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Effects of Cadmium and Zinc on the Growth, Food Consumption, and Nutritional Conditions of the White Shrimp, *Litopenaeus vannamei* (Boone)

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White shrimp, *Litopenaeus vanname* is one of the important aquacultured prawn species, not only in North, Central, and South America but also in Asia. The direct use of seawater from coastal areas without processing for maintaining and rearing shrimp in farms always entails some risks, since coastal waters can very easily become contaminated by many kinds of pollutants. Heavy metals are the most common pollutant due to human activities (Paez-Osuna and Tron-Mayen 1996). Aquaculture provides a substantial portion of aquatic products to humans and the results of successful cultures may produce huge economic values. Therefore, the presence of heavy metals in aquaculture systems may have deep impacts on humans consuming food, farmers, and even the global aquaculture industry. One of the immediate adverse effects on aquaculture caused by heavy metals is the growth retardation of cultured organisms. Also, growth is a sensitive sublethal indicator at the level of the individual organism (Rinderhagen et al. 2000). Some studies have pursued the effects of heavy metals on the growth of fish, such as rainbow trout *Salmo gairdneri* (Waiwood and Beamish 1978) and yellow perch *Perca flavescens* (Kearns and Atchison 1979), but relatively less effort has been expended on prawn culture species, such as *L. vannamei*.

Therefore, the major objective of the present paper was to study the effects of the heavy metals cadmium (Cd) and zinc (Zn) on the growth of *L. vannamei*. Then, with the preliminary results from the growth experiments, we expanded our interests to other related topics, including their effects on food consumption and biochemical parameters such as triacylglycerol, cholesterol, and total soluble protein levels within the hepatopancreas, to determine the effects of Cd and Zn on the nutritional conditions of the animals studied.

MATERIALS AND METHODS

A single batch of postlarval *L. vannamei* was obtained from a commercial hatchery in Ilan, northeastern Taiwan and kept in an incubator for acclimation with water conditions as follows: temperature of 25 °C, salinity of 15 p.s.u., DO of 5.8~6.5 mg/l, pH 7.15~7.87, and Eh of 32~152 μS/cm, under a 12: 12-h light-dark regime with continuous aeration; these were the same conditions used during the experimental period. Animals used for each experimental treatment

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Table 1. Different size of *Litopenaeus vannamei* used for each experiment in this present study.

Experiment Item	Animal Size			
	Weight (g)		Length (cm)	
	Mean	S.D.	Mean	S.D.
Effects of Cd on Growth	0.10	0.01	3.17	0.02
Effects of Zn on Growth	0.04	<0.01	2.02	0.05
Effects of Cd and Zn on Food Consumption	0.49	0.07	4.43	0.22
Effects of Cd and Zn on Nutritional Status	0.28	0.12	3.75	0.19

differed in size, as presented in Table 1. During all experimental periods, animals were regularly fed commercial shrimp pellet feeds twice a day; test solutions as well as control seawater were renewed twice a week.

To determine the effects of Cd and Zn on growth after metal exposure, *L. vannamei* were divided into eight groups, each of which contained 10 shrimp that were exposed to concentrations of either 0.1, 0.2, or 0.4 mg Cd/l as $CdSO_4$, or 0.05, 0.2, or 0.6 mg Zn/l as $ZnSO_4$, and one control set for each metal as well. Each treatment was repeated two times. The body weight and length of each individual *L. vannamei* were carefully measured on days 0, 7, 14, 21, and 28. Body length was measured from the tip of the rostrum to the end of the telson.

To measure the food consumption rate of normal shrimp as well as treated ones, concentrations of either 0.1, 0.2, or 0.4 mg Cd/l, or 0.05 or 0.6 mg Zn/l were used, in addition to the control set. Each treatment contained five shrimp, each of which was kept within a separate 1000-mL beaker with continuous aeration; each treatment was run in triplicate. During the experimental period, shrimp were fed commercial shrimp pellet feeds weighed 1/5 the weight of the animals twice a day, and the uneaten residual was removed after 2 h. All test solutions as well as control seawater were renewed every 3 days. Measurements were taken on days 1, 14, and 28. The food consumption rate (R, g/g body weight/day) for each measurement was calculated as follows: $R = \Delta F/W/T$, where R is the amount of feed (ΔF, g) consumed in the interval (T, 2 h or 0.08 days) by the individual with a wet body weight (W, g).

