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On the dynamics of exploited populations of *Tisbe holothuriae* (Copepoda, Harpacticoidae)

IV. The toxicity of cadmium: Response to lethal exposure

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ABSTRACT: A total of 90, weekly exploited populations of the harpacticoid copepod *Tisbe holothuriae* Humes were exposed to 148, 222, 333, 500, 750 or 1125 μg Cd^{++} l^{-1}, combined with exploitation rates of 10, 30, 50, 70 or 90 % under conditions of surplus food supply at 22° C and 30 ‰ S. All populations exposed to concentrations down to 500 μg Cd^{++} l^{-1} and 3 populations (out of 15) exposed to 333 μg l^{-1} became extinct within the experimental period of 30 weeks. Survival time significantly depended on concentration. A recovery phase from an initially high mortality preceded eventual population extinction after adding 500 μg Cd^{++} l^{-1}. In the initial phase, higher nauplii mortality prevailed. During these experiments on acute intoxication, no relationship could be established between survival and exploitation rate. However, experiments on the effects of stepwise increases in Cd concentration (results not yet published) produced evidence of such a relationship. In spite of increased mortality, no significant numbers of dead copepods were detected in weekly samples because of their rapid decomposition and cannibalism, which depends on the amount of food available. Sampling regimes of 5 times per week yielded significant numbers of dead individuals.

INTRODUCTION

Recently published investigations on brook trout survival and testicular injury (Sangalang & O'Halloran, 1973), findings of Bengtsson et al. (1975) on vertebral damage in *Phoxinus phoxinus* and examination of reproduction in *Jordanella floridae* (Spehar, 1976) have shown a high sensibility of fishes to cadmium poisoning. Concentrations of Cd^{++} as low as 5–10 μg l^{-1} are found to have a deleterious effect.

In *Daphnia magna* Biesinger & Christensen (1972) have reported a 16 % reproductive impairment after 3 weeks exposure to 0.17 μg Cd^{++} l^{-1}. In general, crustaceans have been found to be most sensitive to Cd^{++} (cf. Eisler, 1971). For further literature on the effects of cadmium on aquatic organisms the reader is referred to a recently published paper by von Westernhagen & Dethlefsen (1975). Extensive reviews of the cadmium pollution problem have been presented by Friberg et al. (1974), Lymburner (1974), Nordberg (1974) and Hiatt & Huff (1975).

Because of the particular relevance of tests applied to populations and the proven high toxicity of Cd^{++} to crustaceans, it appeared reasonable to conduct experiments with an appropriate species in order to study the responses at this level. Previous investigations (Hoppenheit, 1975a, b; 1976) have shown that *Tisbe holothuriae* can easily be handled in the laboratory. This copepod offers the possibility of studying several components of the dynamics of laboratory populations. Results of supplementary investigations on the partitioning of cadmium into different compartments of the elected experimental system, which complete the present paper, will be published in a subsequent report (Sperling & Hoppenheit, in preparation).

MATERIAL AND METHODS

The original material of *Tisbe holothuriae* Humes was supplied by Uhlig, who had observed this species, initially, in the open air swimming pool on the island of Helgoland (Uhlig, 1965).

In the experiments conducted the populations were kept in 200 ml of non-aerated sea water in 500 ml volume wide-mouth, flat-bottom flasks. At weekly intervals the populations were exploited at rates of 10, 30, 50, 70 or 90 %. The cultures were harvested by stirring the water and removing the desired percentages of specimens together with the medium. In samples of 10 ml drawn before the weekly harvests, the numbers of live and dead nauplii and live and dead adults plus copepodids were determined. The method used was that described by Dressel et al. (1972) using vital staining with neutral red followed by fixation with 5-sulfosalicylic acid.

The populations were maintained at 22 ± 1°C in a room having 12 h light per day. At the time of weekly exploitation, 90 % of the medium was renewed; at exploitation rates less than 90 % specimens were retained by a fine-meshed sieve (mesh size: 25 μm).

The specimens were fed dried mussel flesh (mantle) once a week following exploitation. The amount of food was proportioned in such a way that after one week some pieces of mussel flesh were still available.

The sea water was filtered through a Seitz filter (K5) and heated for 12 h at 80° C, and was then diluted with a distilled water solution containing the required experimental concentrations of cadmium, in a ratio of 11 : 1; this resulted in a salinity of about 30 ‰. Stock solutions of $CdCl_2$ in distilled water (water free, Baker) had been made up earlier. The experimental sea-water medium was then stored in cadmium pre-equilibrated 5 l glass containers until use.

