The Effect of Temperature on the Uptake of Copper and Cadmium by Penaeid Shrimp

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Summary: Penaeid shrimp (*P .monodon* and *P. penicillatus*) among dominant cultured shrimps were analysed to assess the uptake potentialities of copper and cadmium at different temperature. It is observed that the survival is directly proportional to the intensity of temperature. Uptake of Cu at 25 °C on 100 μg/l exposure was to be 2.34-2.60 μg/g dry wt. and Cd was 1.56-1.65 μg/g dry wt. The concentrations of these metals determined by AAS at 30 °C and 40 °C were 8.45 μg/g and 7.20 μg/g for Cu and 7.25 μg/l and 6.27 μg/g of dry wt for Cd in *P. monodon*. The same amount was determined in *P. Penicillatus* respectively. The uptake of these metals increased with the increase in temperature and gradually variable at every larval stage of both species depending upon size of these specimens. The survival rate was found to be directly proportional to uptake of metals with increased toxicity of copper and cadmium.

Introduction

It has been recognized that heavy metals in the marine environment (such as Cd, Cu and Pb) have a particular significance in the eco-toxicology, since they are highly persistent and can be toxic in traces [1].

Temperature strongly influences the survival, growth and metal uptake of marine organisms [2]. Many marine invertebrates even require specific temperature at different developmental stages. There are several biotic factors, which may influence copper and cadmium toxicity causing changes in the survival of marine organisms. The most important of these factors are the type of organisms and stage of development. The effect of temperature has already been documented in embryonic cleavage, larval development and growth [3]. But there is no comprehensive study to elaborate the effects of temperature on each specific larval stage of shrimp with reference to toxicity of copper and cadmium. Crustaceans are generally regarded as being more sensitive to any metal than other groups such as fish or molluscus [4]. The purpose of the present study is to examine the uptake of copper and cadmium at different temperatures and the effects of altered temperatures on the survival of each larval stage of both species of penaeid shrimp.

Results and Discussion

There are very few post larvae which remained alive at 10 °C however, all nauplii, zoea and M1 died within 4 h at 10 °C. The 48 h LC_{50} value was obtained at 20 °C and there is little variation in mortality after 24 h and 48 h at 20 °C which indicate that survival of all larval stage shrimp is different significantly among temperatures, [5] (Fig. 1, $P < 0.01$). The effect of temperature varied

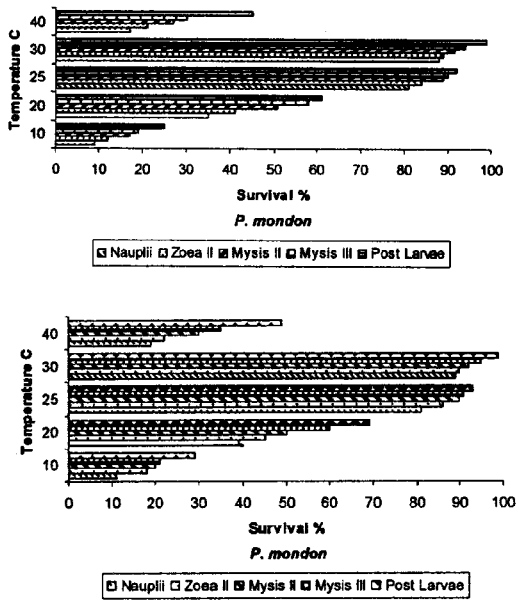

Fig. 1: Effect of Temperature on survival of penaeid shrimp.

