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Acute CO₂ tolerance limits of juveniles of three marine invertebrates, *Sepia lycidas*, *Sepioteuthis lessoniana*, and *Marsupenaeus japonicus*TAKASHI KIKKAWA^{1*}, YUJI WATANABE², YOUICHI KATAYAMA¹, JUN KITA^{3**} & ATSUSHI ISHIMATSU⁴¹ Central Laboratory, Marine Ecology Research Institute, 300 Iwawada, Onjuku, Isumi, Chiba 299–5105, Japan² The General Environmental Technos Co., Ltd., 1–3–5 Azuchimachi, Chuo-ku, Osaka 541–0052, Japan³ Research Institute of Innovative Technology for the Earth, 9–2 Kizugawadai, Kizu, Soraku, Kyoto 619–0292, Japan⁴ Institute for East China Sea Research, Nagasaki University, 1551–7 Taira, Nagasaki 851–2213, Japan

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Abstract: CO₂ ocean storage is proposed as a possible measure to mitigate climate changes caused by increasing atmospheric concentrations of the gas. The feasibility of the measure has been intensively investigated, yet its biological impact on marine animals is still largely unknown. We investigated the acute CO₂ tolerance of juveniles of three marine invertebrates; the cuttlefish, *Sepia lycidas*, the squid, *Sepioteuthis lessoniana*, and the prawn, *Marsupenaeus japonicus*. Median tolerance limits of CO₂ were 8.4% (24 h) for the cuttlefish, 5.9% (24 h) and 3.8% (48 h) for the squid and 14.3% (72 h) for the prawn. Comparison of these and previously reported data suggests an inverse relationship between O₂ requirement and CO₂ tolerance among marine animals.

Key words: CO₂ tolerance, cuttlefish, hypercapnia, juvenile, prawn, squid.

Emissions of anthropogenic greenhouse gases and aerosols continue to alter the atmosphere in ways that have been unequivocally warming the climate system (IPCC 2007). As potential mitigation methods, carbon capture and storage (CCS) technologies are now being intensively investigated, although there are significant environmental, technical, and political uncertainties relating to these technologies (Anderson & Newell 2004, Caldeira 2005). 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 (Barry et al. 2004, Carman et al. 2004, Ishimatsu et al. 2004, Kita & Ohsumi 2004, Pörtner et al. 2004). CO₂ storage in the deep sea will create CO₂-rich plumes; the maximum pH depression near the injection point has been estimated to be –3.0 (fixed pipe) to –1.0 (moving ship, Caldeira 2005), corresponding to >50% to ca. 1% CO₂ under atmospheric conditions. However, biological impacts of such high-CO₂/low-pH plumes have hardly been investi-

gated. As the most fundamental parameter to allow assessment of the acute impacts of this technology, data must be urgently accumulated on the lethality of elevated CO₂ concentrations for various biota. Although the use of deep-sea organisms is desirable for this purpose, it is not always feasible to capture, maintain and use them in evaluation experiments. An alternative idea is to use shallow-water organisms and extrapolate obtained data to deep-sea species after applying appropriate corrections for temperature and pressure effects. Decapod crustaceans and cephalopods inhabit from the ocean surface to great depth (Childress 1995), thus making them suitable animal groups to investigate the CO₂ sensitivities of deep-sea animals. Therefore, we examined the acute CO₂ toxicity of juveniles of three shallow-water invertebrates, kisslip cuttlefish *Sepia (Acanthosepion) lycidas* Gray (Cephalopoda: Sepiida), oval squid *Sepioteuthis lessoniana* (Lesson) (Cephalopoda: Teuthida) and kuruma prawn *Marsupenaeus japonicus* (Bate) (Crustacea: Decapoda).

Juveniles of the cuttlefish (mean mantle length 9.2±0.6 mm (SD), n=20) were obtained from broodstock that spawned egg capsules on 24 April 2002 at the Marine World Uminonakamichi, Fukuoka, Japan. Juveniles of the squid (mean mantle length 7.0±0.3 mm (SD), n=20) were obtained from broodstock that spawned egg sacs on 6 May 2002 at the Institute for East China Sea Research of Na-

