with round net-covered windows cut in the sidewall was submerged in each tank, and used as a container for the ephyrae. Water temperature was maintained at 15°C. Water pH was measured immediately before the trials and subsequently every 24 h with a pH electrode (GST-5721C, DKK-TOA Corporation, Japan) and a pH meter (HM-60G, DKK-TOA). Seawater salinity was determined with a salinometer (601MK III, Yeo-Kal Environmental Electronics, Australia) before each trial. Nine or ten ephyrae were transferred into each bottle placed in the tank with minimum volume of seawater at the start of each experiment. To count pulsation of the ephyral arms, ephyrae were individually transferred to a 100 mL beaker filled with water having the same pCO_2 as in the exposure tanks, and observed for one minute under a stereomicroscope. Only movements in which all 8 arms contracted simultaneously were counted irrespective of the position of the ephyra in the beaker (in the water column or on the bottom). Erratic movements, such as contraction of only a few arms or irregular arm movements, were not included in the counts. After counting, the ephyrae were returned to the bottles. Pre-exposure pulsation rates were determined in the same way using 10 ephyrae randomly sampled from the stock aquarium. Counting was done every 24 h for each 96 h trial. Pulsation rates at each observation time were compared between groups (one control and two CO_2 groups) using the one-tailed Shirley-Williams multiple comparison test (Shirley 1977). The water pH, temperature and salinity during the trials are shown in Table 1.

Table 1. Sea water pCO_2, pH, temperature and salinity adopted in the present study

Trial	pCO_2 (μatm)	pH*1	Temperature (°C)*2	Salinity*3
A	380 (control)	8.098±0.012	15.0±0.2	34.9
	10,000	6.869±0.028	14.9±0.2	34.9
	30,000	6.366±0.021	14.9±0.2	34.9
B	380 (control)	8.124±0.024	15.0±0.2	35.2
	5,000	7.113±0.018	14.9±0.2	35.2
	50,000	6.152±0.010	15.0±0.2	35.2

*1: mean±SD of repeated measurements with a 24-h interval under each set of conditions.
*2: mean±SD, recording interval: 1 min.
*3: measured at the beginning of each exposure.

No mortality occurred in any of the trials. This judgment was based on the fact that there was no single ephyra that completely stopped arm pulsation. The pulsation rates were significantly lower than the corresponding control values at 24 h (1.4 Hz) and 96 h (1.2 Hz) in the 5,000 μatm condition, while the rates nearly halved after 48 h (0.59 to 0.86 Hz) in the 10,000 μatm condition ($P<0.025$, Fig. 3). Pulsation almost instantly stopped upon exposure to 30,000 and 50,000 μatm, and remained at 0.11 to 0.22 Hz (30,000 μatm) and 0.0033 to 0.043 Hz (50,000 μatm). The arms were strongly convoluted in the 30,000 and 50,000 μatm conditions, and became increasingly more convolute with time (Fig. 1b). These ephyrae were unable to position themselves in the water column, and descended to the bottom of the bottles shortly after the onset of the exposure. Some individuals exposed to the 50,000 μatm condition, but not those exposed to the 30,000 μatm condition, showed strong dorsal inversion of the ephyral arms when transferred back to normocapnic seawater (Fig. 1c).

These results demonstrate that ephyrae of *Aurelia* are highly tolerant of elevations of environmental CO_2, showing no mortality in 50,000 μatm pCO_2 conditions within 96 h. In contrast,

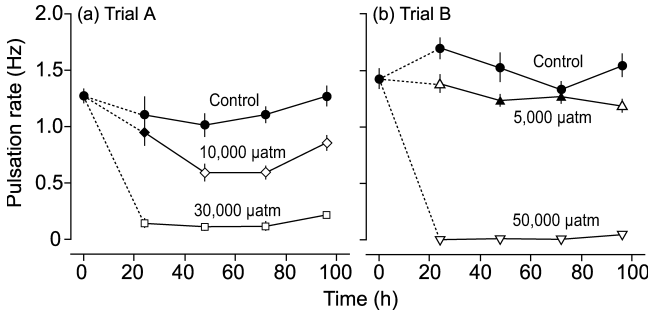

Fig. 3. Effect of 5,000–50,000 μatm pCO_2 on the pulsation rate of *Aurelia* ephyrae. Bars show standard error of the mean. $N=9$ for 10,000 μatm and $N=10$ for the others. Open symbols represent significant difference from the control determined at the same observation time ($P<0.025$; the Shirley-Williams multiple comparison one-tailed test).