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with the concentrations of metal. Maximum survival (98. 8 %) occurred at 25 °C and 10 μg/l concentration of Cu as well as Cd at all stages of larvae, curves for nauplii, zoea, mysis and postlarvae are given (Figs. 2 and 3) as representative pattern of uptake. Metal contents have been expressed as microgram cadmium and copper per gram of dry weight of every larval stage. The uptake pattern of Cu and Cd was considerably different in both of species *i.e. P. monodon* and *P. penicillatus*, and uptake amount determined by atomic absorption spectrometry was found to be directly proportional to the temperature. As temperature rose from 20 °C to 30 °C the accumulated amount also increased but at 10 °C and 40 °C results are quite upset because no sequence was observed. In all experiments uptake of either cadmium or copper in each stage is approximately linear with time. Higher copper and cadmium concentration were found at higher temperatures *i.e.* 35-40 °C. Therefore, mortality was also observed to be increased along with temperature. Analysis of variance (ANOVA) shows that there is no significant difference between regression lines for 25 °C and 30 °C and those for 20 °C and 30 °C are significantly different. At 100 μg/l concentration, and temperature 30 °C, the rate of uptake is approximately 2 times greater than that at 20 °C. All larvae did not show any significant differences in their uptake of copper and cadmium, when temperature maintained at 25 °C.

Effect of concentration level on metal uptake by larval stage:- Uptake of metal by nauplii, zoea, mysis and postlarvae had been plotted in Figs. 2 and 3. The uptake of copper and cadmium was greater at higher temperature and higher concentrations, but some time this principle was not exactly followed The uptake in average at 20 μg/l being about 3 times lesser than that at 200 μg/l (Tables-1 and 2 for both species). Nauplii represents increased uptake amount of cadmium as compared to that determined in zoea and mysis. In control samples certain amount of cadmium and copper was also determined by AAS even without exposure of these metals (Tables-1 and 2). Larvae had a narrow range of dry weight and then varied greatly in dry weight as they grew (Table-3). Variation in final weights tended to be greater at higher temperatures [6]. In all experiments the Lc50 value increased by about 55 % for each 10 °C water temperature increment.

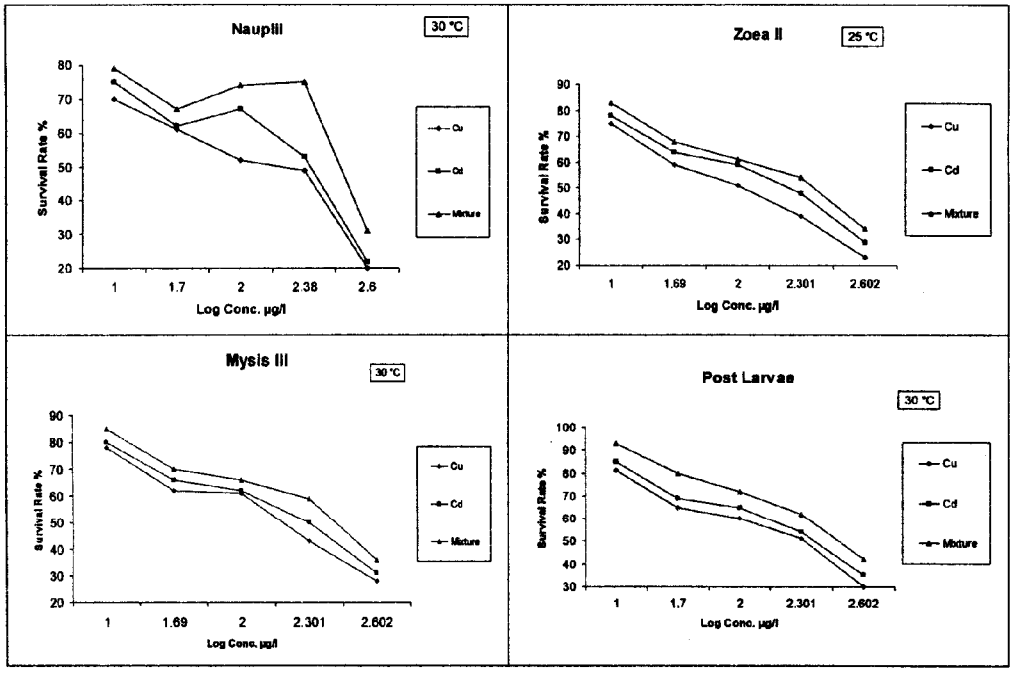

Fig. 2: Effect of exposed metal concentration on survival rate of *P. monodon* at various temperatures.

