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Acute effects of cadmium and copper on survival, oxygen consumption, ammonia-N excretion, and metal accumulation in juvenile *Exopalaemon carinicauda*

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ABSTRACT

Ridgetail white prawn (*Exopalaemon carinicauda*), a commercially important species in China, is a potential candidate for evaluating impairments caused by environmental pollutants in coastal and estuarine areas. The main purpose of the present study was to investigate the acute effects of cadmium (Cd) and copper (Cu) on survival, oxygen consumption, ammonia-N excretion, and metal accumulation in *E. carinicauda*. The feasibility of using this species for pollution monitoring was also evaluated. Results showed that the median lethal concentrations (LC_{50}) for 24 h, 48 h, 72 h, and 96 h were 0.66 mg/L, 0.379 mg/L, 0.343 mg/L, and 0.258 mg/L for Cd, and 0.932 mg/L, 0.748 mg/L, 0.725 mg/L, and 0.712 mg/L for Cu. Cd exposure (0.66 mg/L) caused an inhibition in oxygen consumption of 21.1 percent and an increase in ammonia-N excretion of 47.1 percent, thereby decreasing the atomic ratio of oxygen consumed to nitrogen consumed (O:N ratio) of 46.32 percent relative to the control. Cu exposure (0.932 mg/L) also resulted in an inhibition in oxygen consumption of 34.8 percent and a decrease in the O:N ratio of 23.9 percent in relation to the control, but the ammonia-N excretion was not influenced by the Cu exposure. Concentration-depended accumulation was observed in the experimental animals, which a maximum of 244.8 folds and 1.1 folds increase of mental concentration was measured upon exposure to 24 h LC_{50} of Cd and Cu for 24 h, respectively. The change in O:N ratio indicated an alteration in energy utilization. Based on its sensitivity to heavy metals and its availability all year round, *E. carinicauda* can be used as a test organism to monitor for metal pollution.

1. Introduction

Acute toxicity tests provide quick, relatively inexpensive and reproducible estimates of the toxic effects of chemicals. The most common acute effect measured in aquatic life is lethality or mortality (Chinni et al., 2002). The median lethal concentration (LC_{50}) is one of the more commonly used parameters to describe the acute lethal toxicity of pollutants. From an ecotoxicological point of view, this parameter is often estimated in the more sensitive species or life stages of a particular aquatic community, and the assessment of mortality is a potential tool to establish safe concentrations of pollutants in the environment. Crustaceans have been widely used in toxicological bioassays, with the larval, postlarval, and juvenile stages being particularly sensitive to several pollutants, including heavy metals (Frank and Robertson, 1979; Papathanassion, 1983; Wong et al., 1993; Vanegas et al., 1997; Chinni et al., 2000, 2002; Santos et al., 2000; Wu and Chen, 2004; Barbieri, 2007, 2009; Barbieri and Paes, 2011).

Exposure to heavy metals in the aquatic environment produces many physiological changes in crustaceans, including alternations in the metabolic activities (Barbieri et al., 2005; Barbieri, 2009). Respiration and excretion, the most fundamental physiological activities of animal energy metabolism, are considered to be a good indication of the overall metabolic state of an animal and are useful indicators to evaluate the toxicant effects caused by heavy metals (Chinni et al., 2000, 2002; Wu and Chen, 2004; Barbieri et al., 2005; Barbieri, 2007, 2009; Barbieri and Paes, 2011), detergents (Barbieri et al., 2002), and aromatic compounds (Lemaire et al., 1996).

The ridgetail white prawn *Exopalaemon carinicauda* (Decapoda, Palaemonidae) that inhabits the brackish waters of the Indo-West Pacific, Korea, and China (Holthuis, 1980), is an important economic species in China. Xu et al. (2010) reported that this species contributed one third of the gross production of polyculture ponds in eastern China. This prawn is widely distributed in the coastal zone in China, especially in the Yellow and Bohai Seas as well as in estuaries. Because of human activities, water in coastal and

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estuarine areas is often contaminated by kinds of pollutants, including heavy metals (Chua, 1992; Wang and Wang, 2007). Heavy metal contamination in aquatic systems is reportedly one of the most critical environmental issues (Páez-Osuna, 2000). Hence, evaluations of toxic effects of heavy metals are necessary and urgent.

