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UPTAKE, RETENTION AND LOSS OF CADMIUM BY CRANGON CRANGON

Volkert Dethlefsen
Bundesforschungsanstalt für Fischerei
Institut für Küsten- und Binnenfischerei
Laboratorium Cuxhaven
Niedersachsenstraße
2190 Cuxhaven

Abstract
After their first moult in the laboratory Crangon crangon (50 mm) were exposed to Cd-concentrations of 0.005 - 0.100 mg/l in static tests for accumulation periods of up to 40 days. Loss and retention experiments were conducted for 7 to 12 days after an uptake period of 20 days in the resp. Cd-concentration. The uptake-curves showed two clearly distinguishable areas; a rapid shorttime accumulation characterized by the building of an early plateau and a long term accumulation characterized by a linear increase of the total body load.
The first rapid uptake is interpreted as dominating adsorption process finding a plateau after the saturation of available binding sites; the linear uptake is interpreted as absorption processes which mainly means incorporation of Cd in body tissues.
For no concentration of Cd a final equilibrium between body load and water concentration was encountered.
Concentration of Cd in the exuvia of Crangon crangon who were kept in Cd-concentration until their second moult was not significantly lower than that in the organism.
Retention of Cd, as measured in 7 - 12 days clearance experiments, was very effective depending on the exposure concentration; after exposure to 5 µg Cd/l for 20 days, no loss of Cd from the shrimps could be measured in a period of 7 days.
Accumulation factors calculated for the lowest concentration employed (5 µg/l) were 2400 and identic to those calculated for Crangon crangon living in the Elbe estuary.

I. Introduction

The role of Cadmium as a coastal and estuarine pollutant has two different aspects. On the one hand the possible impact through acute toxicity to marine and estuarine organisms is relatively low. This is due to high/low - low/high interaction between Cadmium and salinity or hardness of water (WESTERNHAGEN and DETHLEFSEN,1975; WESTERNHAGEN et al,1974; 1975; DETHLEFSEN et al, 1975; BROWN,1968).

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In coastal waters and estuaries with relatively high salinity the acute toxicity of cadmium to organisms is low (EISLER,1971), but in freshwater organisms are extremely susceptible to cadmium exposure (PICKERING and HENDERSON,1966; BALL,1967; CEARLEY and COLEMAN,1974).

It has been attempted to ascribe this phenomenon to the interaction between cadmium and calcium ions (MALJKOVIC and BRANICA, 1971; WRIGHT,1977), which possibly compete for bindings sites on tissue surfaces or cell membranes (WESTERNHAGEN et al,1975).

Present concentrations of cadmium in the German bight area were found to be in the range of 0.1 µg/l (SCHMIDT,1976).

Relatively susceptible early life stages of estuarine fishes displayed acute toxic effects when exposed to concentrations in excess of 0.5 mg/l Cd (garpike eggs, WESTERNHAGEN et al, 1975), and 1.0 mg/l Cd (flounder eggs, WESTERNHAGEN and DETHLEFSEN,1975). Thus the probability that present concentrations of cadmium in the Elbe estuary will produce acute harm to estuarine organisms is low.

In spite of its low acute toxicity to marine and estuarine organisms cadmium deserves special attention due to its bioaccumulative potential. Cadmium has been shown to accumulate in a variety of aquatic organisms such as oysters (THROWER and EUSTACE,1973), crabs (THURBERG et al,1973; HUTCHESON,1974), whelks (STENNER and NICKLESS,1974), euphasiids (BENAYOUN et al, 1974), early life stages of estuarine fishes (WESTERNHAGEN and DETHLEFSEN,1975; WESTERNHAGEN et al,1974; 1975; ROSENTHAL and SPERLING,1974), adult fishes (EISLER,1972; JAAKOLA,1971; CEARLEY and COLEMAN,1974).

Information on the dynamics of the uptake, retention and elimination of cadmium by marine organisms is scarce (HUTCHESON, 1974; BENAYOUN et al,1974).

The present paper deals with these aspects, the test organism was Crangon crangon, an estuarine organism with high economical and ecological importance.