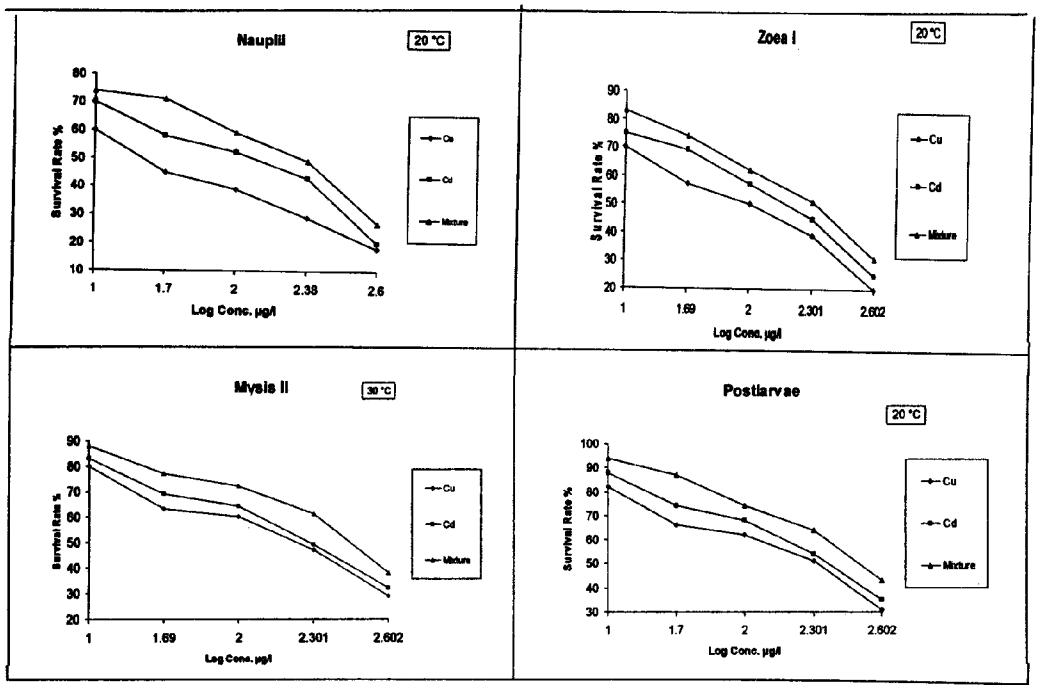

Fig. 3: Effect of exposed metal concentration on survival rate of *P. penicillatus* at various temperatures.

Table-1: *P. mondon* mean uptake amount of Cu and Cd determined by AAS at each larval stage under various temperatures after exposure at different concentrations.

S. No.	Larval Stage	Temperature °C	Exposed metal and Conc. µg/l		Uptake amount determined by AAS (µg/g) Cu	Cd
1.	Nauplii	20	Cu	100	2.3451	1.2412
2.	Nauplii	25	Cd	10	1.5627	3.5906
3.	Zoea I	nd	-	-	-	-
4.	Zoea II	30	Cu	200		
5.	Zoea III	nd	-	-	-	-
6.	Mysis I	25	Cd	10	0.5130	1.5530
7.	Mysis I	40	Mix	100	0.3966	0.1578
8.	Mysis I	30	Cd	100	-	1.8772
9.	Mysis I	25	Cu	200	0.2985	0.0918
10.	Mysis I	25	Mix	200	6.5200	0.6800
11.	Mysis I	25	Cd	10	0.3017	0.1336
12.	Mysis I	25	Cu	400	5.3913	1.9130
13.	Mysis I	30	Cd	20	1.5761	0.3095
14.	Mysis III	25	Cd	100	1.3544	3.7088
15.	Mysis III	25	Mix	10	31.034	2.7580
16.	Mysis III	25	Cu	400	0.2654	0.0442
17.	Post Larvae	40	Cd	200	0.4676	0.2148
18.	Post Larvae	25	Cd	10	2.4753	0.3477