exposure to the same pCO_2 resulted in 40 to 100% mortalities in embryos and larvae of the marine teleosts *Pagrus major* (Temminck & Schlegel) and *Sillago japonica* Temminck & Schlegel within 24 h (Kikkawa et al. 2003), and 100% mortality in juveniles of the squid *Sepioteuthis lessoniana* (Lesson) within 48 h (Kikkawa et al. 2008). To elucidate the observed differences in CO_2 tolerance between animals, we hypothesized an inverse relationship between the O_2 requirement and the CO_2 tolerance among marine animals: active animals with a high O_2 demand, such as fish and cephalopods, are more susceptible to acutely increased ambient CO_2 than inactive species like prawn and lugworm (Kikkawa et al. 2008). If this hypothesis is correct, the ephyrae of *A. aurita* sensu Mangum et al. (1972) are expected to have a low O_2 requirement. In fact, Mangum et al. (1972) reported an O_2 consumption rate of 0.108 mL g wet weight^{-1} h^{-1} on newly detached ephyrae of *Aurelia* sp. at 22°C, which is comparable to the O_2 consumption rate (0.11 to 0.16 mL g wet weight^{-1} h^{-1}) of the prawn *Marsupenaeus japonicus* (Bate), which is one of the most CO_2 tolerant marine animals known (the 24-h median pCO_2 tolerance limits $>$150,000 μatm, Kikkawa et al., 2008). In addition, the lugworm *Perinereis aibuhitensis* Grube shows similarly very high CO_2 tolerance, and even though O_2 consumption rate has not been directly determined for this species, it probably has a low O_2 consumption rate as inferred from the value (0.056 to 0.081 mL g wet weight^{-1} h^{-1}) reported for another lugworm *Arenicola marina* (Linnaeus) (Toulmond 1975). In contrast, the O_2 consumption rates of CO_2-sensitive species are much higher, e.g., 0.16 to 0.45 mL g wet weight^{-1} h^{-1} for *P. major* and 0.20 to 0.37 for *S. lessoniana* (Kikkawa et al. 2008). Contrasting with our hypothesis, Seibel & Walsh (2003) proposed that deep-sea organisms are highly sensitive to elevated ambient CO_2 due to their low capacities for pH buffering (mainly by proteins) and pH restoration through ion transfer processes, which are thought to have evolved as a result of the relative environmental stability and low metabolic rates needed in the deep sea. Clearly, this issue needs further examination.

Since exposures to $\geq$30,000 μatm lead to the cessation of movement of the ephyrae almost immediately, the affected animals would be displaced from their optimal depth range if they encounter such CO_2 conditions in the water column. Our previous studies have demonstrated that a sudden drop of pCO_2 can produce rapid death of fish and cuttlefish that had survived high CO_2 conditions (Kikkawa 2004, Kikkawa et al. 2006a, Kikkawa et al. 2006b, Kikkawa et al. 2008). Even though no ephyrae died within 24 h when transferred back to normocapnic water after the high CO_2 trials, the ephyral arm inversion observed for some individuals (Fig. 1c) is a symptom preceding ephyral death after exposure to environmental stressors (unpublished). Therefore they would have died if the observation had been prolonged. Although the ephyrae survived the exposure period of 96 h, the observed inhibition of swimming activities, which persisted under subsequent normocapnic conditions, can cause sublethal effects leading to reduced prey capture and escape response. It is therefore possible that high CO_2 conditions resulting from CO_2 seepage from geological formations will potentially alter the food web structure through both lethal and sublethal impacts on marine organisms.

Considering the great abundance of gelatinous zooplankton in the ocean realm, more studies on these animals are needed to evaluate the biological impacts of CO_2 storage. Only recently have concrete examples been reported of the effect of seawater acidification on jellyfishes and the detrimental cascade that could result (Lindsay et al 2008). In particular, investigations focusing on early development, reproduction and sublethal, long-term impacts should be of high priority.

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