II. Material and Methods

Experimental animals were caught in the Elbe estuary. Their size was selected to be in range of 50 ± 2.0 mm (all female). They were acclimated to experimental conditions for 2 - 3 days and then individually kept (avoid cannibalism) until their first moult. Exposure of the shrimps was started at the morning after the first moult in the laboratory. More than 95 % of the moults occurred at night. From moulting to the beginning of exposure some hours passed.

Two sets of experimental conditions, both static test methods, were employed.

1. Individual shrimps were kept in 1 l aerated seawater (glas containers) until the occurrence of the next moulting. Total body load of cadmium was measured after termination of experiments.

2. Up to 12 shrimps were kept simultaneously in 10 l aerated seawater (glas containers) seperated by plastic compartments. For measurement of accumulation shrimps were sacrificed in regular intervals and processed individually.

After removal of shrimps for analysis, test containers were restocked to 12 experimental animals whenever possible.

Data were obtained from the analysis of 3 to 10 shrimps per concentration and exposure time.

For elimination experiments shrimps were kept for 20 days in the respective concentration and afterwards transferred into clean seawater.

Elimination experiments were terminated after 7 - 12 days.

In all experiments water was exchanged in 48 h-intervals.

Cadmium was added to the seawater as $CdCl_2$ in concentrations of 0.005, 0.010, 0.020, 0.050 and 0.100 mg/l.

Shrimps were fed on dry food prior to the exchange of water, dry food was excepted readily.

Measurements on °C, pH, NO_2, S ‰ and O_2 were carried out daily.

Some results of these measurements are given in Table I.

Table I: Experimental conditions for experiment 76/I

	NO_2	pH	S ‰	°C	O_2
n	10	24	24	24	24
$\bar{x}$	0.025	8.42	30.49	14.99	7.97
Sx ±	0.016	0.17	0.75	0.15	0.24
S$\bar{x}$ ±	0.005	0.03	0.15	0.03	0.04

Samples for analysis of concentrations of cadmium in test containers were taken in 48 h-intervals immediately before and after the exchange of water.

For chemical analysis 50 μl of seawater were acidified with $(NH_4)\ S_2O_8$ and after dilution with double distilled water 20 μl were injected into the graphite furnace (HGA 72) of the Perkin Elmer 300 equipped with electrodeless discharge lamp, D_2-corrector, automatic sampler and printer.

Sacrificed shrimps were deep frozen at the end of the accumulation or elimination time, dried for 24 hours at 60°C and homogenized in an achate mortar mill. Two 5 mg subsamples from each individual shrimp were weighed into 1.5 ml polypropylene bottles, 100 μl of a 1 to 4 mixture HNO_3+$HClO_4$ were added and the mixture was subjected to 60°C for 24 hours.

After dilution with double distilled water 20 μl were injected into the graphite furnace. Each sample was analysed twice, for verification of 1 value at least 4 measurements were carried out. Concentrations are referred to as mg/kg total body dry weight. Concentrations are representing averages plus standard deviation out of results on 3 to 10 individual shrimps.

III. Results

1. Concentrations of Cd in test water.
   Results for seawater analysis are given in Table II. Concentrations measured varied in relatively narrow ranges. There were no significant differences in Cd-concentrations in test water samples immediately before and after exchange of water.

2. Cd-levels in control animals.
   Cd-concentrations measured in freshly caught specimens (Table III) varied considerably and there seemed to be a tendency of decreasing Cd-concentrations from 1975 to 1977. But this finding is far from being significant because sampling was not carried out systematically and locations of catches were often different.

   Control shrimps kept in seawater without any added cadmium showed fairly constant cadmium-concentrations during the experiments. No uptake of cadmium could be measured in periods of 30 to 40 days (Table IV and V). Also in those experiments where the continuous uptake was measured no increase of total Cd-body loads could be found in control animals.