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gasaki University, Japan, as well as from wild egg sacs collected from the Kamogawa coast, Chiba, Japan on 29 June 2002. Both cuttlefish and squid juveniles were transferred to the Marine Ecology Research Institute (MERI), and held in indoor tanks with filtered running natural seawater regulated at 22–23°C, without aeration of the tank water or feeding. Cuttlefish and squid juveniles were used within 4 and 5 days after hatching, respectively. Prawn juveniles were donated by the Chiba Prefecture Marine Industries Promotion Corporation, Japan, where the nauplii of the prawn hatched on 18 June 2002. They were reared as above except with aeration and feeding (Marine Sigma, Nisshin Marinetech Co., Ltd.) at ca. 24°C at MERI. The prawns were tested 35–66 days after hatching (mean total length 17.8 ± 2.8 mm (SD), $n=82$). The test apparatus used in this study was basically the same as the one previously reported for an experiment with fish (Kikkawa et al. 2003). Two PVC tanks (capacity 14 L), one for CO₂ exposure and the other for a control, were placed in a water bath (100 L), the water temperature of which was regulated to 23.1 ± 0.4 °C. The PVC tanks were filled with 11 L of seawater bubbled with gas mixtures of CO₂ (3–15%) and O₂ (20.95%) balanced with N₂ (CO₂ exposure group), supplied by a gas mixing flowmeter (GF-3/MP, Cameron Instrument Company, Texas, USA) or with air (the control group), at a flow rate of 400 mL min⁻¹. The seawater was continuously bubbled throughout the exposure tests. The test tanks were covered with aluminum foil during the experiments to avoid visual disturbance to the animals. Twenty-four individuals were used for all cuttlefish and squid tests, while 49, 44, and 20 prawns were used for 3, 5 and 7–15% CO₂ exposures, respectively, for the CO₂ exposure groups. The numbers of control animals were 16 (cuttlefish), 6–12 (squid), 30 (prawn 3%), 27 (prawn 5%), and 10 (prawn 7–15%). The exposure durations were up to 72 h and the number of survivors was counted at 6 h and subsequently every 24 h. Median tolerance limit (TL_m) of CO₂, the CO₂ concentration at which 50% of the test organisms survived after a specified time of exposure, was calculated by the method of the Japanese Industrial Standard (Japanese Standards Association 1998). The mean seawater pH values were 8.123 ± 0.030 (SD) and 8.142 ± 0.014 (SD, $n=14$) at the start and the end of experiments, respectively. Initial mean salinity was 34.2 ± 0.5 (SD, $n=14$, final values not determined). Seawater pH equilibrated with different CO₂ levels in this study was represented by the equation: $\text{pH} = -0.981 \log \text{CO}_2 (\%) + 6.859$, $r^2 = 0.999$.

In the control groups no mortality occurred except that one squid died in the 10% CO₂ test and one prawn in the 5% CO₂ test. Mortalities of the CO₂ exposure groups increased with CO₂ concentration and exposure duration (Fig. 1). Cuttlefish and squid showed low (<10%) mortalities during the first 6 h period. All cuttlefish died at CO₂ concentrations of 10% and above by 24 h, and all squids died at 5% and above 7% CO₂ by 48 and 24 h, respectively. Prawns were far more tolerant and nearly 80% of the test individu-

