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Larval Growth in Postlarvae of *Penaeus indicus* on Exposure to Lead

S. Chinni,[1] R. N. Khan,[2] P. R. Yallapragada[1]

[1] Department of Zoology, Andhra University, Visakhapatnam 530 003, India
[2] Department of Biology, Armstrong Atlantic State University, 11935 Abercorn Street, Savannah, GA 31419, USA

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Heavy metals are regarded as serious pollutants to marine ecosystems. Elevated concentrations in coastal regions result from natural and anthropogenic processes. Industrial effluents containing heavy metals are routinely released into commercial waterways. Such common practices warrant additional monitoring of anthropogenic agents and their effects on aquatic biota. A great deal of information is available on the effect of heavy metals and their toxicity on adult and juvenile shrimp (Bombang et al. 1995; Lin and Tin 1993). However, there is a limited amount of research work on growth rates of shrimp postlarvae (PL) in relation to metal exposure, although the PL are very sensitive to changes in the environment. Among adult individuals, Ahsanullah and Ying (1995) observed a decrease in growth of *Penaeus merguiensis* and *P. monodon* exposed to copper. The effect of cadmium on the growth of tiger shrimp *Penaeus monodon* was studied after 14 days of exposure and a loss of growth was reported by Karlson (1994). Liao and Hsieh (1990) noticed a reduction in growth of *Macrobrachium rosenbergii* exposed to three heavy metals, copper, cadmium and zinc. While studying the toxicity of heavy metals, Liao and Hsieh (1988) also observed growth effects in *Penaeus japonicus* when exposed to copper, cadmium and zinc. McClurg (1984) studied the chronic toxicity of fluoride, cadmium and mercury, and assessed their toxic effect by using growth of young *Penaeus indicus* as the criterion. The effect of copper on growth of juvenile *Penaeus indicus* was reported by Carmel et al. (1983). *Penaeus indicus*, one of the important commercial crustacean breeds in the backwaters and estuaries but some of these areas are being contaminated by heavy metals and lead is one of the major heavy metals reported (Sarma et al. 1996). Earlier studies on PL of *P. indicus* indicated a reduced metabolic rate when exposed to a sublethal concentration (1.44ppm) of lead (Chinni et al. 2000). In the present investigation, the growth rate of *P. indicus* PL was monitored in the laboratory by determining their length, wet and dry weights on sublethal exposure to lead.

MATERIALS AND METHODS

The postlarvae (PL) of *P. indicus* were collected from Gosthani estuary of Bheemunipatnam (Lat $19^0$54' N and Long $83^0$28' E), which is an unpolluted area but lies in close proximity to Visakhaptnam on the east coast of India. They were

Correspondence to: P. R. Yallapragada

acclimatized for 48 hrs to laboratory conditions at ambient salinity (20 ppt) and temperature (29 ± 1°C) prior to experimentation. The stock solution of lead was prepared by dissolving lead acetate [$(CH_3COO)_2$ Pb, $3H_2O$] (analytical grade) in distilled water. Random samples of similar sized PL (about 10mm) were exposed to test solutions in triplicate. Plastic tanks of 5 L capacity were used and 4 L of seawater (20 ppt) was added to each tank containing 150 to 200 PL. Appropriate amounts of stock solution were added to each tank in order to get a sublethal concentration of 1.44ppm which is $1/5^{th}$ of the LC_{50} value for 96 hrs (Satyavathi 1999). PL were subjected to test solution for a period of 30 days. Parallel controls were run without the toxicant. Sampling was done for both exposed and control at intervals of 24hrs, 48hrs, 96hrs, 10days and 30days. At each interval, 30 individuals (intermoult) were isolated and their total length (from the anterior tip of the rostrum to posterior tip of the telson) and wet weights were taken. Thereafter, the animals were dried individually in an oven at 70°C for 48 hrs and their dry weights were also recorded. The test solutions were renewed daily at noon and the PL were fed with commercial larval feed (Lux Water Base, Nellore, India) twice a day (10:00 and 16:00hrs) based on 20% of body weight per day. Uneaten food particles were discarded daily around the same time when the test solutions were renewed. The total length was measured with a mm scale, and a digital balance of 0.1mg sensitivity was used for determining the wet and dry weights. Filter paper was used to remove water adhering to the PL before determining wet weights. The regression (b) and correlation (r) coefficients for length and weight were calculated by linear regression (Sokal and Rohlf 1995).

