Research Paper

Effects of Cadmium on Oxygen Consumption and Tissue Accumulation in Tiger Shrimp *Penaeus monodon* Fabricius

*Nongnud TANGKROCK-OLAN and Voravit CHEEVAPORN**
Department of Aquatic Science, Faculty of Science, Burapha University,Thailand

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Abstract

Tiger shrimps (*Penaeus monodon*) were exposed to various concentrations of cadmium as cadmium chloride ($CdCl_2 \cdot 2½H_2O$) for 96 hours. Survival rates and the lethal concentration fifty (LC_{50}) of shrimps with cadmium concentrations of 0.0, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mg/l within 96 hours were 100%, 100%, 100%, 90%, 67%, 17%, 13% and 0%, respectively, and the LC_{50}-96 hours was 2.42 mg/l. Cadmium also reduced rates of oxygen consumption (Mo_2). The rates of oxygen consumption of shrimps reared in seawater for 1 month with cadmium concentrations of 0, 0.1, 0.5 and 1.0 mg/l were 6.21 ± 1.53, 6.92 ± 1.21, 4.87 ± 1.24 and 4.37 ± 1.28 µmol/g/h, respectively. The concentration of cadmium in the reared shrimps was found to be higher in the head region than that found in the body. The concentrations found in both regions increased with the concentration of cadmium. From the results, it is indicated that shrimps did not abruptly die after exposure to cadmium. But the cadmium may accumulate in shrimps and result in changes to their physiology. The changes in the rates of oxygen consumption may reflect the changes in environmental pollution which occur in habitats where shrimps live.

Key words: Peneaus monodon, shrimp, cadmium,oxygen consumption:

*Author for correspondence: Dr. Voravit Cheevaporn, Department of Aquatic Science, Faculty of Science, Burapha University, Chonburi, 20131, Thailand. Telephone: +6638-745900 Ext. 3023 ; Fax: +6638-393491; E-mail: voravit@buu.ac.th

Introduction

Heavy metals occur naturally in seawater in very low concentrations. However in many marine environments heavy metal concentrations may exceed natural levels. High concentrations of metals can enter the coastal environments as the result of human activities. Investigations of heavy metals in aquatic organisms are an important aspect of environmental pollution control since anthropogenic activities progressively increase the concentrations of metal in the marine environment. Metals from industrial discharges can also be deposited in a wide variety of ecosystem components such as water, sediment and suspended particulate matter in the water column, and biota. Helz et al. (1975) indicated that concentrations of cadmium and other metals in water might decrease rapidly downstream from a wastewater treatment plant discharging into an estuarine environment, whereas high concentrations of metals may occur in the sediment all the way from the discharge point to the mouth of the estuary.

Aquatic organisms experience direct waterborne contact with heavy metal pollutants. Several studies of aquatic invertebrates revealed a high degree of variability in the uptake and accumulation of heavy metals (Wong and Rainbow 1986, Moore and Rainbow 1987, Rainbow and White 1989, 1990, Donker and Bogert 1991). The burrowing and feeding activities of some benthic invertebrates such as shrimps may lead to the mobilization of metals from the sediments. The utility of marine shrimp as bioindicators of heavy metal pollution for environmental monitoring studies has been emphasized by several investigators (Clark et al. 1987, Darmono 1990, Mizutani et al. 1991, Palmer and Presley 1993, Dallinger 1994).

The tiger shrimp *Penaeus monodon* is sediment-dwelling and detritus- feeding shrimp found abundantly in mangrove and estuarine environments. This shrimp species is also one of the most economically important shrimps in Thailand. In an attempt to predict some of the possible biological effects of heavy metal pollution on this shrimp, cadmium was used as a heavy metal substance for this study. The objectives of the present investigation were (1) to determine the acute toxicity of cadmium in juvenile tiger shrimps, (2) to study the effect of sublethal cadmium concentrations in seawater on the oxygen consumption capacity of the shrimp, and (3) to determine the level of cadmium in tissues of the shrimp.