A population was considered to be extinct when samples which contained no specimens had been taken for three successive weeks. For further details on material and methods the reader is referred to Hoppenheit (1957a, b; 1976).

RESULTS AND CONCLUSIONS

A total of 90 populations were tested and survivors were maintained for 30 weeks. All populations were observed for at least 27 weeks before the addition of Cd^{++}.

Table 1

Results of experiments on acute intoxication induced by Cd^{++} in exploited populations of *Tisbe holothuriae*. (For explanation see text)

Exploitation rate (%)	Cd^{++} ($\mu g\ l^{-1}$) 148	222	333	500	750	1125	Number of populations
10	+ + +	+ + +	— + +	— — —	— — —	— — —	18
30	+ + +	+ + +	+ + +	— — —	— — —	— — —	18
50	+ + +	+ + +	— — +	— — —	— — —	— — —	18
70	+ + +	+ + +	+ + +	— — —	— — —	— — —	18
90	+ + +	+ + +	+ + +	— — —	— — —	— — —	18
Total	15	15	15	15	15	15	90
Mean survival time (weeks)			8.7	6.7	5.1	3.3	

Groups of three replicates each were subjected to six Cd^{++} concentrations in combination with five exploitation rates, according to the scheme shown in Table 1. Each plus sign represents a population that survived for a period of 30 weeks; each minus sign indicates a population that became extinct in the course of the experiment. The last line of Table 1 gives the mean survival times in relation to the Cd^{++} concentration in those populations that died during the observation time of 30 weeks. Survival time means the period between the addition of Cd^{++} and the first appea-

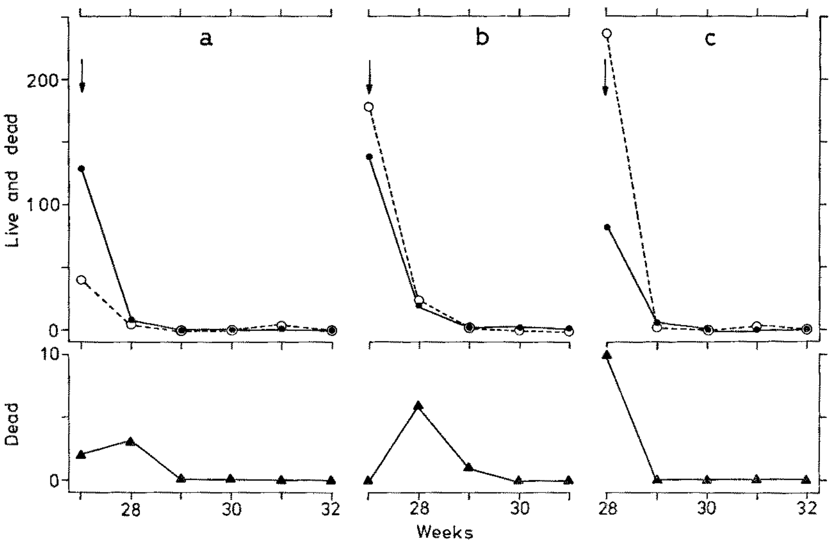

Fig. 1: *Tisbe holothuriae*. Population density of adults plus copepodids (●) and nauplii (○) and number of dead specimens per sample (▲) after addition of 1125 $\mu g\ Cd^{++}\ l^{-1}$ (indicated by an arrow) in weekly exploited populations. Exploitation rates are 10 (a), 50 (b) and 90 % (c)

rance of a "zero value" in three successive samples of 10 ml taken at weekly intervals. By multiple comparison of the medians of the non-normally distributed survival data using the non-parametric S_J-test of Jonckheere (cf. Lienert, 1973; p. 277 ff.) can be shown that the differences among mean survival times are highly significant ($\alpha < 0.001$). No relationship could be established between survival and exploitation rate in this experiment on the effects of acute intoxication. A subsequent paper (Hoppenheit, in preparation) will show that exploitation rate has an influence on survival when populations are exposed to stepwise increasing Cd^{++} concentration.

Three examples of decrease of population density following addition of 1125 μg l^{-1} of Cd^{++} to the medium are presented in Figure 1. The graphs indicate rapidly declining numbers of nauplii and adults plus copepodids. The lower parts of the figure show that no significant numbers of dead specimens could be found in the samples.

