Note

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

TAKASHI KIKKAWA1,*, YASUSHI MINOWA2, YUKIO NAKAMURA2, JUN KITA2 & ATSUSHI ISHIMATSU3

1 Central Laboratory, Marine Ecology Research Institute, 300 Iwawada, Onjuku, Chiba 299–5105, Japan
2 Demonstration Laboratory, Marine Ecology Research Institute, 4–7–17 Arahama, Kashiwazaki, Niigata 945–0017, Japan
3 Institute for East China Sea Research, Nagasaki University, Taira 1551–7, Nagasaki, Nagasaki 851–2213, Japan

Received 29 November 2008; Accepted 9 March 2010

Abstract: Ephyrae of the scyphozoan jellyfish, *Aurelia*, were exposed to hypercapnic seawater ($pCO_2$ 5,000 to 50,000 $\mu$atm) 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 $\mu$atm $pCO_2$ compared to control animals, and immediately ceased in animals exposed to $\geq$30,000 $\mu$atm. When returned to normocapnic seawater ($pCO_2$ 380 $\mu$atm) after 96 h exposure to 50,000 $\mu$atm $pCO_2$, 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 $\mu$atm, 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 [120x 45x50 (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 $\mu$atm, see below) and April 25 (Trial B, 5,000 and 50,000 $\mu$atm trials). Water temperature was 15.8°C and 16.4°C on April 19.
and 25, respectively, when the sampling was made. Mean bell diameter of 10 individuals was $1.5 \pm 0.1$ (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.

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,
exposure to the same pCO2 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 CO2 tolerance among animals, we hypothesized an inverse relationship between the O2 requirement and the CO2 tolerance among marine animals: active animals with a high O2 demand, such as fish and cephalopods, are more susceptible to acutely increased ambient CO2 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 O2 requirement. In fact, Mangum et al. (1972) reported an O2 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 O2 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 CO2 tolerant marine animals known (the 24-h median pCO2 tolerance limits >150,000 ppm, Kikkawa et al., 2008). In addition, the lugworm Perinereis aibuhitensis Grube shows similarly very high CO2 tolerance, and even though O2 consumption rate has not been directly determined for this species, it probably has a low O2 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 O2 consumption rates of CO2-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 CO2 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 \(\geq 30,000 \text{ ppm} \) 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 CO2 conditions in the water column. Our previous studies have demonstrated that a sudden drop of pCO2 can produce rapid death of fish and cuttlefish that had survived high CO2 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 CO2 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 CO2 conditions resulting from CO2 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 CO2 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.

Acknowledgements

We thank Dr. S. Ueno of the National Fisheries University, Dr. N. Omoto of the Hokkaido Electric Power Co., Inc, Mr. Z. Aoyama and the other staff members of the Marine Ecology Research Institute for providing their valuable comments and supports.

References