Materials And Methods

Animals and Maintenance

Juvenile tiger shrimps, *P. monodon*, with body length 4-5 cm and weighing between 0.6-1.4 g, were used in this study. All shrimps were bought from a shrimp farm in Chachoengsao province. They were then transported to the Department of Aquatic Science, Burapha University and acclimated to the laboratory conditions in well-aerated seawater (20 ppt in salinity) under a natural photoperiod at seasonal temperature for at least 1 week before testing. During this period they were fed every day with shrimp pellets.

Survival study

Before performing the oxygen consumption experiments, it was necessary to know whether shrimps had the ability to survive in a given cadmium concentration. Therefore, the rates of survival of shrimps were determined after 96 hours of exposure to eight different concentrations (0, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mg/l) of cadmium solutions at 25°C. Cadmium solutions were prepared using $CdCl_2 \cdot 2½H_2O$ and seawater (20 ppt in salinity) and its concentration is expressed as that of $CdCl_2 \cdot 2½H_2O$. Three experimental treatments with ten individuals were maintained in each experimental cadmium concentration.

A mortality count was taken after 24, 48, 72 and 96 hours in all tested concentrations. Shrimps were determined to be dead as they stopped moving and lay on the bottom. The ability of shrimps to survive in different concentrations of cadmium was expressed as percentage survival. LC_{50} for 96 hours was also calculated.

Oxygen consumption study

Measurements of oxygen consumption rates were done in shrimps which reared for 30 days in three different concentrations of cadmium (0.1, 0.5 and 1.0 mg/l) and in one control. The highest concentration (1.0 mg/l) of cadmium solution prepared was the concentration in which all shrimps could survive up to 96 hours. The oxygen consumption rates of 10 individuals from each treatment were determined on day 30. Each individual was placed in a closed respiratory chamber of 750 ml capacity filled with sterilized seawater of 20 ppt salinity. The chamber was placed in a water bath to keep the temperature at 25°C. Shrimps were acclimated for three hours and the oxygen concentration in the seawater was measured by means of an oxygen electrode of about 1 hour at a temperature of 25°C. Oxygen consumption rate was expressed as micromole of oxygen per hour.

Prior to each experiment, shrimps (n = 10 shrimps at each concentration) were rinsed thoroughly to remove or to kill any exterior microorganisms. After measuring the oxygen consumption rate, shrimps were towel-dried to remove surface water and weighed for wet weight. Weight specific oxygen consumption (Mo_2) was then calculated.

Tissue accumulation study

Cadmium concentrations in the tissues of *P. monodon* were investigated in shrimps which had been used in the oxygen consumption study. Shrimps were killed by freezing, freeze-dried and stored at -80°C until investigation. Prior to analysis, four shrimps were dissected into two parts: head and body. Sample preparation and analysis were carried out according to the procedure described by Bernhard (1976). Each body part was dried at 105°C for 48 hours and weighed for dry weight. The dried material was then digested with a mixture concentrated nitric acid and concentrated perchloric acid (2:1 v/v) at 120°C for 3 hours. After dilution of the reaction mixture, the cadmium content of each part was measured by an atomic absorption spectrophotometer.

Data analyses

The survival rate of shrimps in different concentrations of cadmium was expressed as percentage survival. Data analysis of survival was performed by the Log-probit plots of percentage survival at 96 hours against the cadmium concentration.

Mass-specific metabolic rates are illustrated by plotting against body mass in a double logarithmic regression. Differences in mean Mo_2 among treatment groups were tested by analysis of covariance and including mass as a covariate. Pairwise comparisons among treatment were made using Scheffe (post hoc) test.

Comparison of cadmium in tissue was made by ANOVA. Subsequent multiple comparisons of means were performed using Scheffe pairwise comparisons method.