For examination of the biochemical parameters, *L. vannamei* individuals were exposed to concentrations of either 0.1, 0.2, or 0.4 mg Cd/l, or 0.05, 0.2, or 0.6 mg Zn/l, in addition to the control set, with each treatment containing at least 15 shrimp. Each treatment was repeated three times. Three shrimp in each treatment were taken on days 7, 14, and 28. On each occasion, shrimp were removed and sacrificed, then the hepatopancreas was dissected out, freshly weighed, and homogenized in 20 volumes of cold distilled water using a pellet pestle (Kontes Glass Company, Vineland, NJ, USA). Two 50-µl aliquots of each homogenate were dried in a preweighed glass jar at 80 °C for 24 h to determine the dry weight of the organ. After the homogenate was centrifuged at 10,000 x*g* for 20 min at 4

°C, the supernatant was used to examine the concentration of triacylglycerols, cholesterols, and total soluble proteins, which were determined using the Merck triacylglycerol and cholesterol diagnostic kits (Cat. nos. 1.14856.0001 and 1.14830.0001, respectively; Darmstadt, Germany) and a Sigma micro protein determination kit (procedure no. 610) (Sigma Chemical, St. Louis, MO, USA), while calculations were carried out using the Merck calibrator (Cat. no. 1.19720.0001). One-way ANOVA or Duncan's *t*-test was used to statistically determine the difference between treatments and controls.

RESULTS AND DISCUSSION

Both Cd and Zn caused very obvious growth retardation in *L. vannamei*, according to our results as shown in Fig. 1 and 2. Compared to the control shrimp, growth retardation of *L. vannamei* exposed to Cd was apparent after 14 days with both the 0.1 and 0.2 Cd/l treatments, and after 7 days with the 0.4 Cd/l treatment (Fig. 1). Zn showed milder effects on the growth of *L. vannamei* compared to Cd. Even though very significant growth retardation was only observed in shrimp exposed to 0.6 Zn/l for 7 days, the average body weights and lengths of shrimp exposed to 0.2 and 0.6 Zn/l were generally lower than those of control shrimp (Fig. 2). Scope for growth is a measure of the energy budget, that is, the difference between the energy absorbed from food intake and energy metabolized, and gives an indication of the metabolic condition of an organism (Rinderhagen et al. 2000). Moore and Farrar (1996) reported that growth rates significantly decreased with reduced food rations in the amphipod *Hyalella azteca*. It is very reasonable that growth retardation may result from a decrease in food intake. Furthermore, a decrease in feeding activity was also observed in another amphipod species, *Gammarus pulex,* exposed to the freshwater pollutants of copper, lindane, and 3,4-dichloroaniline (3,4-DCA) according to a review article by Rinderhagen et al. (2000). Therefore, after demonstrating growth retardation in *L. vannamei* exposed to heavy metals in this study, we further examined the effects on food consumption caused by Cd and Zn.

According to the results shown in Fig. 3, significant decreases in food consumption rates only appeared in treated shrimp exposed to 0.2 mg Cd/l for 28 days, while those of almost all other treatments showed no significant difference with the controls. Growth retardation caused by both Cd and Zn appeared much earlier than the appearance of a significant decrease in food consumption. Although animal size difference might be probably one of the causes, we believed there could have been other causes that resulted in the growth retardation in addition to the decrease in food consumption. Besides, food consumption rates of shrimp exposed to 0.05 mg Zn/l for 14 days showed a significant increase compared to the control. Since a trace amount of Zn is nutritionally essential for organisms like *L. vannamei* obtaining Zn not only from the diet but also from water, it may have positive effects on metabolism, tissue mineralization, growth, and food consumption (Davis and Lawrence, 1993).

Stuck et al. (1996) concluded that biological changes in the hepatopancreas, located in the head, reflect growth and development more than those of the total