By observation of the decomposition of adult specimens killed by slight heating, it could be established that, after 48 hours, decomposition had already reached such a degree that it became difficult or impossible to differentiate between dead and disintegrated specimens, and exuvies or the remains of exuvies.

In a series of experiments, 50 dead adult specimens and copopodids were added to 50 live copepods; no intact dead specimen could be detected after 24 hours. The situation changed when food was offered; all dead copepods could be found after 24 hours. In the performance of the population experiments, therefore, a higher degree of cannibalism has to be taken into account with deficiency of food; this situation may well be expected to occur particularly at the end of the chosen interval of one week and at high rather than at low population densities.

To show the influence of population density on the appearance of dead specimens in the samples, experiments with daily exploitation were performed. Two groups of three populations each with daily removal of 5 and 35 % of specimens, respectively, were observed for 33 days. It was found that a relatively higher number of dead copepods occurred at lower population densities. A daily exploitation of 5 % of specimens, for example, resulted in a mean value of 4.5 % dead copepods at population densities lower than 100 specimens per sample and in a value of 1.2 % dead copepods at population densities higher than 175 specimens per sample (based on 33 samples in each case). Assuming a constant mortality rate or – more probably – a higher mortality rate at higher population densities, the findings indicate a relationship between population density and extent of cannibalism where – most likely as a consequence of food shortage – a higher population density results in a higher degree of cannibalism.

Due to the fact that one would not expect to find large quantities of dead copepods with an exploitation, feeding and sampling regime of once a week, further experiments with removal and enumeration of 5 % of the specimens five times a week were performed. Three populations each were exposed to 1125 and 500 μg l^{-1} of Cd^{++}, respectively. The patterns of decline of the density of two populations exposed to 1125 μg Cd^{++} l^{-1} are shown in Figure 2. The populations became extinct on the 22nd and 26th days, respectively, following addition of Cd^{++}. The percentages of dead specimens are presented in Figure 3. Immediately after the addition of

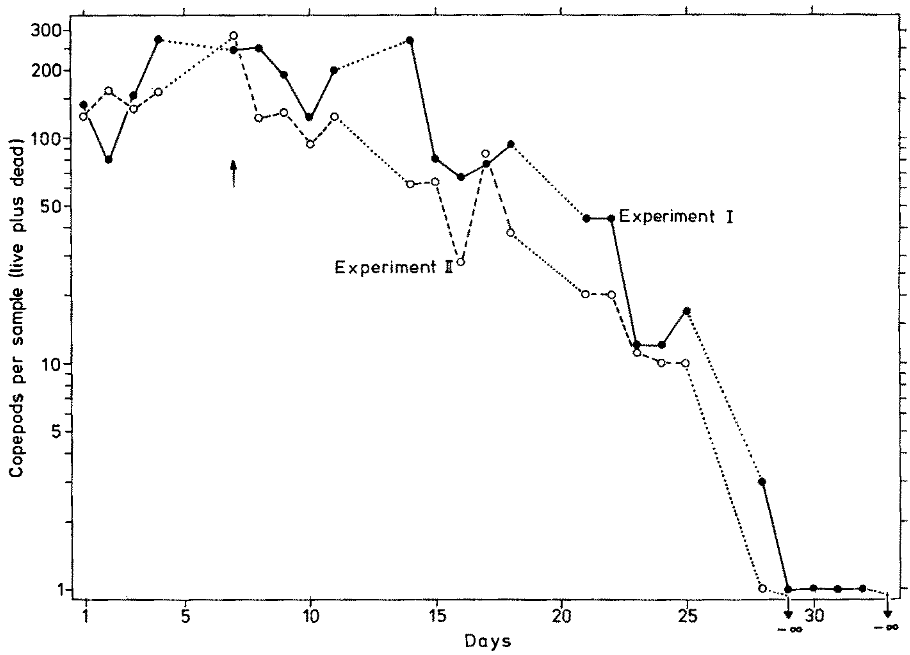

Fig. 2: *Tisbe holothuriae*. Population density before and after addition of 1125 μg Cd^{++} l^{-1} (indicated by an arrow) in two exploited populations. Removal of 5 % of specimens five times a week