Among several abiotic factors which affect the biotoxicity of heavy metal, the temperature is an important one. Response of estuarine animals to Cd pollution was reviewed and concluded that acute Cd toxicity is strongly modified by temperature [3]. Similarly the effects of temperature and salinity on the toxicity of Cu to marine and esturine invertebrates were also reviewed [7]. Temperature directly affected the cellular and sub-cellular processes and indirectly affected the organisimal activity through effects on feeding [8]. Since penaeid larvae, when newly metamorphosed and they start to feed at stage of zoea then both environmental and genetic variability affect their growth and consequently the amount of variation in size increased. The results of the present study indicate that as temperature of water raised the uptake amount of copper and cadmium also increased. The same findings was recorded by M. Fabiano and *et al.* [4]. It has been documented that in general, increase in temperature increase the toxicity and bio-accumulation of Cd in marine biota. The optimal temperature was found 25-30 °C for the maximum survival and growth of all larvae. In other words, only a few larvae were able to survive at 10 °C without consideration of exposed concentration of either copper or cadmium. Since during all experiments salinity was kept constant *i.e.* 30 °C no interaction was observed as temperature raised because it was expected that increase in uptake along with temperature may be balanced by increase in salinity [9]. At

Table-2: *P. penicillatus* mean uptake amount of Cu and Cd determined by AAS at each larval stage under various temperatures after exposure at different concentrations.

S. No.	Larval Stage	Temperature °C	Exposed metal and Conc.	μg/l	Uptake amount determined by AAS (μg/g) Cu	Cd
1.		25	Cd	100	7.7824	3.8075
2.	Nauplii	20	Cu	100	2.3414	0.4567
3.	Nauplii	30	Cu	200	1.1544	0.0795
4.	Nauplii	40	Cu	200	3.500	9.350
5.	Nauplii	25	Cd	100	2.073	0.5129
6.	Nauplii	25	Mix	400	0.5191	2.0400
7.	Nauplii	40	Cd	10	6.8530	1.1880
8.	Nauplii	25	Cd	10	0.1070	0.0026
9.	Nauplii	30	Con	-	19.342	1.0526
10.	Zoea I	40	Cu	20	40.7612	0.4013
11.	Zoea I	30	Cu	100	0.3269	0.0336
12.	Zoea I	30	Con	-	7.0202	1.5151
13.	Zoea III	25	Cu	10	2.600	-
14.	Zoea III	40	Cu	20	1.7149	0.2657
15.	Mysis I	20	Mix	400	4.3089	0.4092
16.	Mysis I	ND	-	-	-	-
17.	Mysis I	30	Cu	20	0.8750	0.0400
18.	Mysis I	30	Cu	100	0.3278	0.0102
19.	Mysis II	30	Cd	20	1.6533	0.3247
20.	Mysis II	20	Cu	100	4.0140	8.2660
21.	Mysis II	30	Cu	200	2.0730	0.5129
22.	Mysis II	40	Cd	200	0.3773	0.6839
23.	Mysis II	30	Mix	20	0.272	0.0336
24.	Mysis II	40	Cd	20	0.3062	0.2824
25.	Mysis III	40	Cu	10	9.8122	1.4319
26.	Mysis III	30	Cu	100	1.200	0.6200
27.	Mysis III	30	Mix	10	46.2130	2.0118
28.	Mysis III	30	Mix	100	0.3956	0.0775
29.	Post Larvae	30	Mix	100	0.3578	-
30.	Post Larvae	40	Cd	10	0.6037	0.2558
31.	Post Larvae	20	Cu	10	0.5171	0.3250
32.	Post Larvae	40	mix	400	0.0617	0.1574

Table-3: Mean dry weight and standard deviation (+ SD) in grams of total weight of *Penaeus Penicillatus* and *Penaeus monodon* with hours of duration of exposure in the laboratory.