Growth indices were calculated using the respective dry weights of control and exposed PL and the formula described by Winberg (1971) was followed. The mean specific rate of growth was calculated by the equation:

$$g = (\ln W_{n+1} - \ln W_n) / t$$

where g = the mean specific rate of growth; W_{n+1} = the mean dry weight of PL at a particular interval (n+1); W_n = the mean dry weight of PL at the beginning (n) and t = the number of days between n and (n + 1). The specific rate of growth was incorporated into the formula:

$$G = (e^g - 1)$$

to quantify the fractional weight gain (G) for the PL under control and exposure conditions. G was multiplied by the replicate dry weights to produce values of daily weight gain (WG) for PL at different time intervals. Growth rates (WG) of control and lead exposed PL were used to produce values for daily production of energy by incorporation in to the formula:

$$P = (WG \times TC) + 0.10\ (WG \times TC)$$

where P = the gross daily energy production in Joules; WG = daily weight gain ($\mu g\ day^{-1}$); TC = the mean caloric values for dry tissue of PL of shrimp (25.69 x 103 Joules per µg dry weight) (Anderson 1977); and 0.10 (WG X TC) = a correction factor for the average energy loss for marine crustaceans by shedding

of exuvia (McKenney 1982). Daily maintenance energy was determined from the formula:

$$R = VO_2 \text{ X } 24 \text{ X } W \text{ X } OC$$

where R = daily expenditure in Joules for maintenance; VO_2 = the respiration (μg O_2 mg^{-1} dry wt hr^{-1}); and OC = the calorific coefficient in Joules for carnivores that utilize ammonia as their chief excretory product (13.556 X 103 J μg^{-1} O_2) (Elliot and Davison 1975). Net growth efficiency (K_2) (Winberg 1971) was derived by the formula:

$$K_2 = P / (P + R)$$

where K_2 = the net growth efficiency; P = daily production in Joules and R = the daily expenditure in Joules for maintenance. All the values are presented as mean ± standard deviation. Statistically significant differences between control and exposed larvae were calculated by Student's 't' test (Sokal and Rohlf 1995).

RESULTS AND DISCUSSION

The sublethal effect of lead on total length, and wet and dry weights of PL of *P. indicus* is shown in Figure 1. Initially, the growth was not significantly different ($P>0.05$) between control and exposed PL at 24, 48 and 96 hrs. Thereafter, a significant ($P<0.05$) decrease in the length and wet weights was observed from the 10^{th} day onwards. Additionally, a significant decrease in dry weight was noticed in PL exposed for 30 days. For 10 days exposure, the percent decrease was in the order of wet weight (13.5%)>total length (10%)>dry weight (4%). For the 30 day period, the order of decrease was changed to wet weight (55%)>dry weight (39%)>total length (30%). Several investigators reported similar reduction in growth on toxic exposure of PL, juveniles and adult prawns. Chen and Tu (1991) observed a reduction in weight (72%) and length (48%) in the PL of *P. monodon* exposed to ammonia for 8 weeks. Chen and Lin (1992) reported a reduction of 40-60% growth at lower concentrations of ammonia (2mg L^{-1}). Chen and Lin (1992) noticed a growth inhibition of 64-68% in *Penaeus pencillatus* juveniles on exposure to 20mg L^{-1} ammonia for 60 days. Ahsanullah and Ying (1995) reported that 25 μg Cu L^{-1} resulted in a loss of 10% growth over a 2 week period in *Penaeus merguiensis*. Chen et al. (1996) observed that after 36 days of exposure to saponin, the weight of *Penaeus japonicus* was significantly reduced at higher (1-2mg L^{-1}) than lower (0.1-0.5mg L^{-1}) concentrations. In *Penaeus monodon*, growth was reduced by 4.4% in low concentrations of ammonia (3ppm) whereas at higher concentrations (6ppm), 65% inhibition was observed (Noor et al. 1994). Exposure to the sublethal concentration of lead reduced the growth efficiency in postlarvae. In exposed PL, the values of net growth efficiency (K_2) were found to decrease significantly ($P<0.05$) when compared with their respective controls at all time intervals (Figure 2). The daily weight gain showed an increase with increasing time both in control and exposed PL (Figure 3) and the increase was greater in control than exposed PL. At all intervals, the daily weight gain of exposed PL was significantly ($P<0.05$) lower than their respective controls. However, the maximum decrease (almost 3 times) was noticed at 30 days for exposed PL.