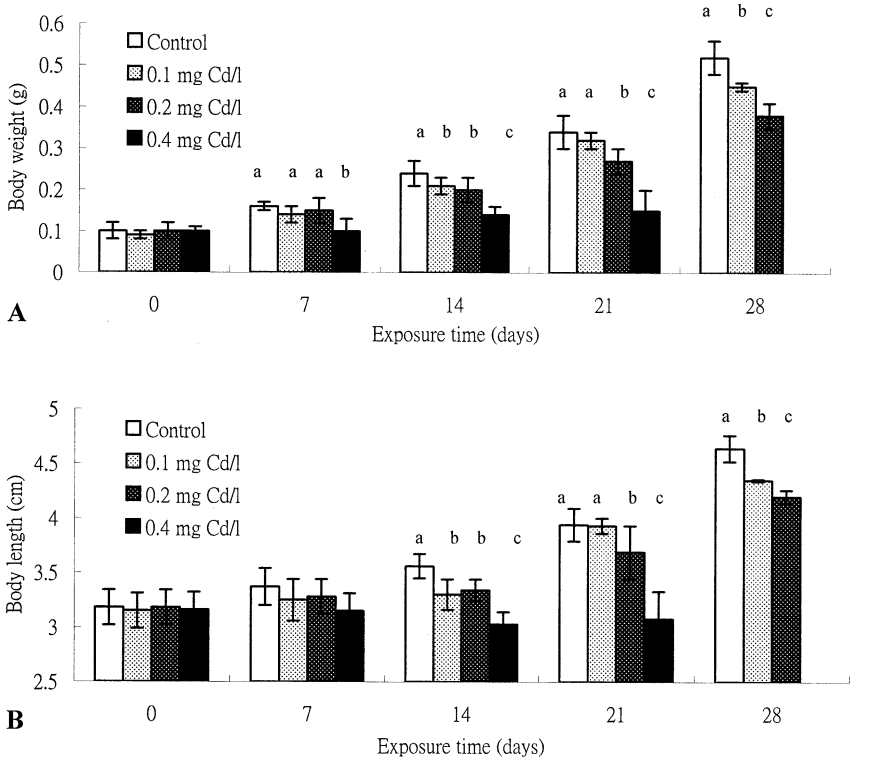

Figure 1. (A) Body weights and (B) body lengths of *Litopenaeus vannamei* exposed to different concentrations of cadmium for 28 days. Values are expressed as the mean ± S.D. of all measurements. Means with different letters significantly differ ($p<0.05$). Results of treated animals after exposure to 0.4 mg Cd/l for 28 days are unavailable since animals died due to metal toxicity.

body. After exposure to the heavy metals Cd and Zn, total soluble protein (TSP), triacylglycerol (TAG), and cholesterol levels in the hepatopancreas of *L. vannamei* changed with different behaviors as shown in Fig. 4. TSPs of all treated shrimps exposed to Cd and Zn for 7 and 14 days were higher than those of the controls (Fig. 4A). Detoxification enzymes, such as glutathione S-transferase (GST) and cytochrome p450, and metallothioneins (MTs) have been reported to be elevated in the liver or hepatopancreas of organisms exposed to heavy metals (Roesijadi 1992, James and Boyle 1998, Wright et al. 1998, Bainy 2000). However, after exposure for 28 days, TSPs of Cd-treated shrimps had decreased and were lower than those of the controls. Cytotoxicity has been shown towards hepatocytes exposed to cadmium (Koizumi et al. 1994). Therefore, the de novo synthesis of proteins in the hepatopancreas should be influenced, since there is a close relationship between the total amount of Cd and the reduction in protein synthesis of cells (Hogstrand and Haux 1991). In addition, morphological and functional alterations in the intestine were also reported in aquatic organisms treated with heavy metals (Crespo et al. 1986). Organisms utilize their protein reserves as energy sources during prolonged nutritional stress, when they are