Cadmium (Cd), a typically nonessential metal to living organisms, is an very common and persistent heavy metal in aquatic environments and is known to be highly toxic to marine and estuarine crustaceans (Wu and Chen, 2004; Barbieri and Paes, 2011). Copper (Cu) is an essential metal that participates in normal physiological process of crustaceans but becomes toxic at high concentrations (Santos et al., 2000; Valavanidis and Vlachogianni, 2010). Accordingly, the present study investigates the acute effects of Cd and Cu on the mortality, oxygen consumption, ammonia-N excretion, and metal accumulation in *E. carinicauda*. The feasibility of using this species in pollution monitoring was also evaluated.

2. Materials and methods

2.1. Shrimp collection and maintenance

All experimental animals were obtained through artificial breeding at Nantong Juhao Aquaculture Co. Ltd., Jiangsu, PR China. The juvenile shrimp were 40 day-old postlarvae (PL40) cultured in an outdoor earth pond. A total of 2000 shrimp with body weights of 0.73 ± 0.05 g were transferred into a 5 m^3 (350 cm × 180 cm × 110 cm) cement pool with a salinity of 10 ± 0.5‰ and natural temperature ranging from 24 °C to 25 °C. After 7 d acclimation, only active and intermoult shrimp were selected and used as experimental animals.

Standardized photoperiod (12 h:12 h, light:dark) and continuous aeration were maintained during the entire period of acclimation. The shrimps were fed thrice daily (7:00, 15:00, and 23:00) at 10 percent of their wet weight biomass to satiation using a commercially formulated diet (crude protein 40 percent; Guangdong Evergreen Fishery Feed Co. Ltd., Zhanjiang, China). Water was exchanged at 80 percent/d after removal of wastes, exuviae, and unconsumed food by siphoning (Zhang et al., 2008). The physico-chemical parameters of the experimental seawater (i.e., pH value varied from 8.4 to 8.5, dissolved oxygen (DO) concentration varied from 6.83 mg/L to 7.56 mg/L, and ammonia-N (AN) concentration varied from 0.01 mg/L to 0.03 mg/L) were maintained within the optimal range recommended for *E. carinicauda* (Liang et al., 2012a, 2012b). Dissolved oxygen, pH, salinity, and temperature were monitored using an oxygen and pH meter (Model HQ40d, Hach Co, Colorado, USA), a portable refractometer (Model master-s 10 M, Atogo Co, Tokyo, Japan), and a mercury thermometer, respectively.

2.2. Metal solution and analysis

The stock solutions of Cd and Cu were freshly prepared by dissolving $CdCl_2 \cdot 2.5H_2O$ (2.03 g) and $CuSO_4 \cdot 5H_2O$ (3.99 g) in deionized water (1 L). Both compounds (Sinopharm chemical reagent Co. Ltd, Shanghai, China) were analytical grade. The solutions were serially diluted to get the experimental concentrations for the toxicity test.

The test concentrations of Cd and Cu were measured using an atomic absorption spectrophotometer (AAS, Thermo SOLAAR, USA). Cd and Cu were measured according to a previously described method "The specification for marine monitoring—Part 4: seawater analysis" (AQISQ/ROC, 2007) using a graphite furnace AAS. The detection limits were 0.01 μg/L for Cd and 0.02 μg/L for Cu.

2.3. Survival experiments

Static renewal bioassays were conducted to determine the acute toxicity of Cd and Cu on juvenile shrimp of *E. carinicauda* as per standard procedure (FAO, 1977). Six Cd concentrations (0.1 mg/L, 0.2 mg/L, 0.5 mg/L, 1 mg/L, 2 mg/L, and 5 mg/L) and five Cu concentrations (0.1 mg/L, 0.2 mg/L, 0.5 mg/L, 1 mg/L, and 2 mg/L) were tested. Test solutions were prepared by adding a calculated volume of stock solution to 30 L seawater in a 50 L plastic container. A parallel control was maintained without metal toxicant. Each plastic container held 20 individuals at a particular concentration, and three replicates were set up for each test concentration. A total of 720 juveniles were used in the static renewal bioassays.

The tests lasted for 96 h. The test solutions were changed almost entirely every morning to maintain the metal concentration. Dead shrimp were removed and mortality was recorded every 24 h. The experimental animals were not fed and a constant aeration was supplied during the exposure to heavy metals. The water temperature, salinity, and photoperiod were similar to the acclimation period.

2.4. Metabolic test

According to the data of tolerance test at 24 h, four Cd concentrations (LC_5, 0.140 mg/L; LC_{10}, 0.179 mg/L; LC_{25}, 0.349 mg/L; LC_{50}, 0.660 mg/L) and four Cu concentrations (LC_5, 0.374 mg/L; LC_{10}, 0.458 mg/L; LC_{25}, 0.641 mg/L; LC_{50}, 0.932 mg/L) were chosen as the experimental concentrations for the metabolic test. A separate control without toxicant was maintained.