Table II: Concentrations of cadmium in test water during two experiments (µg/l)

	1976/I intended			1976/II concentrations			
	Control	10 µg/l	20 µg/l	Control	5 µg/l	10 µg/l	20 µg/l
n	25	25	25	30	30	30	30
$\bar{x}$	0.94	10.50	21.22	1.27	6.32	10.20	21.09
Sx $\pm$	0.36	1.24	1.30	0.52	0.40	1.10	1.50
S$\bar{x}$ $\pm$	0.07	0.25	0.26	0.13	0.07	0.20	0.27

Table III: mg $Cd^{++}kg^{-1}$ dry weight in freshly caught Crangon crangon from the Elbe estuary

	Sept.75	April 76	June 76	Aug.76	Sept.76	April 77	Mai 77
$\bar{x}$	0.97	1.06	0.95	0.82	0.71	0.41	0.37
Sx $\pm$	0.24	0.14	0.24	0.36	0.36	0.12	0.10
S$\bar{x}$ $\pm$	0.12	0.04	0.12	0.18	0.18	0.06	0.05

Table IV: Uptake of Cadmium by Crangon crangon after 30 days of exposure in 0.04 and 0.1 mg Cd/l. Total body load mg/kg dry weight (1975/I)

	Control	surviving	dead	moulted animal	moulted exuvia
n		4	2	6	6
$\bar{x}$		0.81	1.61	1.19	1.21
Sx ±		0.27		0.49	0.17
S$\bar{x}$ ±		0.13		0.25	0.09
n	0.040	6	2	4	4
$\bar{x}$		8.34	5.91	10.44	9.46
Sx ±		1.14		0.52	1.17
S$\bar{x}$ ±		0.46		0.27	0.54
n	0.100	8	2	2	2
$\bar{x}$		16.11	14.90	14.30	15.30
Sx ±		2.60			
S$\bar{x}$ ±		0.92			

Table V: Uptake of Cadmium by Crangon crangon after 40 days of exposure in 0.05 and 0.1 mg Cd/l. Total body load mg/kg dry weight (1975/II)

	Control	surviving	dead	moulted animal	moulted exuvia	days time to moult
n		5	6	2	2	
$\bar{x}$		1.30	1.36	1.29	0.75	21
Sx ±		0.41	0.28			
S$\bar{x}$ ±		0.18	0.13			
n	0.05	9	2	2	2	
$\bar{x}$		14.66	14.14	14.15	10.38	24
Sx ±		2.70				
S$\bar{x}$ ±		0.90				
n	0.1	5	8	2	2	
$\bar{x}$		22.00	18.48	23.08	22.50	26.5
Sx ±		5.10	4.37			
S$\bar{x}$ ±		2.28	1.78			

Uptake

Results of the first two experiments are depicted in Table IV and V.

Concentrations of cadmium in Crangon crangon reached at the end of 30 and 40 days respectively were depending on the time of exposure . and on the concentrations employed.

Highest medium concentrations reached were 22.00 mg Cd^{++} per kg dry weight of total body after 40 days of accumulation in 0.1 mg Cd/l.

Test animals found dead during these experiments had slightly lower body loads of Cd possibly due to the shorter time of exposure. This is also true for shrimps that moulted during the experiment, these animals were sacrificed after their second moult. Cadmium concentrations in the exuvia were slightly lower than in the animals.

Mortality of test animals in all cadmium concentrations was not significantly different from that encountered in controls.

Short time accumulation

The accumulation of cadmium by Crangon crangon in the first hours after the beginning of the exposure followed the same general pattern in all uptake experiments (Fig. 1 and 2). A rapid uptake in the first 2 to 8 hours in the experiment is followed by a preliminary plateau and a loss of cadmium after 24 to 48 hours. In most cases a minimum body load was found after 3 days of the experiments.

Long term accumulation

After these three days an increase of cadmium concentrations in shrimps was found in all experiments, the rate of increase was lowest in water concentrations of 5 µg Cd/l (Fig. 3) and highest after exposure to water concentrations of 20 µg Cd/l (Fig. 5).

Highest body loads of cadmium reached were 2.7 mg Cd/kg total body dry weight in the 5 µg experiment, 4.0 mg/kg in the 10 µg experiment (Fig. 4) and 7.32 mg/kg in the 20 µg experiment.

For no Cd-concentration employed a final equilibrium between Cd body loads in the shrimps and Cd-concentration in the sea water was reached. The individual variations of the body loads as depicted in Fig. 1 - 8 were highest in the 20 µg experiments and seemed to increase with increasing accumulation time.

Retention and loss

After 20 days of exposure to the respective cadmium concentration test animals were transferred to seawater without additional cadmium. Test conditions remained unchanged. Total body concentrations were measured 2, 4, 8, 24, 48 hours up to 12 days after the termination of the exposure.