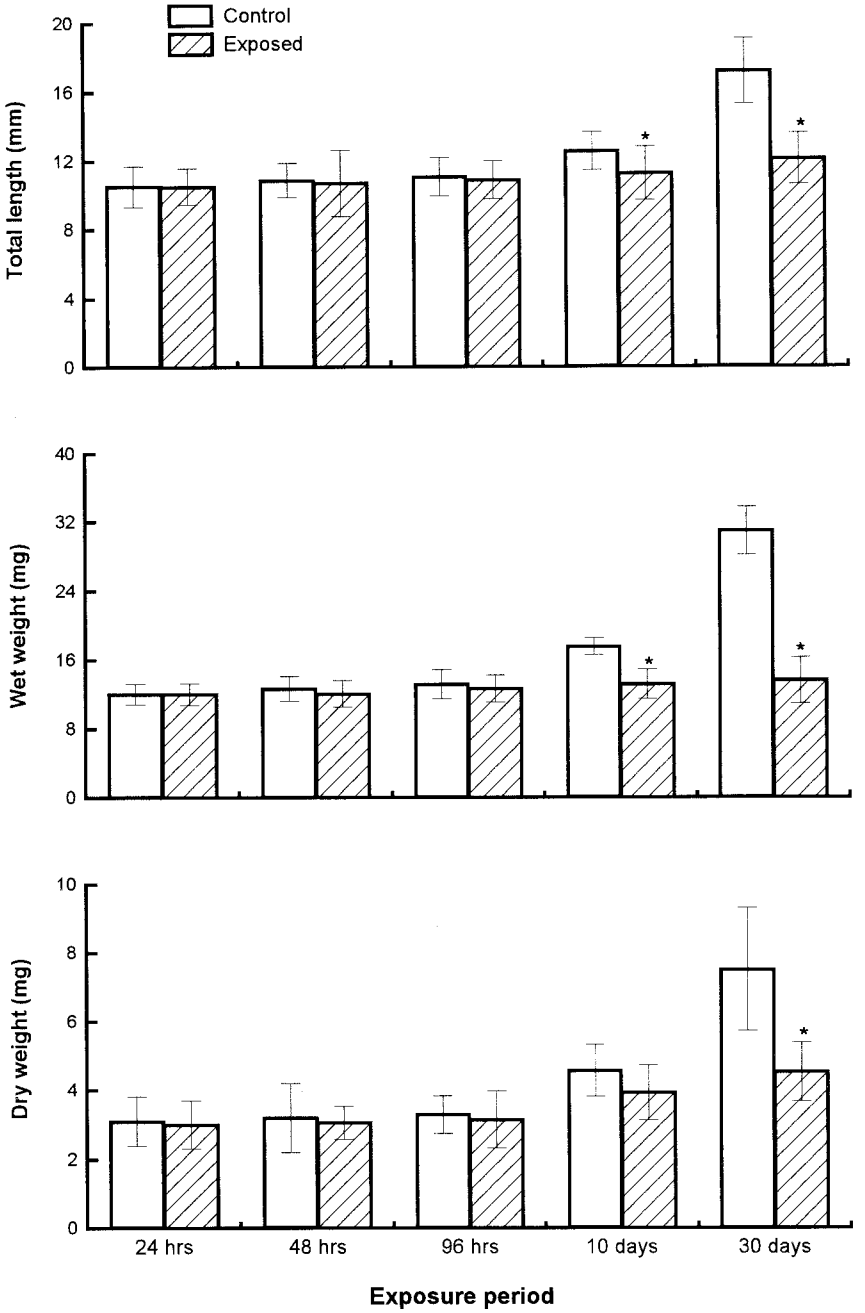

Figure 1. Total length, Wet and Dry weights of *P. indicus* PL exposed to sub-lethal concentration of lead. The vertical lines represents standard deviation and * represents values significantly different from their respective controls at $P<0.05$, N=30.

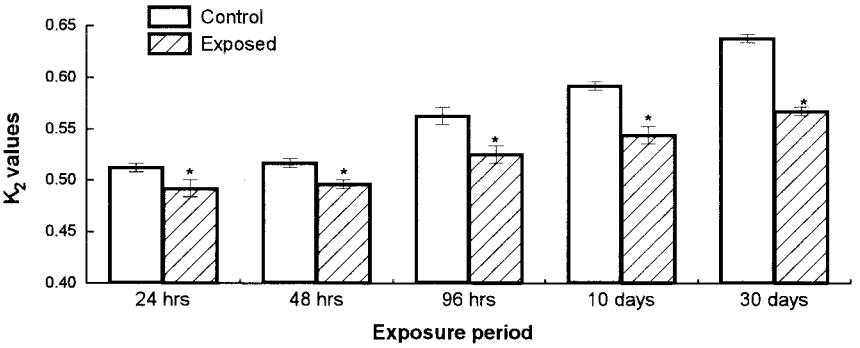

Figure 2. K_2 values in control and exposed PL to sublethal concentration of lead. The vertical lines represent standard deviation and *represents values significantly different from their respective controls at P<0.05, N=30.