After 7 d acclimation, the shrimp were individually examined to determine their molting stage according to the previous description by Robertson et al. (1987) and Promwikorn et al. (2004). Only shrimp in the intermoult stage were used for subsequent metabolic experiments. The selected shrimp were left to acclimatize for an additional 24 h to attenuate the handling stress as well as empty the stomach and intestine of the experimental animals. The shrimp were not fed during the additional 24 h and standard metabolic rates measurement. To avoid cannibalism, every two selected shrimp were segregated using a hatching cage (15 cm × 15 cm × 20 cm) with mesh wall and bottom (mesh diameter, 1 mm). Once a shrimp died, the surviving shrimp in that same cage was also discarded. A total of 45 juveniles were used in the metabolic test.

Prior to the experiment, 54 glass bottles (1.135 ± 0.003 L) with airtight covers were filled with the test solution and placed in a bathing container, with the water temperature kept at 25 °C using a temperature controller (Model OKE-6710HF, I'm Tech Co. Ltd., Korea). The experimental shrimp were individually transferred into the bottles, which were subsequently sealed. A total of 45 bottles (5 replicates per metal concentration) were used as experimental chambers, and 9 bottles were used as blank chambers (no animal present); one blank for each concentration. Prior to the metabolic test, the animals were maintained in the bottle for at least 1 h to attenuate the handling stress.

At the beginning of the metabolic experiment, 10 mL of the water sample in each bottle was removed by a transferpettor (maximum volume=5 mL) for subsequent measurement of ammonia-N (AN) concentration. The dissolved oxygen (DO) concentration was then measured directly in the bottle, and then another 10 mL of the experimental water was added to the bottle and sealed again. The metabolic experiment lasted for 3 h, preliminary results indicated that for this experimental duration, the oxygen concentration remained above 60 percent of the original values. The body weights of the experimental shrimp were measured at the end of the metabolic experiment using an electronic balance (Model LT202B, 0.001 g precision, Tianliang Instrument Co., Changshu, China). Water samples were taken through siphoning at the end of the experiment. DO values were measured using a DO meter and electrode probe (Model LDO101, HACH Co., Colorado, USA), and AN values were measured through the salicylate method (Hach, 2012). For each water sample, the readings of DO and AN were repeated thrice, and the mean of these three replicates was calculated as the final result in this sample. Data were corrected for changes in DO and AN measured in the blank chambers. Slight changes (< 1 percent) in DO and AN concentration that resulted from the sampling and addition of water were dismissed.

2.5. Accumulation experiments

Juvenile shrimp were exposed to the same metal concentration as the metabolic tests (LC_5, LC_{10}, LC_{25}, and LC_{50}), and a parallel control was maintained without metal toxicant. Twenty individuals were placed in a 50 L plastic container as a concentration treatment, and three replicates were set up for each test concentration. A total of 540 juveniles were used in the accumulation experiment. After 24 h of exposure, five shrimp were sacrificed from each metal concentration group and control group and used as a metal sample (5 shrimps per sample). All samples were stored at −20 °C for subsequent measurement of metal accumulation. Whole body samples in both control and exposed shrimp were kept in hot air oven at 80 °C for 48 h, after which the dried material was homogenized into a fine powder (Chinni et al., 2002). A known quantity of dried tissue powder was microwave-digested with nitric acid and hydrogen peroxide in teflon digestion tank, then the digestion solution was used for metal estimation by inductively coupled plasma mass spectrometry (ICP–MS) method (AQISQ/ROC, 2008). The detection limits were 0.001 mg/kg for both metals.

2.6. Data calculation and statistical analysis

The standard metabolic rate was evaluated in terms of oxygen consumption (OCR), ammonia-N excretion rate (AER), and oxygen consumed to nitrogen consumed ratio (O:N ratio). These indices were calculated as

$$\mathrm{OCR\ (mg/g/h)} = \{(DO_B - DO_E) \times V\}/(t \times W)$$

$$\mathrm{AER\ (mg/g/h)} = \{(AN_E - AN_B) \times V\}/(t \times W)$$

$$\mathrm{O:N\ ratio} = = (17 \times \mathrm{OCR})/(16 \times \mathrm{AER})$$

where DO_B is the oxygen concentration in the water of experimental chamber at the beginning of the metabolic experiment (mg O_2/L), DO_E is the oxygen concentration in the water of the experimental chamber at the end of the metabolic experiment (mg O_2/L), V is the volume of the respiration chamber (L), t is the