In the 5 µg experiment there was virtually no loss of cadmium after the 20 days exposure period. Cadmium body concentrations remained constant over a period of 7 days in the range of 2.25 mg Cd/kg (Fig. 6).

The same was found in the 10 µg experiments where the cadmium body concentrations remained constant in the range of 2.6 mg Cd/kg over a period of 12 days (Fig. 7).

Only in the 20 µg experiment there was significant loss of cadmium in the first 3 days after the termination of the exposure, after this period body concentrations again were constant in the range of 4.3 mg Cd/kg (Fig. 8).

IV. Discussion

The shape of the uptake curves found in my experiments indicates the action of two different mechanisms of uptake.

The first part of the uptake curve suggests a superimposed adsorption and absorption curve.

In experiments on the accumulation of Cd by eggs of fishes the high initial uptake of Cd was interpreted as adsorption of Cd to the surface of the chorion of the fish eggs due to complexation (WESTERNHAGEN and DETHLEFSEN,1975; WESTERNHAGEN et al,1974; 1975) PENTREATH (1976) found high initial concentrations of ^{65}Zn adsorbed to eggs of Pleuronectes platessa. The rapid initial uptake was always followed by a period of steady loss of the metal or radionuclide from the fish eggs. After hatching a linear uptake of Cd of ^{65}Zn in the larvae could be measured.

In the first few hours after the beginning of the exposure the uptake processes are dominated by adsorption (Physicochemical sorption, BRYAN,1971).

For Callinectes sapidus it could be shown that metal concentrations on the carapace reached a certain level and remained high possibly representing a saturation of binding sites (HUTCHESON,1974). This concentration of cadmium on the exoskeleton is agreed to be due to adsorption (BRYAN,1971).

In my experiments early equilibria of the total Cd body loads of the shrimps were reached after 8 - 24 hours. After a rapid uptake and the building of an early plateau in all experiments a loss of cadmium from the shrimps occurred. This loss might be due to the fact that an incipient oversaturation of available binding sites is reduced in the further course of the experiments.

Minimum concentrations were reached at day 1 (5 µg/l) and day 3 (10 and 20 µg/l) after the beginning of the experiments.

The assumption that the first part of the accumulation curve is represented by predominating adsorption processes is supported by the fact that maximum body loads were the same in the 10 µg/l and 20 µg/l experiments and were not increasing with increasing exposure concentrations.

After the initial uptake the absorption seems to begin which in all experiments was linear fitting the equation y = a+bx, (Table VI) coefficients of correlation were highly significant.

Table VI: Uptake equations

	5 µg/l	10 µg/l	20 µg/l
		experiment 76/I	experiment 76/I
linear uptake	y = 1,14+0.05x	y = 0.79+0.08x	y = 0.60+0.17x
correlation coefficient	r = 0.97	r = 0.95	r = 0.97
$t = r\sqrt{\frac{n-2}{1-r^2}}$	t = 9.77	t = 6.8	t = 8.92
	p = <0.0010	p = <0.0010	p = <0.0010
		experiment 76/II	experiment 76/II
linear uptake		y = 1.68+0.08x	y = 0.63+0.22x
correlation coefficient		r = 0.92	r = 0.99
$t = r\sqrt{\frac{n-2}{1-r^2}}$		t = 5.75	t = 17.19
		p = <0.0010	p = <0.0010

The moulting cycle is of major importance to many physiological processes in crustacea. DJANGMAH,(1970)and DJANGMAH and GROVE (1970) found that blood protein and blood copper levels in Crangon crangon were closely correlated to the intermoulting cycle.

Much of the variations in the total Cd body loads of the shrimps encountered during the uptake experiments could be due to differences in the time between moulting and beginning of exposure.

The hardening of the exoskeleton and other physicological processes could contribute significantly to the uptake of cadmium not only into the exoskeleton.

When shrimps were exposed during their intermoulting phase as in my experiments the cadmium concentrations in the exuvia after the second moult were about the same as in the organisms accounting for 10 - 15 % Cd of the total body load.

From this finding no conclusions can be drawn as to the role of moulting and exuvia to the transfer of cadmium through Crangon crangon, because shrimps were not kept for periods long enough to allow for 2 or 3 moults during the exposure time.