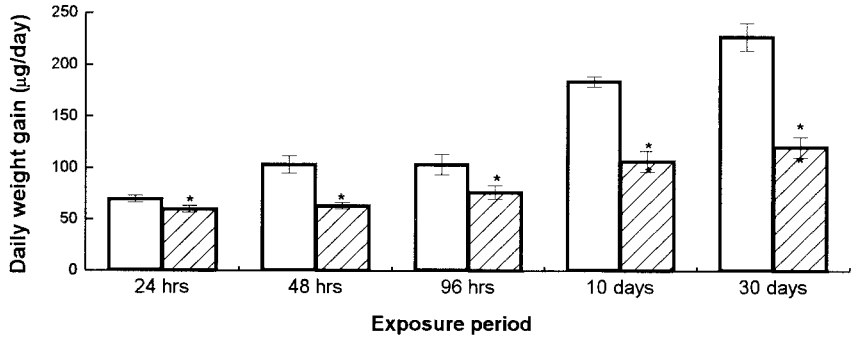

Figure 3. Daily weight gain in control and exposed PL to sublethal concentration of lead. The vertical lines represent standard deviation and *represents values significantly different from their respective controls at P<0.05, N=30.

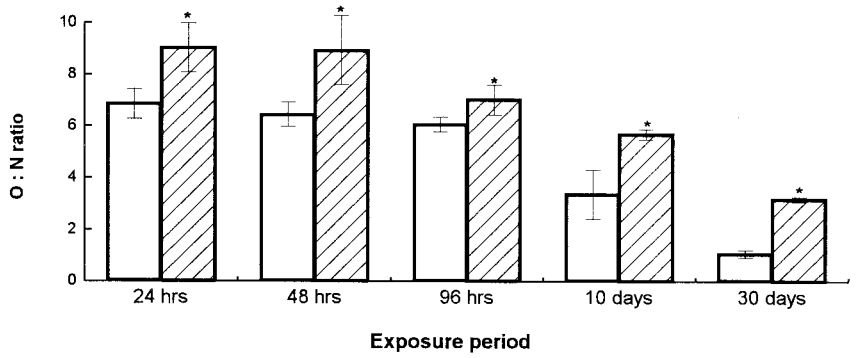

Figure 4. Oxygen : Nitrogen ratios in control and exposed PL to sublethal concentration of lead. The vertical lines represent standard deviation and *represents values significantly different from their respective controls at P<0.05, N = 30.