Results

Survival study

Shrimps exposed to 0.0, 1.0 and 1.5 mg/l cadmium concentrations survive 100% for 96 hours. Mean (±SD) values of percentage survival of juvenile tiger shrimps in 20 ppt seawater after exposure to 2.0, 2.5, 3.0, 3.5 and 4.0 mg/l cadmium concentrations for 96 hours were 90%, 67%, 17%, 13% and 0%, respectively (Fig. 1). The percentage survival of shrimps at 96 hours gave a 96-hours LC_{50} of 2.42 mg/l (Fig. 2).

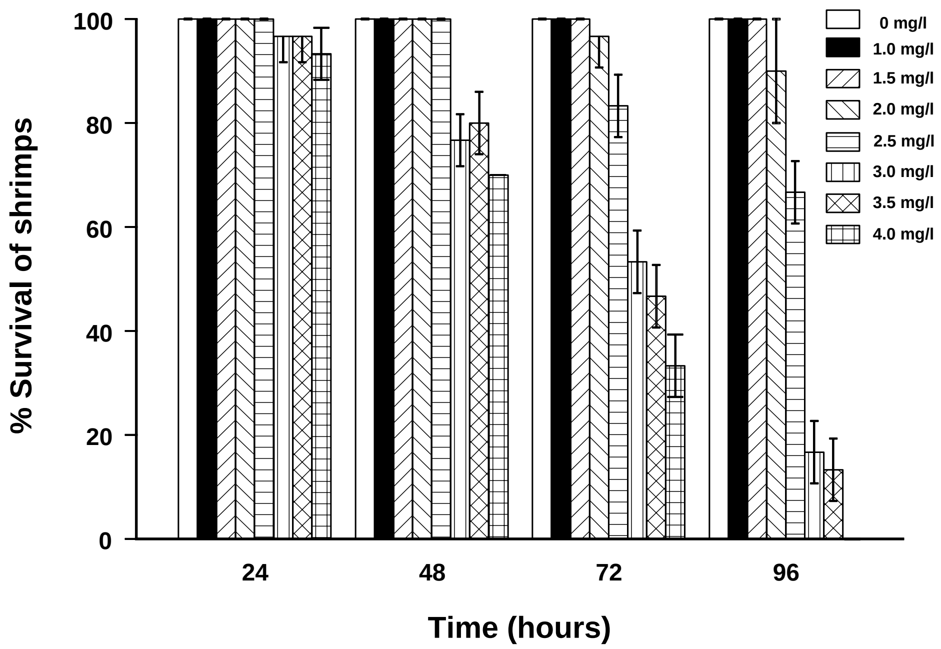

Figure 1 Mean (±SD) values of percentage survival of juvenile tiger shrimps in seawater of 20 ppt in salinity after exposure to different concentrations of cadmium for up to 96 hours at 25°C (n = 30 for each bar).

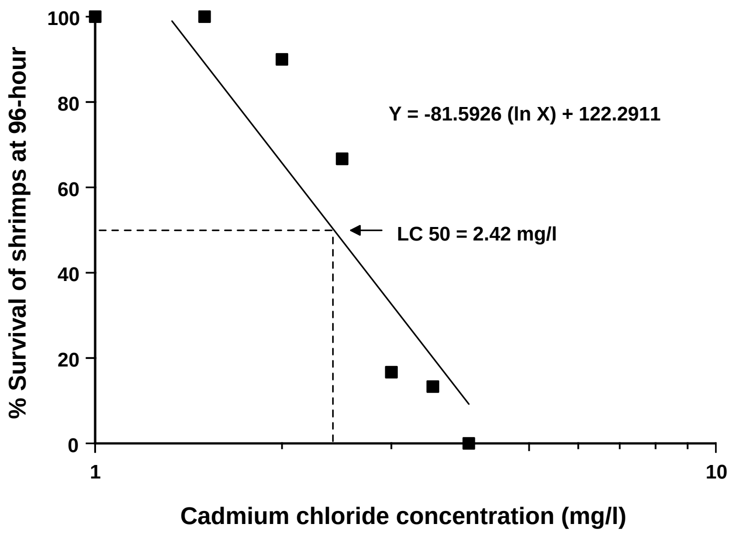

Figure 2 Log-probit plots of the percentage survival at 96-hours against (versus) the cadmium concentration. The LC_{50} value of the cadmium is 2.42 mg/l.

