TOXICITY OF HEAVY METAL CADMIUM ON THE ONTOGENY OF THE ESTUARINE HERMIT CRAB *CLIBANARIUS LONGITARSUS* (DE HAAN)

ABSTRACT

The 96 hr LC_{50} value for cadmium was found to be 270 ppb. Presently larval development was studied in 9 sublethal concentrations (10, 20, 30, 40, 50, 75, 100, 150 and 200 ppb). Two series of experiments were conducted. In the first series effect of cadmium on whole development was studied. In the second series effect of this metal on individual zoeal stages was studied. In both the series of experiments as the test concentration increased survival rate of larvae decreased. The survival rate was comparatively low in the second series. In the first series larval development was completed in all the test concentrations, but in the second series, development proceeded only upto 75 ppb concentration in II zoea, upto 100 ppb in III zoea and upto 40 ppb in IV zoea. Previous history of larvae was thus found to have a definite say on the survival rate. When the larvae were raised right through in test concentrations of pollutants, they develop a resisting capacity and fared comparatively better than the larvae introduced into the medium afresh. The overall time required for the completion of zoeal development increased with increase in test concentrations in the first series. In the second series, the intermoult duration in some lower test concentration was found to be shorter than that of control and higher concentrations. All the test concentrations of the first series fitted in with the definition of chronic concentration by Epifanio (1979). In the second series it was not so and it varied in different zoeal stages.

AWARENESS of the toxicity of the heavy metal cadmium has stimulated considerable interest in recent years and the incresing number of metal anlyses performed on marine species firmly established the occurrence of cadmium in the marine environment (Ahsanullah and Arnott, 1978). Not much is known about the effect of heavy metals on the larval stages of decapod crustaceans in Indian waters and the present study was done to elucidate the role of the above heavy metal on the larval development of the estuarine hermit crab *Clibanarius longitarsus* occurring in Vellar Estuary (lat. 11°29'N ; long. 79°49'E).

Material and methods

Ovigerous females were collected from the estuary. As soon as the larvae were liberated by the ovigerous crabs, they were separated in clean 500 ml beakers containing filtered sea water. 1.791 gm of cadmium sulphate dissolved in one litre of deionised water was used as the stock solution and the strength of this solution was 1 ppt *i.e.*, 1 ml contained

10,000 ppb of cadmium. Serial dilutions were made to get required concentrations.

To find out the 96 hr LC_{50} value 20 larvae each were used in different concentrations of cadmium following the method of Ahsanullah and Arnot (1978). The acute toxicity test was carried out in 13 concentrations (50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 and 900 ppb). All the larvae died on the same day in 900, 800, 700, 600, 500 and 400 ppb concentrations and after two days in 300 ppb concentration. In all the other lower concentrations there was survival of larvae after the expiry of 96 hours and the 96 hr LC_{50} value for cadmium was found to be 270 ppb (Fig. 1). Presently larval development was studied in 9 chronic concentrations (10, 20, 30, 40, 50, 75, 100 150 and 200 ppb).

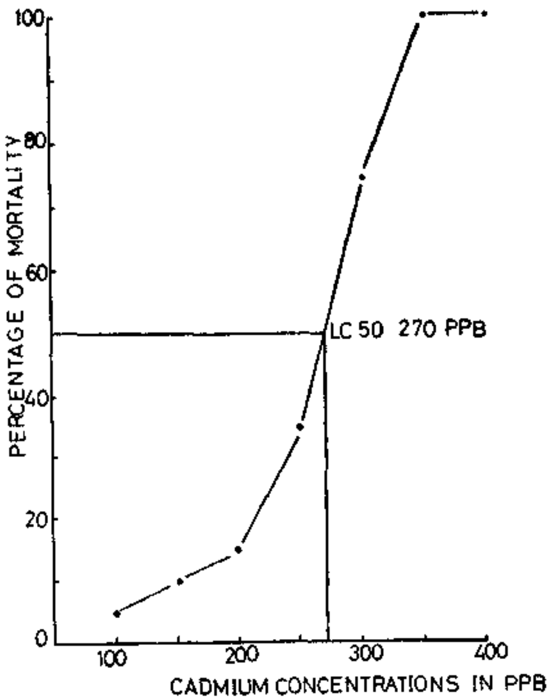

FIG. 1. 96 hr LC_{50} value of cadmium on I zoea of *Clibanarius longitarsus*.