The O:N ratios for control and exposed PL are presented in Figure 4. In control PL, the O:N ratios showed a decreasing trend with time indicating a shift from greater utilization of lipid substrates to more protein usage as has been observed in *Homarus americanus* (Capuzzo and Lancaster 1979), *Hyas araneus* (Anger 1986) and *Palaemonetes pugio* (McKenney and Celestial 1993). A similar trend was noticed in exposed PL but the ratios were found to be significantly ($P< 0.05$) higher when compared with their respective controls at all intervals. This might be due to the interaction of lead with protein metabolism (Satyavathi 1999). These energetic imbalances might have been reflected in the decreased daily weight gain (WG) and reduced net growth efficiency (K_2 values) of *P. indicus* PL exposed to lead.

The influence of lead on percent water content (difference between the wet weight and dry weight multiplied by 100 and divided by wet weight) was determined in PL at different time intervals and the PL showed a gradual decrease with length of exposure (Table 1). However, a significant ($P>0.05$) decrease in percent water content was noticed only at 30 days exposure. According to Rasmussen et al. (1995), one hour exposure to 1mg/L mercury and lead significantly decreased the apparent water permeability (movement of water through membrane) of the shore crab *Carcinus maenas*. A similar but not significant tendency was also observed following exposure to cadmium, copper and zinc. The influence of zinc (500 ug/L) on apparent water permeability of the brown shrimp *Crangon crangon* was also investigated by Rasmussen et al. (1995) and after 24 hrs of exposure to zinc, the apparent water permeability decreased significantly. Rasmussen et al. (1995) concluded that the inhibition in apparent water permeability was due to toxic effects of the metals or it may represent physiological adaptations to minimize the effects of perturbations. This might be the reason for the decrease in the percent water content of exposed PL.

Table 1. Effect of sublethal exposure of lead on percent water content in PL of *Penaeus indicus*.

Exposure period	Percent Water Content	
	Control	Exposed
24 hrs	72.75 ± 3.4	71.53 ± 3.4 (1.68)
48 hrs	74.40 ± 2.8	71.89 ± 4.2 (3.37)
96 hrs	74.40 ± 3.5	72.96 ± 3.8 (1.94)
10 days	71.85 ± 4.0	68.48 ± 3.2 (4.69)
30 days	72.30 ± 4.2	62.65 ± 2.8 (13.3)*

Each value represents the mean ± standard deviation. The values in the parenthesis represent percent decrease over respective control and * represents the values significantly different from their respective controls at $P<0.05$, N=30.

Comparisons of linear regression coefficients for total length versus wet and dry weights of control and exposed PL are presented in Table 2. The regression coefficient values for total length versus wet weight of PL was greater for control than exposed PL while a reverse relationship was observed for dry weight.

The present investigation clearly demonstrates that lead negatively affects the growth of PL in *P. indicus*. As recent reports indicate an increase in heavy metal concentrations, including lead, in the coastal and estuarine environments along Visakhapatnam (Sarma et al. 1996), it would be interesting to extend this study beyond 30 days to document further evidence in the shrimps' growth pattern. Furthermore, these findings may encourage other investigators to examine the possible detrimental effects of other heavy metals on the survival, growth, and development of these crustaceans.

Table 2. The details of exponential relation between length and weight in control and 30 days exposed PL of *Penaeus indicus*.

	Regression coefficient	Intercept	Correlation coefficient
Wet weight			
Control	1.9457	-0.9073	0.9986
Exposed	0.9305	0.1301	0.9384
Dry weight			
Control	1.8801	-1.4351	0.9942
Exposed	3.2769	-2.8774	0.9729

Experimental protocol of lead exposure is given in Materials and Methods.

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