Oxygen consumption rate

Mean oxygen consumption rates for shrimps reared in seawater for 1 month with cadmium concentrations of 0, 0.1, 0.5 and 1.0 mg/l were 6.21 ± 1.53, 6.92 ± 1.21, 4.87 ± 1.24 and 4.37 ± 1.28 μmol/g/h, respectively. However, the regression line between the weight specific oxygen consumption (Mo_2) and body weight showed a decrease of Mo_2 with increase in the body weight of shrimps (Fig. 3). Mo_2 values of one-gram body weight calculated from the regression equation were 5.54, 6.07, 5.71 and 3.99 µmol/h in shrimps reared in cadmium concentrations of 0, 0.1, 0.5 and 1.0 mg/l, respectively. Mo_2 values of shrimps reared in cadmium solutions at the concentrations of 0, 0.1, 0.5 mg/l were found to be similar but higher than for those reared in the cadmium concentration of 1.0 mg/l.

Analysis of covariance and Scheffe's statistical test showed that the mean Mo_2 values of shrimps reared in cadmium concentrations of 0, 0.1 and 0.5 mg/l were significantly higher than for those reared in the cadmium concentration of 1.0 mg/l (Table 1).

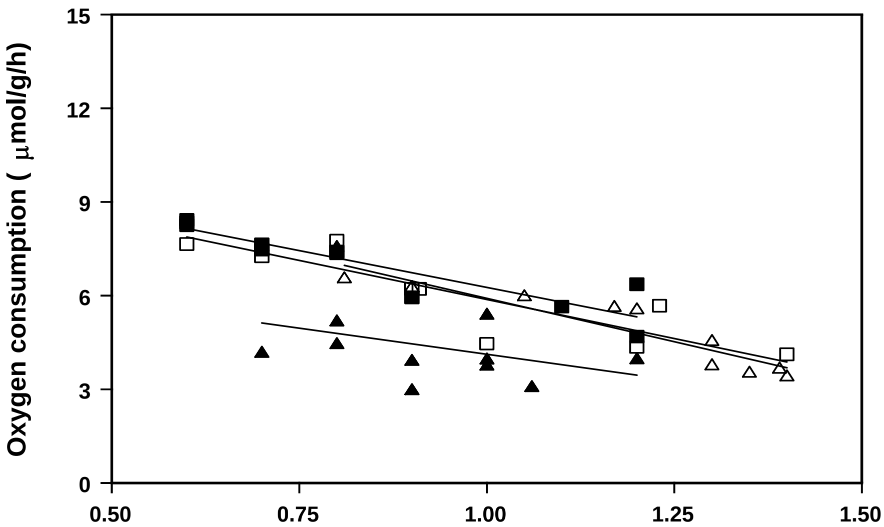

Figure 3 The relationship between weight specific oxygen consumption and body weight of juvenile shrimp, *Penaeus monodon* after being reared in different concentrations of cadmium for 30 days (□ = 0 mg/l, ■ = 0.1 mg/l, Δ = 0.5 mg/l and ▲ = 1.0 mg/l). Measurement was done in seawater of 20 ppt in salinity at 25°C.

Table 1 Regression equations for the weight specific oxygen consumption (µmol/g/h) of juvenile shrimp, *Penaeus monodon* after being reared in different concentrations of cadmium for 30 days. Measurement was done in seawater of 20 ppt in salinity at 25°C.

Concentration of Cadmium (mg/l)	*Regression equation*	r	n
0	$Mo_2 = 5.54\ W^{-0.78}$	0.89	10
0.1	$Mo_2 = 6.07\ W^{-0.63}$	0.90	10
0.5	$Mo_2 = 5.71\ W^{-1.22}$	0.88	10
1.0	$*Mo_2 = 3.99\ W^{-0.66}$	0.39	11

* Asterisk indicates the significant differences ($P < 0.05$) in the mean Mo_2 from the corresponding groups.