Two series of experiments were conducted. In the first series, effect of cadmium on whole development was studied. In the second series effect of this metal on individual zoeal stages was studied. Here one batch was raised upto II zoeal stage and introduced into different test concentrations of cadmium. In the same way zoeal stages III and IV were raised in seawater medium and introduced afresh.

Results

Larval survival

The survival rate of *C. longitarsus* zoeal stages at different test concentrations of cadmium in both the series is given in Tables 1 and 2.

II Zoea

When the larvae were raised right through in test concentrations of pollutants, they seem to develope a resisting capacity and fared comparatively better than the larvae introduced into the medium afresh. In the first series, development proceeded in all the test concentrations and more than 85% of I zoea larvae moulted to the subsequent stage. But in the second series, development proceeded only upto 75 ppb concentration and in 50 and 75 ppb concentrations only 50 and 35% of larvae respectively moulted to III stage.

III Zoea

This zoea fared comparatively better than the previous zoeal stage. In the first series, development took place in all the concentrations and more than 50% of the larvae metamorphosed to the subsequent stage zoea IV. In the second series, development took place only upto 100 ppb concentration and survival rate was less than 50% in 75 and 100 ppb concentrations.

IV Zoea

Among the zoeal stages in the second series, this zoeal stage fared comparatively badly and development took place only upto 40 ppb

TABLE 1. *Mean intermoult duration in days and survival rate in percentage of zoeal stages I-IV of* Clibanarius longitarsus *(along with standard deviation and variance) exposed to different test concentrations of cadmium*

	Stage	control	Test concentrations in ppb									
			10	20	30	40	50	75	100	150	200
I	Zoea										
	Mean	5.36	5.52	5.58	5.71	5.96	6.25	6.79	7.75	7.96	8.41
	S.D.	0.7000	0.7703	0.7755	0.7506	0.6903	0.6757	0.8330	0.8969	0.8245	0.6661
	Variance	0.4900	0.5933	0.6014	0.5634	0.4764	0.4565	0.6938	0.8043	0.6798	0.4437
	N	25	25	24	24	24	24	24	24	23	22
	Survival rate	100	100	96	96	96	96	96	96	92	88
II	Zoea										
	Mean	4.96	5.04	5.59	5.77	6.19	6.90	6.95	7.63	7.78	8.00
	S.D.	0.9097	0.9283	1.0075	0.9726	0.8729	0.7684	0.7592	1.0116	0.9428	0.8944
	Variance	0.8243	0.8617	1.0151	0.9459	0.7619	0.5905	0.5763	1.0233	0.8889	0.8000
	N	25	23	22	22	21	21	20	19	18	16
	Survival rate	96	92	88	88	84	84	80	76	72	64
III	Zoea										
	Mean	5.13	5.23	5.48	5.70	5.90	6.20	6.78	6.88	7.33	7.64
	S.D.	0.9197	0.9223	0.9808	1.0311	0.9679	0.8944	1.0033	0.8575	0.8997	1.0082
	Variance	0.8458	0.8506	0.9619	1.0632	0.9368	0.8000	1.0060	0.7353	0.8095	1.0165
	N	23	22	21	20	20	20	18	17	15	14
	Survival rate	92	88	84	80	80	80	72	68	60	70
IV	Zoea										
	Mean	6.87	7.09	7.30	7.42	7.50	7.94	8.06	8.56	9.08	9.10
	S.D.	0.7570	0.7502	0.7327	0.6925	0.7071	0.8024	0.8269	1.0308	0.9962	0.5676
	Variance	0.5731	0.5628	0.5368	0.4795	0.5000	0.6438	0.6838	1.0625	0.9924	0.3222
	N	23	22	20	19	18	18	17	16	12	10
	Survival rate	92	88	80	76	72	72	68	64	48	40

concentration. In 40, 30 and 20 ppb concentrations, less than 50% of larvae moulted to the subsequent stage-glaucothoe stage. Survival rate was less in the second series when compared to the first series.