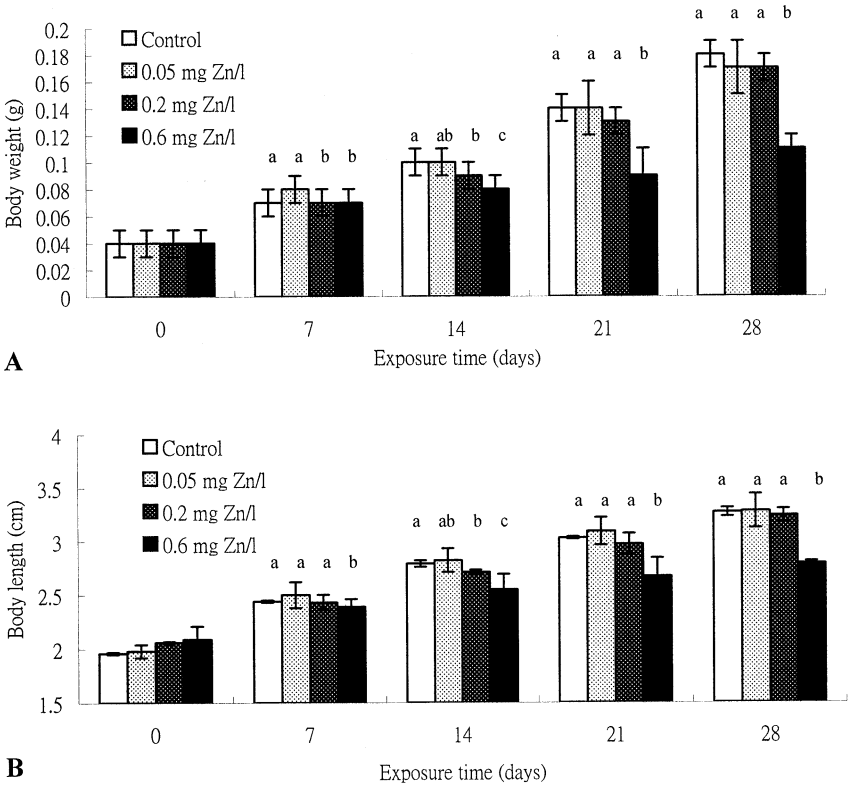

Figure 2. (A) Body weights and (B) body lengths of *Litopenaeus vannamei* exposed to different concentrations of zinc for 28 days. Values are expressed as the mean ± S.D. of all measurements. Means with different letters significantly differ ($p<0.05$).

limited from obtaining energy substrates from food intake (Stuck et al. 1996), and therefore the total protein level decreases. TSPs of 0.05 mg Zn/l-treated shrimp were higher than those of the control after exposure for 28 days. We consider that this was because the exposure dosage and time did not reach the threshold, and the hepatopancreas continued to synthesize de novo proteins as it did during short-term exposure. TSP levels of 0.2 and 0.6 mg Zn/l-treated shrimp after 28-day exposure showed no significant differences compared with those of the control.

TAG levels of treated *L. vannamei* were higher than those of control animals in the 0.4 mg Cd/l treatment for 7 days, in the 0.4 mg Cd/l and 0.2 and 0.6 mg Zn/l treatments for 14 days, and in the 0.2 mg Cd/l treatment for 28 days, while other treatments showed no significant differences compared to the control groups (Fig 4B). Generally, during acute short-term nutritional stress, normal shrimp rapidly utilize TAG reserves as their energy source (Stuck et al. 1996). However, when the liver or hepatopancreas, which possesses similar functions as the liver in crustaceans, is injured or damaged, synthesis of adequate apolipoproteins may be

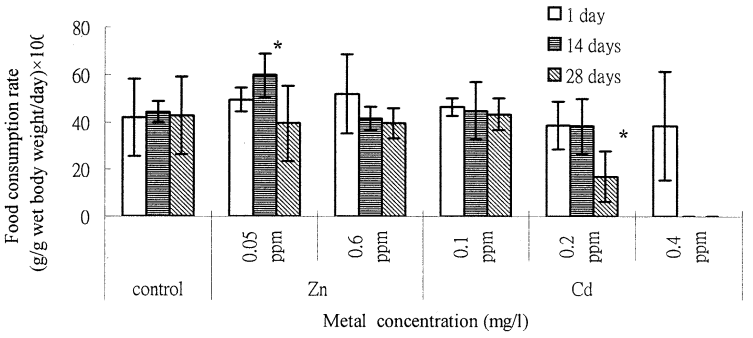

Figure 3. Food consumption rates of *Litopenaeus vannamei* exposed to different concentrations of Cd or Zn for 1, 14, and 28 days. Means with an asterisk "*" significantly differ from the control value ($p < 0.05$).

blocked. Once this happens, endogenously synthesized fat may accumulate in the liver because it is not being transported to peripheral tissues (Mathews and Van Holde 1996) and cannot be utilized as energy substrates.

It is obvious that Cholesterols levels within the hepatopancreas of treated *L. vannamei* were generally lower than those of control shrimp, especially with the 0.6 mg Zn/l treatment for 7 days, with the 0.2 and 0.4 mg Cd/l treatments for 14 days, and with the 0.2 mg Cd/l and 0.2 mg Zn/l treatments for 28 days ($p < 0.05$) (Fig 4C). Related studies on the effects of contaminants such as heavy metals on hepatopancreatic or liver cholesterol levels are very limited, and we do not have very clear evidence for the cause of the decreased cholesterol levels in the hepatopancreas of heavy metal-treated *L. vannamei*. However, based on the metabolism of cholesterol itself in animals, a decrease in the hepatopancreatic or liver cholesterol levels may result from some biochemical alterations that cause the normal balance to break down (Mathews and van Holde 1996). As mentioned, some heavy metals are cytotoxic to hepatocytes which might cause decreased production of low-density lipoprotein (LDL) receptors mediating the uptake of cholesterols from the blood into the liver, and thus limit cholesterol levels within the liver. Also, injury to the liver may trigger some repair mechanisms which may require a fairly large amount of cholesterol, since cholesterols constitutes 25% or more of the lipid content in cell and organelle membranes. If synthesis of high-density lipoproteins (HDLs) were also inhibited due to cell injury in the liver, the major function of HDL, which removes cholesterol from peripheral tissues and returns it to the liver, may also be limited.