Accumulation in tissue

The concentration of cadmium in tissues of shrimp reared in the seawater of 20 ppt salinity at cadmium concentrations of 0, 0.1, 0.5 and 1.0 mg/l for 30 days was shown in Figure 4. The metal levels in both head and body regions were increasingly higher in shrimps exposed to 0.1 and 0.5 mg/l and rose markedly in those exposed to 1.0 mg/l. At every cadmium concentration, the cadmium content in the head region is more than twice higher than that in the body region. Analysis of variance and Scheffe's statistical test showed that the mean cadmium contents accumulated in both the head and the body regions of shrimps reared in all cadmium concentrations were significantly higher than for those in the control group (Table 2). When comparing the values on the analysis of variance and Scheffe's

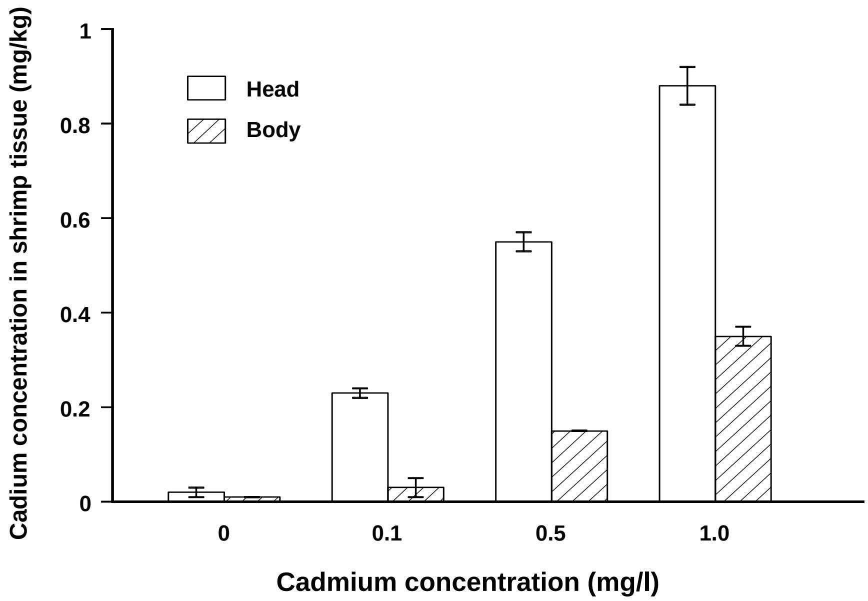

Figure 4 Mean (±SD) values of cadmium concentrations accumulated in the head and body regions of juvenile tiger shrimps, *Penaeus monodon* after being reared in different concentrations of cadmium for 30 days (n = 4 for each bar).

Table 2 Average cadmium concentrations accumulated in the head and body regions of juvenile tiger shrimps, *Penaeus monodon,* after being reared in different concentrations of cadmium for 30 days

Cadmium concentration (mg/l)	Cadmium accumulation (Mean ± SD)		n
	Head	Body	
0	0.02 ± 0.01	0.01 ± 0.00	4
0.1	*0.23 ± 0.01	0.03 ± 0.02	4
0.5	*0.55 ± 0.02	**0.15 ± 0.00	4
1.0	*0.88 ± 0.04	**0.35 ± 0.02	4

*, ** Asterisks indicate the significant differences ($P < 0.05$) in the mean from the control and the corresponding groups.

statistical test, it was also found that the mean cadmium contents in the head regions of shrimps reared in cadmium concentrations of 0.5 and 1.0 mg/l were significantly higher than those found in the body regions (Table 2).

Discussion

There is no evidence that cadmium at any concentration is essential or beneficial to living organisms, but this metal accumulates in the tissues of a wide variety of marine organisms (Eisler 1971, Bjerregaard and Depledge 1994). A number of studies have reported the toxicity of small amounts of this metal to marine animals (McCahon and Pascoe 1988, Sosnowski and Gentile 1978).

