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Studies of Cadmium Tolerance in Two Populations of Grass Shrimp, *Palaemonetes pugio*

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Several investigators demonstrated that tolerance to pollutants can be increased by previous exposure to low sublethal concentrations. Saliba & Krzyz (1976) reported that pre-exposure of brine shrimp (*Artemia salina)* to low levels of copper increased their tolerance to higher levels of copper. Likewise, LeBlanc (1982) demonstrated that *Daphnia magna* pre-exposed to copper, lead or zinc became more tolerant to the toxic effects of these metals.

Acquired tolerance to metal can result from increased synthesis of metallothioneins. Metallothioneins are small (6000-7000 daltons), sulphydryl-rich, metal binding proteins, whose free thiol groups readily bind the heavy metals (Olafson et al. 1979). The purpose of the present study was to investigate the effect of pre-treatment with low doses of cadmium chloride (Cd) and mercuric chloride (Hg) on tolerance to higher levels of cadmium to two populations of grass shrimp, *Palaemonentes pugio*. One was from Piles Creek (PC), a tributary of the Arthur Kill in heavily industrialized Linden, New Jersey. The other population was from Big Sheepshead Creek (BSC), a relatively pristine system near non-industrialized Tuckerton, New Jersey. Piles Creek sediment metal concentrations have been reported to be 11.2, 5.9, 623.5, and 627.9 ug/g of Hg, Cd, Cu, and Zn, respectively; whereas BSC sediment contained 0.054, 0.13, 12.9, and 7.7 ug/g of Hg, Cd, Cu, and Zn, respectively (Khan et al. 1987).

MATERIALS AND METHODS

Adult shrimp were caught during the summer of 1986 by using minnow traps in Piles Creek (PC), Linden, New Jersey, and by using seine nets in Big Sheepshead Creek (BSC), Tuckerton, New Jersey. The salinity in these areas varied between 20 and 25 ppt. Shrimp were brought back into the lab and acclimated for one week in 20 ppt artificial sea water (Instant Ocean, Carolina Biological Supply Company, Burglington, North Carolina) and a light:dark cycle of 14:10 h. After this period of acclimation, shrimp were exposed to

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sublethal concentrations of 0.05 mg/L Cd (as $CdCl_2$) (Fisher Scientific, Sprinfield, New Jersey) or 0.02 mg/L Hg (as $HgCl_2$) (Fisher Scientific, Springfield, New Jersey) for a week to determine if such pre-exposure would stimulate physiological mechanisms which would increase the tolerance of the shrimp to higher levels of Cd. During the acclimation period, those shrimp that molted and those groups which had more than 2% mortality were not used for the experiments. Shrimp were fed ground Purina "trout chow" and were maintained in glass aquaria containing 8 L of sea water at room temperature (23-25^{o}C). The water was changed and redosed daily during the acclimation and pre-exposure periods. After 1 week of pre-exposure, groups of 25-35 shrimps and comparable groups not pre-exposed were exposed to 0, 1.0, 3.0, 5.0, and 10.0 mg/L Cd. All treatments were diluted to the appropriate concentrations by mixing 20 ppt sea water with known stock solution of Cd. In each treatment, five shrimp were held in a plastic (Nalgene) tray containing 2 L of appropriate solution for 96 h. Each treatment was replicated 5-9 times. Shrimp were not fed during the 96-h treatment period, but were checked daily and dead individuals removed. LC_{50} values and 95% confidence limits were calculated on these data using Probit analysis (Huber 1984). The resulting LC_{50} values were used to calculate a resistance factor (RF) for the pre-exposed shrimp.The RF was calculated by dividing the LC_{50} value for the pre-exposed shrimp by the LC_{50} value of the shrimp not pre-exposed. The RF was used to quantitate the degree to which the pre-exposed shrimp had developed resistance (LeBlanc 1982).

RESULTS AND DISCUSSION

The result of acute lethality tests conducted with shrimp pre-exposed to 0.05 mg/L Cd and 0.02 ppm Hg for 1 week, and shrimp not pre-exposed, are presented in Table 1. The LC_{50} values of PC shrimp were 3.28, 6.81, and 2.63 mg/L Cd for not pre-exposed, Cd pre-exposed, and Hg pre-exposed, respectively. LC_{50} values of BSC shrimps were 1.83, 3.89, and 1.68 mg/L Cd for not pre-exposed, Cd pre-exposed, and Hg pre-exposed, respectively (Table 1.)

LC_{50} value of PC shrimp not pre-exposed was significantly higher than BSC shrimp not pre-exposed indicating that PC shrimp were more resistant to Cd (Table 1). The RFs for PC and BSC shrimp that were pre-exposed to Cd were 1.91 and 2.12, respectively, indicating that the pre-exposed shrimp of both populations were nearly twice as resistant to the lethal effects of Cd than shrimp not pre-exposed. However, RFs for PC and BSC Hg pre-exposed shrimp were 0.80 and 0.91, respectively, indicating no increase of tolerance to the lethal effect of Cd. In fact pre-exposure to Hg may actually have made the shrimp slightly more sensitive to the toxic effects of Cd.