FOWLER et al (1972) who measured concentrations of radionuclides of Co, Mn and Zn in freshly cast exuvia of Meganyctiphanes norvegica found 40 to 60 % of the radionuclides in the exuvia and 70 % in the organisms but no relation of the weight of exuvia to total organism is given. They conclude that euphasiid moults are important in the transfer of radionuclides through a marine ecosystem.

No final equilibrium between body Cd concentrations of the shrimps and the water concentrations employed could be found neither for high nor for low cadmium concentrations. This gives rise to the question wether cadmium concentrations in shrimps living in the Elbe estuary reach an equilibrium with ambient Cd levels.

The accumulation factor at the end of the 5 µg experiment (total body concentration in the shrimp on a wet weight basis divided by the concentration in the test water) was found to be 2400.

Corresponding accumulation factors for shrimps living in the Elbe estuary were also 2400. Basis for this figure was a medium body load of 0.5 mg/kg (dry weight) and a concentration of Cd in Elbe water of 1.0 µg/l, measured with the methods applied in this investigation. Average water content of Crangon crangon in my investigation was 79,1 %.

This finding indicates that cadmium body concentrations of shrimps living in the Elbe estuary do not reach a final plateau.

Only in the 20 µg experiment there was a significant loss of cadmium from shrimps who had been exposed to 20 µg Cd/l for 20 days and afterwards were transferred to clean seawater.

But the cadmium levels in the shrimps reached at the end of the experiment were still high representing the level that has been reached after 15 - 17 days of uptake. Loss followed an exponential curve of the type $y = a \cdot e^{-bx}$, which was also found by WILSON et al (1975) and ADEMA and COOPMAN (1972) in experiments on the loss of dieldrin by Crangon crangon.

In the lower concentrations employed in my experiments there was very little loss and in the 5 µg Cd/l experiments virtually no elimination or loss of cadmium from the shrimps was found. This very effective retention of cadmium after exposure to low concentrations of cadmium in water could of course be due to the relatively short duration of elimination experiments.

The reaching of a plateau in the 20 µg experiment after 3 days of loss indicates that mechanisms of cadmium excretion are not effective enough to eliminate cadmium to levels as low as at the beginning of the exposure.

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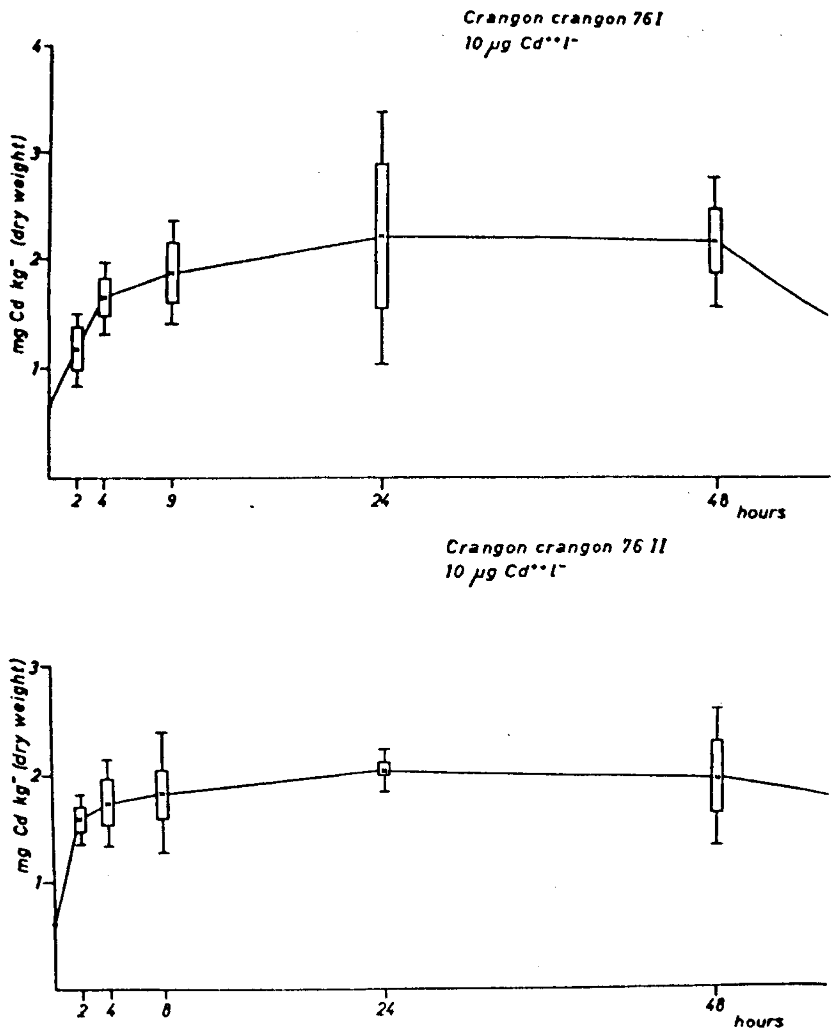