The 96-hour LC_{50} value for cadmium obtained in the present study (2.42 mg/l, in shrimps acclimated to seawater of 20 ppt salinity) is about three to five times lower than the values reported for other euryhaline crustaceans, fishes and bivalve molluscs tested in normal seawater (Eisler 1971, Wright 1977, GESAMP 1985, White and Rainbow 1986, Patel and Anthony 1991) but more than 17 times higher than those found for freshwater or marine amphipods and copepods (Sosnowski and Gentile 1978, Wright and Frain 1981, McCahon and Pascoe 1988, Zanders and Rojas 1992). The cadmium LC_{50} value thus reveals a slightly low tolerance of *P. monodon* to this metal, differing from other decapods.

Shrimps exposed to cadmium appeared to be less active than those in the control group. Inhibition of respiration by cadmium has been reported in mud crabs (Collier et al. 1973). A similar response was observed in shrimps in the present study. A cadmium concentration of 1.0 mg/l appeared to have an influence on the oxygen consumption rate of *P. monodon*. The Mo_2 values for shrimps reared in cadmium at concentrations of 0, 0.1, 0.5 mg/l were found to be similar but higher than for those reared in a cadmium concentration of 1.0 mg/l. The lower Mo_2 values obtained for the shrimps reared in cadmium at a concentration of 1.0 mg/l may be due to damage to their gills and this leads to the respiratory impairment. The reduced Mo_2 values may be caused by other factors, for example, metabolism may be depressed in this shrimp as a result of energy intake limitations.

The levels of cadmium in *P. monodon* were significantly different between the head and the body regions. The cadmium content in the head region was found to be higher than that found in the body region. This is not surprising, since the hepatopancreas and gill tissues are located in the head region and it is well known that the hepatopancreas is the main regulatory organ in crustacean species and as such it would be the prime site for metal storage and detoxification in these animals (Lyon et al. 1983, Thaker and Haritos 1989, Darmono and Denton 1990). Heavy metals mainly accumulate in metabolically active tissues. The hepatopancreas and gill tissues were highly active in the uptake and storage of heavy metals. Crustaceans (shrimp) respond to heavy metal exposure by producing metallothionein, particularly in hepatopancreas (Darmono 1990, Howard and Hacker 1990, Dallinger 1994). High levels of metals found in the head region of shrimp is possibly due to binding of the cadmium to metallothionein proteins in the hepatopancreas. The gill was also a tissue in which active and passive exchanges occurred between the animal and the aquatic environment.

Invertebrates, particularly crustaceans, were very sensitive to heavy metals (Thorp and Lake 1974). A number of studies show that concentrations of cadmium in aquatic organisms depend mainly on their environmental levels (Amiard et al. 1987, Health 1987, Bryan and Langston 1992). Shrimps from clean environments contain very low levels of metals. However they can accumulate substantial quantities of these metals under polluted conditions. Higher metal concentrations were found in the tissues of *Palaemonetes varians* caught in a polluted area (Frenet and Alliot 1985). Zanders and Rojas (1996) reported that accumulation of cadmium in tissues of fiddler crabs is enhanced under conditions of low salinity. Wright and Frain (1981) and McCahon and Pascoe (1988) reported that high salinities (or Ca^{2+} concentrations) diminish the uptake and toxicity of heavy metals, especially cadmium, in crabs and amphipods.

In conclusion, chronic sublethal exposure to waterborne cadmium resulted in physiological changes and accumulation of cadmium in the tissues of shrimp *P. monodon* in this study. Thus, cadmium may not be immediately harmful to shrimp populations, but it may accumulate and have effects (they may become affected) in the long term, such as in changes in metabolic rates. Besides the effects of cadmium uptake on metabolism, other processes may also become affected in the long term; for instance, incipient disturbances of reproduction or molting cycles. Habitat of *P. monodon* are close to the mouth of the river which is the area affected by heavy pollution. Further research using new approaches and alternate methods should be required to understand or to predict the biological impact of cadmium and other metals on this and other aquatic organisms.

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