Both field and laboratory research have shown that in some

Table 1. 96-h LC_{50} values of *P. pugio* of 2 populations comparing shrimp pre-exposed to Cd, or Hg and shrimp not pre-exposed.

Pre-exposed mg/L	Exposed mg/L	LC_{50} mg/L $\bar{X}$ (95% C. L) PC	BSC
0	Cd	3.28 (4.46-2.89)[abc]	1.83 (2.34-1.44)[abc]
0.05 (Cd)	Cd	6.81 (7.38-4.85)[b]	3.89 (4.56-2.95)[b]
0.02 (Hg)	Cd	2.63 (3.94-1.75)[c]	1.68(2.45-1.29)[c]

a: significant difference between PC & BSC.
b: significant difference between Cd pre-exposed and not pre-exposed.
c: no significant difference between Hg pre-exposed and not pre-exposed.

species, previous exposure to low concentrations of a toxicant can produce higher tolerance. The ability to develop increased tolerance to pollutants is of considerable advantage to organisms because it provides a certain degree of protection against toxic effects of pollutants. Our results indicated that the pre-exposure of shrimp to low concentrations of Cd increased their tolerance to higher concentrations of Cd. Pesch & Hoffman (1982) indicated that pre-exposure of the marine polychaete (*Neanthes arenaceodentata)* to sublethal concentrations of Cu increased their tolerance to lethal concentration and showed a significant increase of LT_{50} value. Roesijadi et al. (1982) found that pre-exposure of the marine mussel (*Mytilus edulis)* to low levels of mercury enhanced their tolerance to more toxic levels. Weis (1985) reported that pre-exposure of male fiddler crabs (*Uca pugilator*) to low concentrations of Cd enhanced their resistance to higher concentration of Cd as measured by effects on limb regeneration. However, this phenomenon was not found in females. Dixon & Sprague (1981) reported that *S. gairdneri* pre-exposed to Cu increased their tolerance to Cu.

There have been a number of studies in which pre-exposure of animals to one metal made them more tolerant to other metals. Brown et al. (1986) reported that pre-treatment of freshwater fish (*Salmo gairdneri, Rutilus rutilus*, and *Noemacheilus barbatulus*) to Zn enhanced their resistance to Cd. However, in our experiments pre-exposure of shrimp to Hg did not increase their tolerance to Cd. It would appear that this pre-exposure did not activate a protective mechanism. Similarly, Weis & Kim (1986) reported that pre-exposure of fiddler crabs *(Uca pugilator)* to Cd enhanced Cd tolerance while pre-exposure to Hg did not.

The pre-treatment concentration can have an important impact on the outcome. Rojesijadi et al. (1982)) indicated that pre-exposure of *Mytilus edulis* to 0.05 ug/L Hg increased the subsequent tolerance. However, pre-exposure to 5.0 ug/L did not increase subsequent tolerance. It may be that the one pre-exposure concentration which we selected for Hg pre-exposure was not appropriate to stimulate the inducing mechanism for increasing tolerance to Cd. Green et al. (1976) also reported that pre-treatment of white shrimp (*Penaeus setiferus*) to 0.5 to 1.0 ug/L Hg did not have any effects on LC_{50} or on growth and molting in response to higher concentration of Hg. In this study the pre-exposure levels may also have been in appropriate.

Pre-exposure to Cd of BSC shrimp made them more tolerant to higher concentrations of Cd. Many authors have found that non-tolerant animals from unpolluted sites can be made tolerant by exposure to low levels of metals. Fraser et al. (1978) reported that the freshwater isopod *(Asellus aquaticus)* from a polluted area was much more tolerant to lead than one from a non-polluted area. Later experiments Fraser (1980) indicated that pre-treatment of non-tolerant *A. aquaticus* from non-polluted sites produced tolerance similar to that of tolerant isopods from polluted sites. It was suggested that naturally occurring tolerance may be achieved by acclimation. Similarly in our study, pre-treated BSC shrimp developed tolerance levels comparable to PC shrimp. The PC shrimp were also found to increase their tolerance after pre-exposure to Cd. This indicates that they have additional capacity to increase tolerance. This was not the case for methylmercury in PC shrimp. Kraus (1986) found that pre-exposure of *P. pugio* to methylmercury resulted in decreased rather than increased resistance. Piles Creek is not as heavily contaminated with Cd as with Hg and other metals.

One week pre-exposure of shrimp to 0.05 mg/L Cd produced an increased tolerance to higher concentrations of Cd. This may be due to the production of metal binding proteins in response to Cd. Jennings et al. (1979) found higher levels of metal-binding proteins in crabs in response to Cd exposure.

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