Fig. 1 Short time accumulation of Cd by Crangon crangon exposed to 10 µg Cd/l, mg/kg total body load, dry weight, means, standard deviations and standard errors.

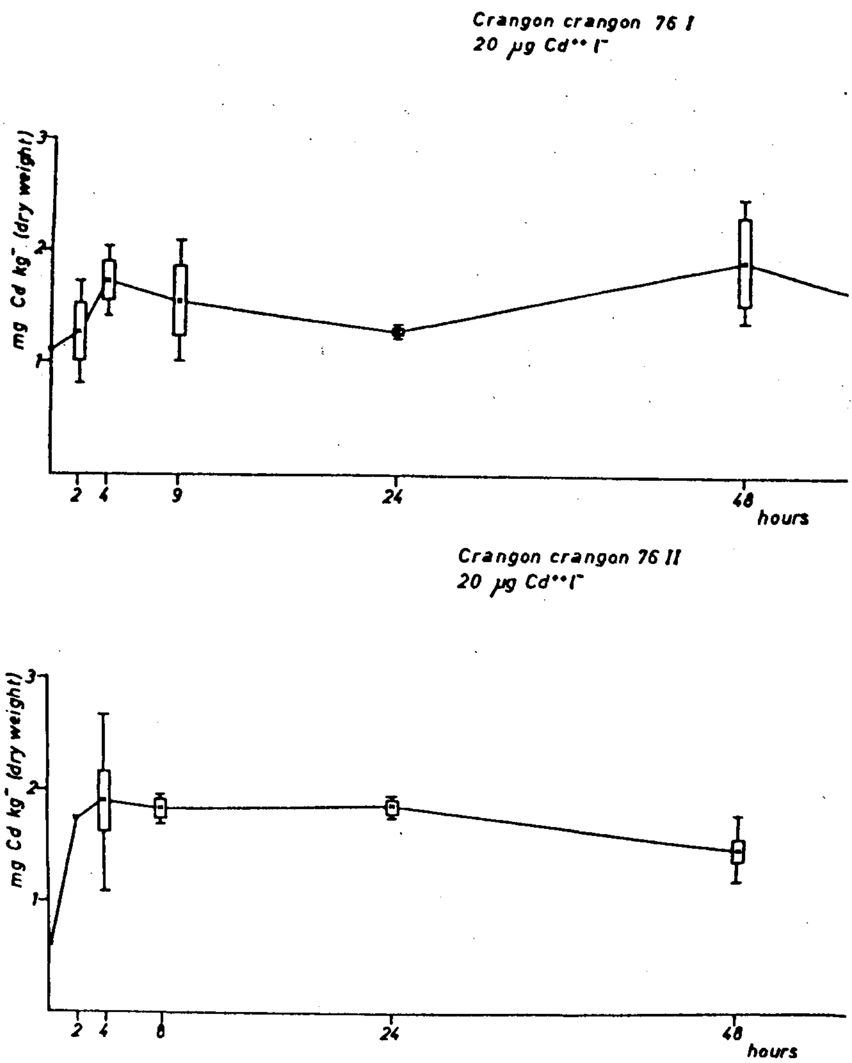


Fig. 2 Short time accumulation of Cd by Crangon crangon exposed to 20 µg Cd/l, mg/kg total body load, dry weight, means, standard deviations and standard errors.