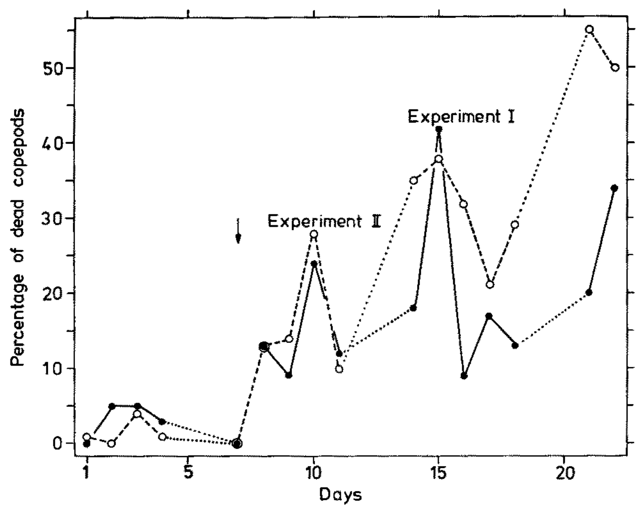

Fig. 3: *Tisbe holothuriae*. Percentages of dead specimens before and after addition of 1125 μg Cd^{++} l^{-1} (indicated by an arrow) in two exploited populations. Removal of 5 % of specimens five times a week

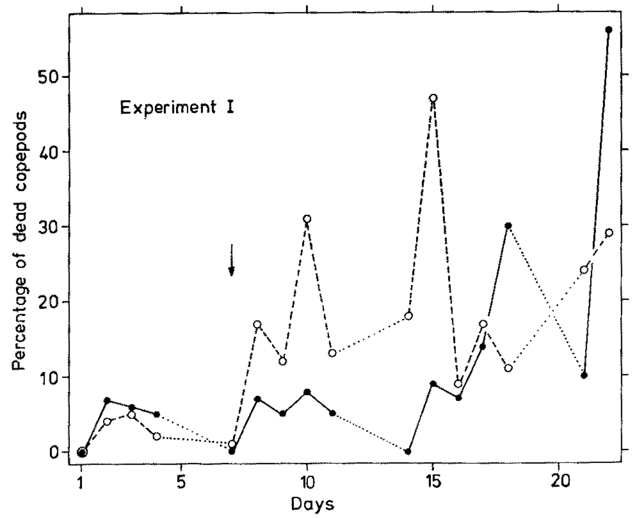

Fig. 4: *Tisbe holothuriae*. Percentages of dead adults plus copepodids (●) and nauplii (○) before and after addition of 1125 μg Cd^{++} l^{-1} (indicated by an arrow) in an exploited population. Removal of 5 % of specimens five times a week

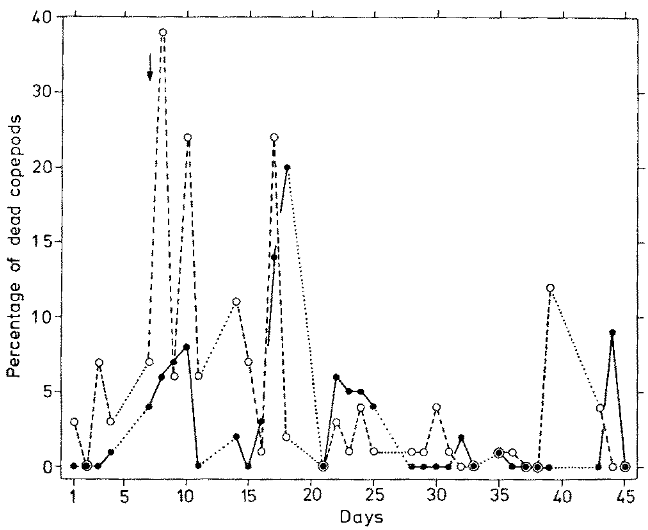

Fig. 5: *Tisbe holothuriae*. Percentages of dead adults plus copepodids (●) and nauplii (○) before and after addition of 500 μg Cd^{++} l^{-1} (indicated by an arrow) in an exploited population. Removal of 5 % of specimens five times a week

Cd^{++} the relative frequency of dead individuals increased indicating an acute mortality; the mortality rate of nauplii is higher than of adults and copepodids during the first days after the addition of Cd^{++} (Fig. 4). Relative frequencies have been calculated up to the 22nd day of the experiment only, because of verv low numbers of specimens in the samples at the end of the experiments. After the addition of 500 μg Cd^{++} l^{-1} an increase in mortality was also observed (Fig. 5). One population became extinct on the 65th day. The other two experimental populations were rejected on the 70th day at population densities of 12 and 2 specimens per 10 ml, respectively.