Development duration

The mean intermoult duration of each zoeal stage in different test concentrations of both the series is given in Tables 1 and 2. Analysis of variance done to find out the difference in the intermoult duration of the zoeal stages in both the series among control and different test concentrations showed significant variations.

II Zoea

Previous history of larvae with pollutant was found to have a say on the intermoult duration also and intermoult duration in the first series was found to be lengtheir than the second series. 't' values calculated showed significant differences in the intermoult duration between the two series in 30, 40 and 50 ppb concentrations.

III Zoea

Intermoult duration in concentrations 30, 75 and 100 ppb was found to be lengthier in the second series. In other concentrations

TABLE 2. *Mean intermoult duration in days and survival rate in percentage of individual zoeal stages II-IV of* Clibanarius longitarsus *(with standard deviation and variance) exposed to different test concentrations of cadmium*

Stage	Control	Test concentrations in ppb						
		10	20	30	40	50	75	100
II Zoea								
Mean	4.70	5.00	5.10	4.50	4.86	5.30	6.57	—
S.D.	1.03	0.82	1.02	0.69	1.23	0.95	1.72	—
Variance	1.06	0.67	1.04	0.47	1.52	0.90	2.95	—
N	20	19	20	20	14	10	7	—
Survival rate	100	95	100	100	70	50	35	—
III Zoea								
Mean	4.92	5.08	5.33	5.36	6.22	5.29	7.00	7.00
S.D.	1.00	0.29	0.65	0.81	0.44	0.76	0.00	0.00
Variance	1.00	0.08	0.42	0.65	0.19	0.57	0.00	0.00
N	20	12	12	11	9	7	2	4
Survival rate	100	60	60	55	45	35	10	20
IV Zoea								
Mean	6.67	8.10	10.00	10.00	12.50	—	—	—
S.D.	0.65	0.32	0.00	0.00	1.73	—	—	—
Variance	0.42	0.10	0.00	0.00	0.00	—	—	—
N	20	10	4	4	4	—	—	—
Survival rate	100	50	20	20	20	—	—	—

(10, 20, 40 and 50 ppb) intermoult duration was lengthier in the first series. 't' values showed significant difference between 50 ppb concentrations of the two series.

IV Zoea

This is just the reverse of zoeal stage II and here intermoult duration in different test concentrations of the second series was found to be lengthier than the first series. 't' values showed significant differences between the concentrations of the two series.

Discussion

The 96 hr LC_{50} value of 270 ppb of cadmium for *Clibanarius longitarsus* was found to be low compared to 490 ppb of cadmium for I zoea of *Paragrapsus quadridentatus* (Ahsanullah and Arnott, 1978). Epifanio (1979) defined the sublethal and acute concentrations of pollutants. According to him sublethal concentrations are those in which there is differential survival with increased concentrations in relation to survival in the control medium and those in which more than 10% of the larvae complete development, whereas acute concentrations are those in which less than 10% of the larvae complete development. These definitions hold good when survival to the crab is high in the control, but not when survival is low (Bookhout and Costlow, 1975). In the present study the overall survival rate of larvae in conrtol was more in both the series of experiments and also in all the test concentrations of the first series. In the second series it varied indifferent zoeal stages. In II zoeal stage, concentrations upto 75 ppb fit in with the definition, in III stage upto 100 ppb concentration and in IV stage upto 40 ppb concentration. In the case of the zoeae of *Rhithropanopeus horrisii* and megalopa of *Callinectes sapidus* a concentration of 150 ppb cadmium was found to be acutely toxic (Rosenberg and Costlow, 1976). Variations in 96 hr LC_{50} or acute toxicity values could be ascribed to differences in (1) test conditions (Salinity, temperature, etc.), (2) duration of observation and (3) the sensitivity of stages and species. But such information is quite essential to compare the sensitivity of the species and toxicity potential of chemicals, to derive safe environmental levels of such toxic chemicals using LC_{50} values and application factors.