Larval Stags	Mean dry weight (g)	No. of specimen	Mean hours of exposure
Nauplii	0.0679 ± 0.34	50	24
Zoea I	0.1018 ± 0.45	40	20
Zoea II	0.1308 + 0.10	40	26
Zoea III	0.1809 ± 0.83	40	28
Mysis I	0.2094 ± 0.69	45	24
Mysis III	0.3632 ± 0.69	30	30
Post Larvae	0.4042 ± 0.69	20	36

40°C maximum accumulated amount was determined by AAS, at the same time lowest survival was observed that showed increased copper and cadmium toxicity as temperature increased. Similar results were reported by many scientists in a variety of marine organisms such as crab and prawn by Theeda *et al.*, and vern berg *et al.* [10, 11]. During present study quite delay development is observed from nauplii till postlarvae, this observation indicates the influence of temperature on the development of penaeid shrimp. By lowering the temperature the survival increases along with lower concentration of exposed metal. Thus it can be stated that lower temporary by reducing toxicity of copper and cadmium may promote a temperatory increase in population density. At 10 °C, mortality of nauplii and zoea was found higher than that of larger size larvae such as mysis and postlarvae, it may not be due to toxicity of metals but due to cold environment in experimental chambers. The present investigation also revealed the effects of temperature on the duration of development. An alternation in the temperature (of the surrounding water) during every exposed concentration experiment also has several physiological effects such as uptake of metal ions, which changes the chemical forms of Cu. It has been observed that uptake (of metal) amount by larvae was not directly proportional to exposed concentration of metal which may be due to regularity of these metals during exposure period. Typically metal uptake rates are beyond the short-term physiological control of individual invertebrates carrying out metal excretion rates. Which can be evident by the regulation strategies may come from laboratory experiments [12].

Experimental

Samples of nauplii, zoea, mysis and postlarvae were collected from a hatchery. All specimens were maintained in sea-water having salinity 27.65°/∞, and allowed to acclimatize for at least 2hours prior to an experiment. Metal solutions were prepared in deionized water from $CuSO_4.7H_2O$ and $CdCl_2$. All of the experiments were carried out with 50 nauplii, 40 individual of Zoea and Mysis and 20 specimens of postlarvae in a 600 ml beaker, containing 500 ml of aerated, filtered sea water with metal solutions of copper and cadmium of concentrations varying between 10 μg/l up to 400 μg/l and their 1:1 mixture at different salinities of 10, 20, 30 and 40. Lower salinities were obtained by dilution with fresh water and higher salinity was achieved by adding sodium chloride by distributing among various temperature (temperature range was 10°C, 20°C, 30°C and 40°C). After completion of each successive larval stage, individuals were removed, blotted and dry weights obtained by oven drying at 80°C for a period of one hour in tarred glass counting vials and for preparation of AAS analysis.

Sample Preparation for AAS

All specimens were digested in closed glass ampoules, using extra pure nitric acid, perchloric acid and hydrogen fluoride (HNO_3:$HClO_3$:HF) in 2:1:2

ratio. These ampoules were placed in a sand bath and heated initially at 90 °C for 2 hours and then at 140 °C for 4 hours. Cooled digests were then diluted with de-ionized (Milli-Q) water to prepare solutions of about 3N HNO_3 for analysis of copper and cadmium by atomic absorption spectrophotometer (AAS). The cadmium was analyzed by graphite furnace AAS, and copper by flame AAS. Blanks and standard reference materials IAEA-140/TM were run along with samples. In addition to the determination of metal concentrations by AAS in specimens of penaeid shrimp, their dry and wet weights, and effect of salinity of water medium on their survival was also investigated.

Statistical Analysis

LC_{50} values were calculated using probit analysis. One way ANOVA was used to compare the effect of temperature on uptake of Cu and Cd in their mixtures as well as t-test was used for comparison between means of each exposure experiments for determination of significance results. In order to determine interactive effects of temperature and survival rate two way analysis of variance was applied. The EC_{50} value, the temperature at which each larval stage survival was reduced up to 5 % was supposed to be minimum acceptable temperature linear regression was used to investigate the relationship between temperature and exposed concentration of metals at each specific temperature.

Conclusion

In conclusion, the optimum temperature for lesser uptake of Cu and Cd causing lesser toxicity and having maximum survival rate temperature was noted as 25 °C. As the temperature raise after 25°C up to 40°C the uptake amount was also increased and that had shown increased toxicity of copper and cadmium. The results for uptake of copper and cadmium during exposure in form of mixture can not be fully explained because it is supposed that in this sequence further detailed experiments are required to completely elucidate the exact mechanism of these two metals in mixture.

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