The graphs, Figures 6 and 7, are examples of the variation of population density after addition of 500 μg Cd^{++} l^{-1} medium at weekly exploitation and sampling. With one exception (Fig. 7b), again no significant mortality could be

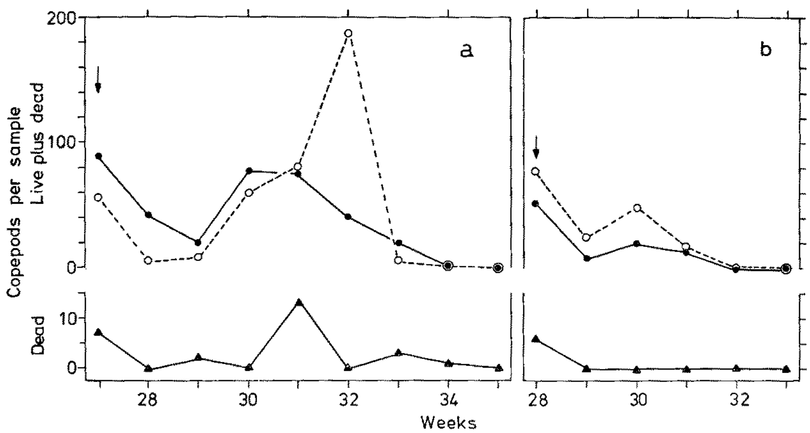

Fig. 6: *Tisbe holothuriae*. Population density of adults plus copepodids (●) and nauplii (○) and number of dead specimens per sample (▲) after addition of 500 μg Cd^{++} l^{-1} (indicated by an arrow) in two weekly exploited populations. Exploitation rates: 10 (a) and 90 % (b)

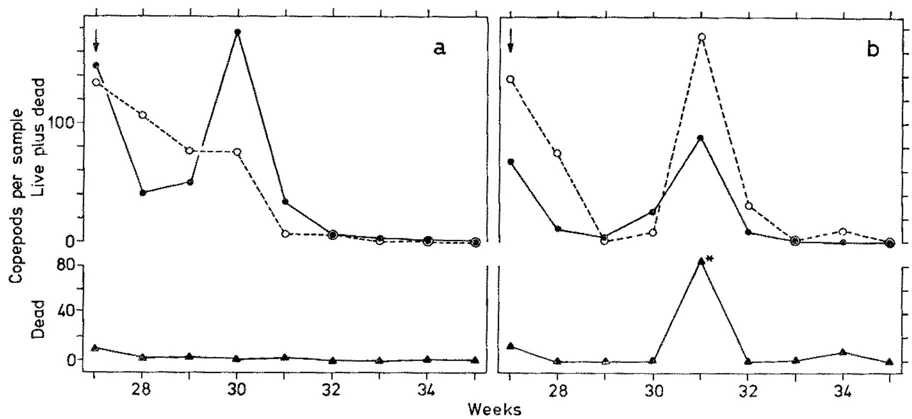

Fig. 7: *Tisbe holothuriae*. Population density of adults plus copepodids (●) and nauplii (○) and number of dead specimens per sample (▲) after addition of 500 μg Cd^{++} l^{-1} (indicated by an arrow) in two weekly exploited populations. Exploitation rate: 50 %, * 75 nauplii

detected at this sampling regime. At this concentration the lethal effect of Cd^{++} on the whole population does not become evident until a phase of recovery following initial mortality. The experiments with exploitation rates of 5 % five times a week have revealed that there are two phases of increased mortality with a period of low mortality between (cf. Fig. 5).

Nauplii were present in nearly all cases until the end of the experiments in the 48 populations that eventually became extinct. In the majority of cases females carrying an egg sac were not detected at the very end of the experiments; thus the observed nauplii must have been at an advanced stage. D'Agostino & Finney (1974) established that inhibition in *Tigriopus japonicus* occurred in the development of ovigerous females and hence in the production of the F_2 generation when 44 μg Cd^{++} l^{-1} were added (as $CdSO_4 \cdot 8\ H_2O$). Due to the fact that *Tisbe holothuriae* adult females have a survival time of up to 43 days (Hoppenheit, 1976), which is close to the mean survival time of 6.7 weeks in populations exposed to 500 μg Cd^{++} l^{-1} (cf. Table 1), it is not possible to establish on the basis of the present experiments whether besides mortality such a mechanism is responsible for the observed extinctions of the populations.

This paper dealing with lethal effects will be supplemented by a subsequent publication on responses to sub-lethal cadmium exposure. The findings of both the present and the forthcoming paper will be discussed in the latter.

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