Presently in both the series of experiments as the test concentration increased survival rate of larvae decreased. Similar results have been reported elsewhere also. When Decoursey and Vernberg (1972) studied the effect of mercury on survival of *Uca ugilator* larvae they found that the number of larvae surviving in higher concentrations of mercury was very few. Shealy and Sandifer (1975) exposed the larvae of grass shrimp *Palaemonetes vulgaris* to different concentrations of mercury for 48 hours and studied their development, the observed reduced survival to the postlarvae in high concetrations of mercury. In another study by Rosenberg and Costlow (1976) on larvae of *C. sapidus* and *R. harrisii*, survival of developmental stages was significantly lower in higher concentrations of cadmium. The reduced survival was believed to be due to the pollutant which was absorbed by the larvae being released into the blood stream causing mortality in some unknown way. Some larval stages are more vulnerable to pollutants than others. While megalopa was found to be more susceptible in some species, both zoeae and megalopa were found to be equally sensitive in another species. Thus different species of crabs show sensitivity to the same pollutant at different stages of development (Bookhout *et al.*, 1972). To compare the sensitivity of different zoeal stages, it is advisable if each zoeal stage is introduced to the test medium freshly. Presently this has been done. When the larvae were raiesd right through in test concentrations of pollutants, they seem to develop a resisting capacity and fared comparatively better than the larvae introduced into the medium afresh. In the first series development took place in all sublethal concentrations. But in the second series development took place only upto 75 ppb concentrations in II zoea, upto 100 ppb in III zoea and upto 40 ppb in IV zoea. Thus the sensitivity of the larvae was found to vary depending upon the situations. Among the zoeal stages, IV zoea appeared to be the most sensitive stage.

In the present study, with increase in test concentration the overall time required for the completion of zoeal development increased in the first series. In the second series also generally the intermoult duration in higher concentrations was found to be lengthier than control and lower concentrations. The above results were treated statistically and the differences were found to be significant. Reports such as concentrations as low as 50 ppb of lead and 50 ppb of zinc prolonged the duration of zoeal development of *R. harrisii* are quite in agreement with the present observations (Benijts and Benijts, 1975). Shealy and Sandifer (1975) also reported delayed moulting and extended development time in the larvae of *P. vulgaris* when the mercury test concentration increased. When the zoeal stages of *R. harrisii* were exposed to cadmium concentration of 50 ppb under non optimal conditions the duration of development was found to be significantly increased. In 150 ppb cadmium also zoea survived, but the duration was significantly prolonged (Rosenberg and Costlow 1976). An increase in the duration of development of *R. harrisii* with an increase in mercury from 5 to 20 ppb was reported by Mc Kenney and Costlow (1982).

Bookhout *et al.* (1972) attributed the increased duration of larval stages in different test concentrations to stress. Stress in test concentrations of pollutants may act in two ways. First an alteration in normal development *i.e.* an increase in number of zoeal stages. As most of the crabs consistently have a definite number of zoeal stages, perhaps both larval moulting and rate of development are controlled by the same mechanism. However, there are two exceptions as in the larvae of *C. sapidus* and *Menippe mercenaria*. According to Costlow (1968) two mechanisms may be involved, one that controls moulting and a second normally synchronized with the first, that regulates the rate of morphological development, for when both the eyestalks of *R. harrisii* were removed, the larvae passed through an extra zoeal stage without morphological development. However, in the present study there was no extra zoeal stage under sublethal test concentrations of cadmium.

Secondly stress in higher concentrations of pollutants may exert an action on the mechanism which regulates morphological and moulting changes and hence speedy development of larvae (Bookhout *et al.*, 1972). Many authors suggested that toxicity of pollutant was associated with the moulting process (Epifanio, 1976). Moulting in crab larvae is known to be a neuroendocrine function (Costlow, 1963, 1966 a, b) and naturally sublethal toxicity of neurotoxins may also interfere with moulting. In the present study, some sublethal concentrations of cadmium in the second series simulated moulting with associated morphological changes in the larvae of *C. longitarsus*. Similar results were reported by Carmel *et al.* (1983). They found that when the juveniles of prawn *Penaeus indicus* were exposed to copper, they moulted at a faster rate than control animals.

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