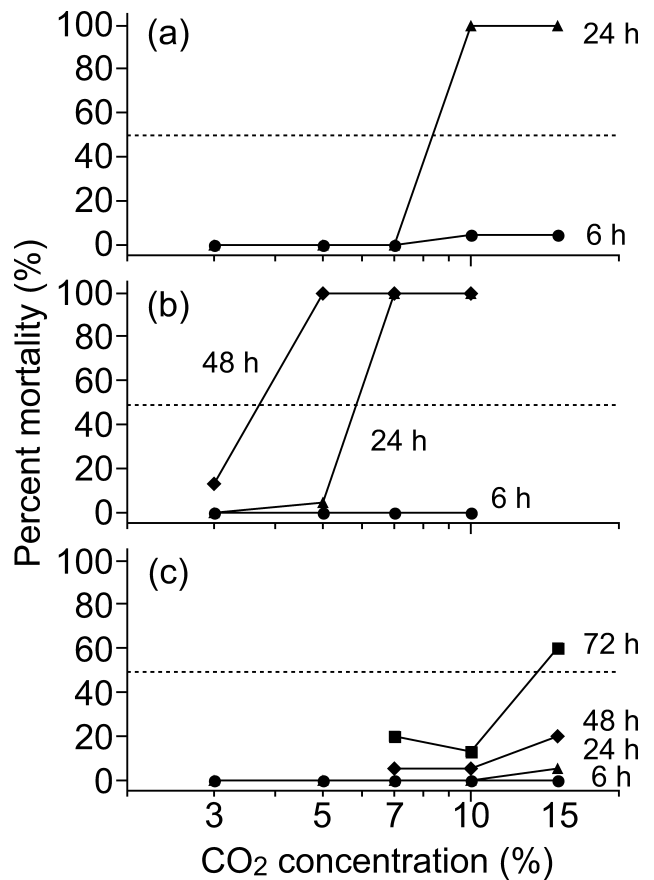


Fig. 1. Lethal effect of CO₂ on juveniles of the cuttlefish *Sepia lycidas* (a), the squid *Sepioteuthis lessoniana* (b) and the prawn *Marsupenaeus japonicus* (c). Symbols show the exposure durations (circles: 6 h, triangles: 24 h, diamonds: 48 h, and squares: 72 h). Dotted lines show the 50% survival level.

als survived 15% CO₂ exposure for 48 h. TL_ms of CO₂ were calculated at 8.4% (24 h) for the cuttlefish, 5.9% (24 h) and 3.8% (48 h) for the squid, and 14.3% (72 h) for the prawn. Upon transfer to CO₂-enriched seawater, the cuttlefish often raised their bodies off the bottom during the exposure to >5% CO₂, while they lay flat on the bottom in the control and 3% conditions. The squid responded to the onset of CO₂ exposure by violent swimming and large, rapid respiratory movements. Thereafter, both cephalopods showed blanching from their normal brown body color, loss of equilibrium, and convulsion, with the tentacles extended and flaccid. The only abnormality observed in prawn was loss of equilibrium.

All the cuttlefish that survived the >5% CO₂ exposures died within a few minutes after returning to air-equilibrated seawater. Some prawns also died upon transfer to air-equilibrated seawater after the 15% CO₂ test. A similar rapid mortality has also been observed for two fish species, i.e., embryos of *Amphiprion frenatus* Brevoort (Kikkawa et al. 2006a) and juveniles of *Sillago japonica* Temminck & Schlegel (Kikkawa et al. 2006b), upon return to normocapnic conditions following CO₂ exposure.

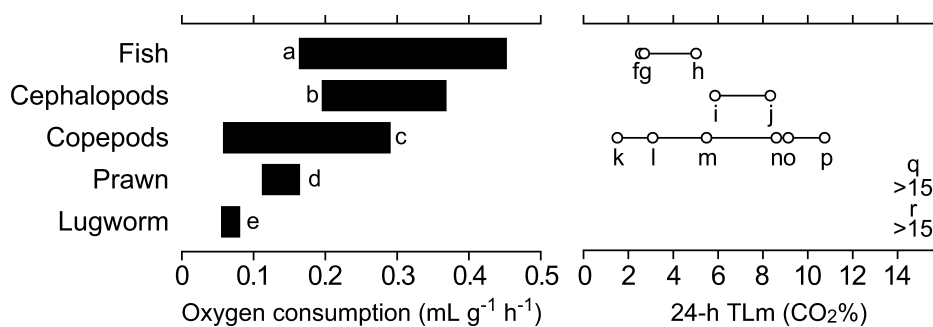


Fig. 2. Mass-specific oxygen consumption (left) and acute CO₂ tolerance (right) among fishes, cephalopods, copepods, a lugworm, and prawn. The oxygen consumption rates were standardized to a wet body weight of 1 g and temperature at 20°C. Note that the ranges of oxygen consumption rates are derived from the application of a temperature quotient of 2 to 3 and mass exponent of 0.65 to 0.86 (see text). a: *Pagrus major* (Oikawa et al. 1991), b: *Sepioteuthis lessoniana* (Segawa, 1995), c: average of 35 species (Ikeda et al. 2001), d: *Marsupenaeus japonicus* (Egusa 1961), e: *Arenicola marina* (Toulmond 1975), f: *P. major* (Kikkawa et al. 2003), g: *Sillago japonica* (Kikkawa et al. 2003), h: *Paralichthys olivaceus* (Kikkawa et al. 2003), i: *S. lessoniana* (this study), j: *Sepia lycidas* (this study), k: subarctic epipelagic species, l: *Paracalanus parvus*, m: subtropical epipelagic species, n: *Stephos* sp., o: subtropical mesopelagic species, p: *Tisbe gracilis* (TLms of copepods (k–p) calculated from the mortality data by Watanabe et al. (2006)), q: *M. japonicus* (this study), r: *Perinereis aibuhitensis* (Kikkawa et al. unpublished data).