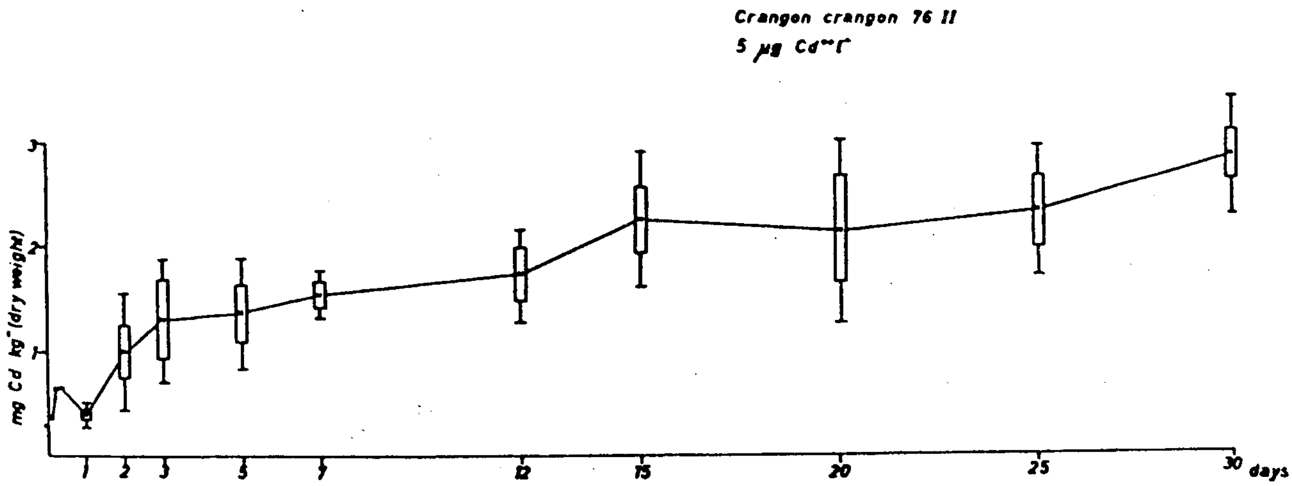
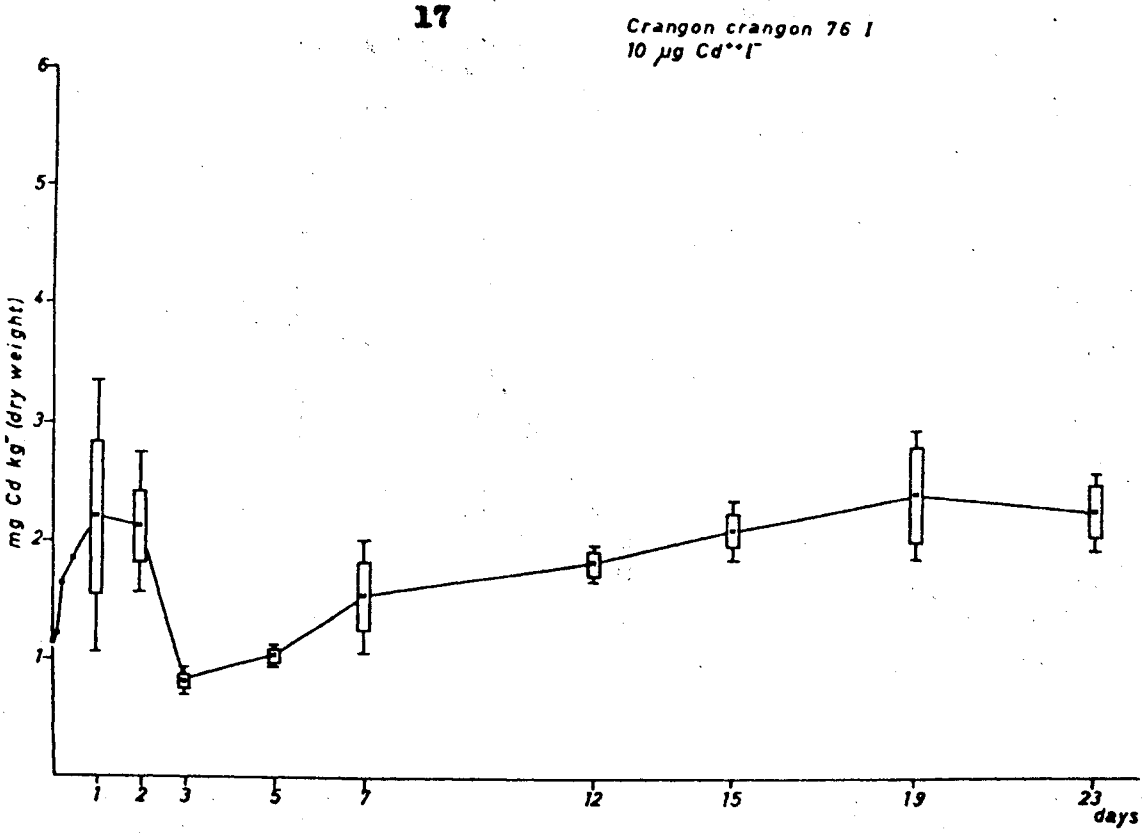