In conclusion, we demonstrate that Cd and Zn cause growth retardation in *L. vannamei*, which might be related to the decrease in their food consumption rates as well as changes in nutritional conditions, since alterations in levels of or unavailability of biochemical and nutritional materials within the hepatopancreas were observed. These findings not only have significance on basic researches of environmental toxicology and crustacean biology, but also show that these parameters possess potentials to be developed as biomarkers to monitor the

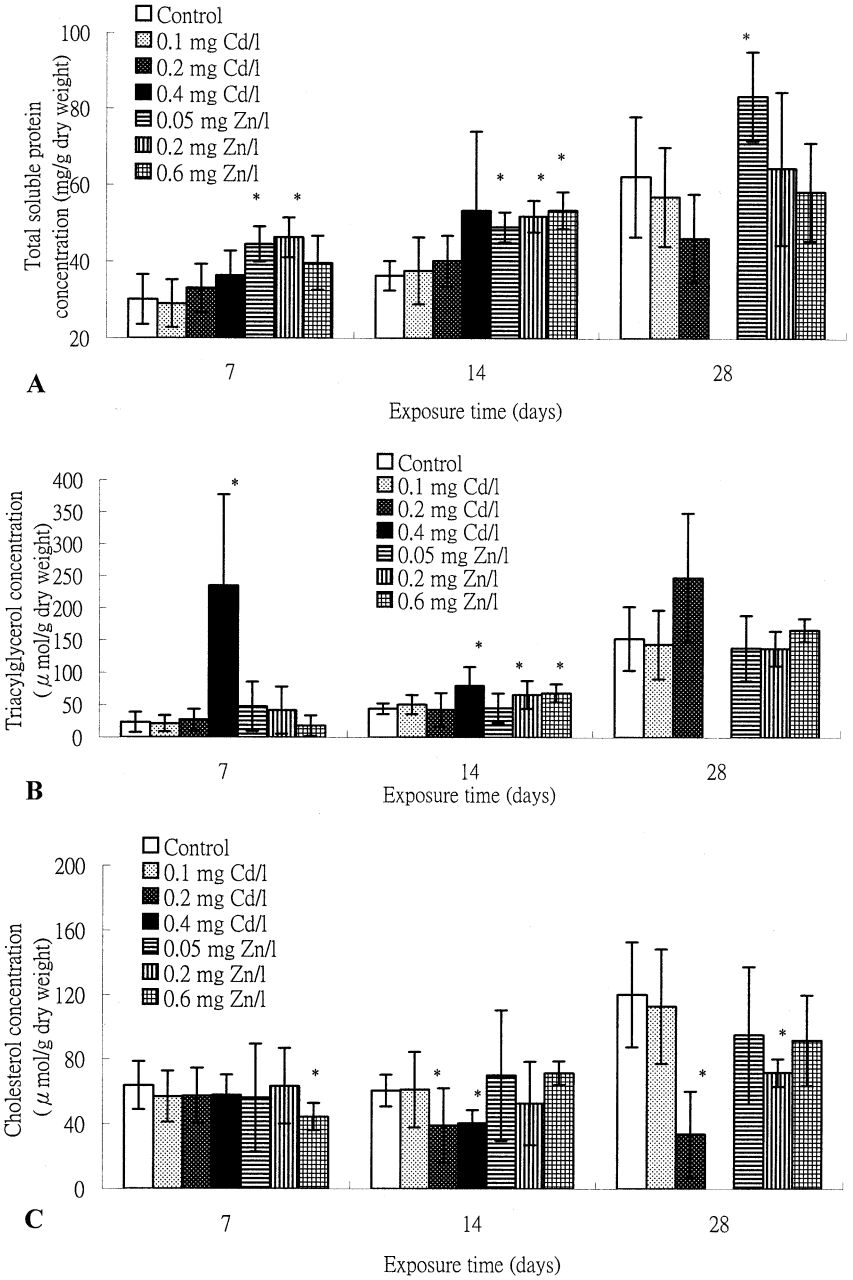

Figure 4. (A) Total soluble proteins (TSPs), (B) triacylglycerols (TAGs), and (C) cholesterol levels within the hepatopancreas of *L. vannamei* exposed to different concentrations of the heavy metals cadmium (Cd) and zinc (Zn) for 28 days. Mean values of treated groups with an asterick (*) significantly differ from those of control groups ($p<0.05$). Results of treated animals after exposure to 0.4 mg Cd/l for 28 days are unavailable since animals died due to metal toxicity.

existence of heavy metal contamination in aquaculture system.

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