Of the two cephalopods used in this study, the squid was less tolerant than the cuttlefish to hypercapnia. The squid were more active than the cuttlefish, continuously swimming in rearing tanks under captivity. In contrast, the cuttlefish tended to remain still on the bottom. Pörtner & Reipschläger (1996) pointed out that a rise of CO₂ partial pressure (*p*CO₂) in water by more than 1.5 mmHg would reduce blood oxygen transport and thereby lead to a reduced scope for activity in the pelagic squid *Illex illecebrosus* (LeSueur). A rise of more than 5 mmHg would cause asphyxiation. In contrast, a four-fold elevation of ambient *p*CO₂ (20 mmHg) would be needed to elicit similar physiological disorder in the less active coastal squid *Loligo pealei* (LeSueur). Among fish, the highly active pelagic species, *Seriola quinqueradiata* Temminck & Schlegel was shown to be more sensitive to hypercapnia than the sluggish demersal fish *Paralichthys olivaceus* (Temminck & Schlegel) (Hayashi et al. 2004); the routine oxygen uptake rate of the former species is 4.6 times higher than that of the latter (Hishida et al. 1998). The 24-h TLms found for the two cephalopods (8.4% for cuttlefish and 5.9% for squid) are slightly higher than those previously reported for marine fish, e.g., 5.3% for *Pagrus major* (Temminck & Schlegel) larvae, 5.0% for *P. olivaceus* juveniles and 4.8% for *S. japonica* larvae (Kikkawa et al. 2003).

To further ascertain a possible relationship between CO₂ tolerance and activity of animals, we calculated the oxygen consumption under standardized conditions among fish (*P. major*, Oikawa et al. 1991), squid (*S. lessoniana*, Segawa 1995), copepods (35 species, Ikeda et al. 2001), prawn (*M. japonicus*, Egusa 1961) and lugworm (*Arenicola marina* (Linnaeus), Toulmond 1975). Note that oxygen consumption data are not available for all the species in which TLms are known (Fig. 2). Temperature was standardized to 20°C using a temperature quotient of 2 to 3 (Randall et al. 1997). Body weight was standardized to 1 g using a mass exponent

of 0.65 to 0.86 between oxygen consumption rate and wet body mass (Cech 1990). In the case of copepods, wet body mass was estimated from reported dry body mass assuming a water content of 80% (Mizdalski 1988). The calculation for the copepods is potentially more prone to inaccuracy because the primary body weight is considerably smaller, i.e., ca. 500 µg. Although the purpose of the analysis shown in Fig. 2 is only to view the general relationship between oxygen consumption and CO₂ tolerance, the oxygen consumption rates of tolerant animals such as the lugworm and prawn are much lower than those of animals that were less tolerant like the fish and the squid (Fig. 2). On the basis of this apparent relationship, we hypothesize that active species are more sensitive to the elevation of ambient CO₂ than inactive ones, as previously pointed out by Pörtner & Reipschläger (1996). In contrast, Seibel & Walsh (2003) hypothesized that deep-sea animals characterized by low metabolic rates are probably highly vulnerable to high CO₂ conditions. They demonstrated that the low metabolic rates of these animals are correlated with their low capacities for pH buffering and ion transport, two important mechanisms for survival in an acidified environment. However, this has not yet been experimentally verified. The possible relationship between CO₂ tolerance and metabolic rate must clearly be tested by more rigorous experimentation using a standardized protocol and a larger number of species. Possible confounding factors include changes in CO₂ tolerance related to developmental stage, effects of feeding regimes on CO₂ tolerance, and acclimation to high CO₂ environments (Kikkawa et al. 2006b). Infaunal animals such as the lugworm are expected to have higher CO₂ tolerance as they are more regularly exposed to oscillating CO₂ environments (Pörtner & Reipschläger 1996, Pörtner et al. 2004). Currently, it is not possible to discern whether the observed high CO₂ tolerance of the prawn is due to its partial (day-time) infaunal existence or to relatively low O₂ demand or

both.

We consider that research priority must be placed on active, pelagic deep-sea animals to evaluate the biological impacts of CO₂ storage in the deep sea, because these active species may be affected by CO₂ to a larger extent than sluggish animals. Currently, it is unknown how low temperatures and high pressures, which characterize the deep-sea environment, affect CO₂ sensitivities of marine animals. Therefore, experiments recreating realistic deep-sea conditions need to be carried out. Furthermore, long-term impacts on the deep-sea ecosystem must be fully understood before implementation of deep-sea CO₂ storage.

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