Fig. 3 Long term accumulation of Cd by <u>Crangon crangon</u> exposed to 5 µg Cd/l, mg/kg total body load, dry weight, means, standard deviations and standard errors.



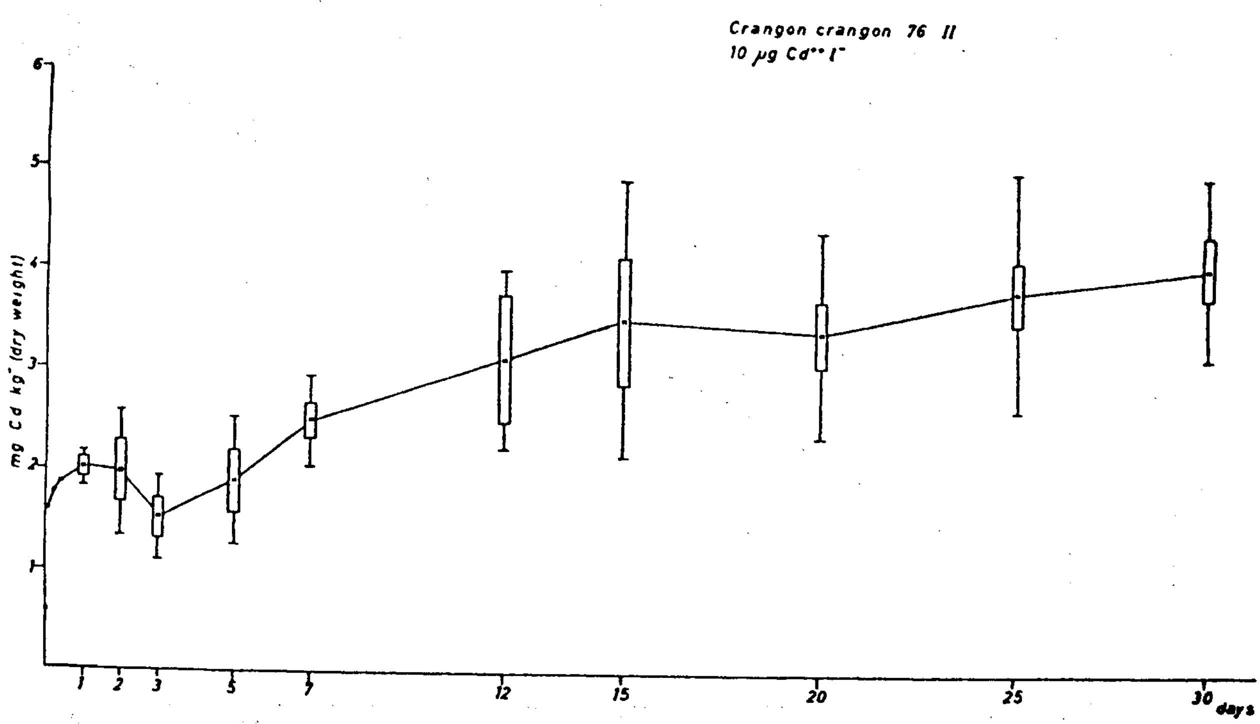


Fig. 4 Long term accumulation of Cd by <u>Crangon crangon</u> exposed to 10 µg Cd/l, mg/kg total body load, dry weight, means, standard deviations and standard errors.

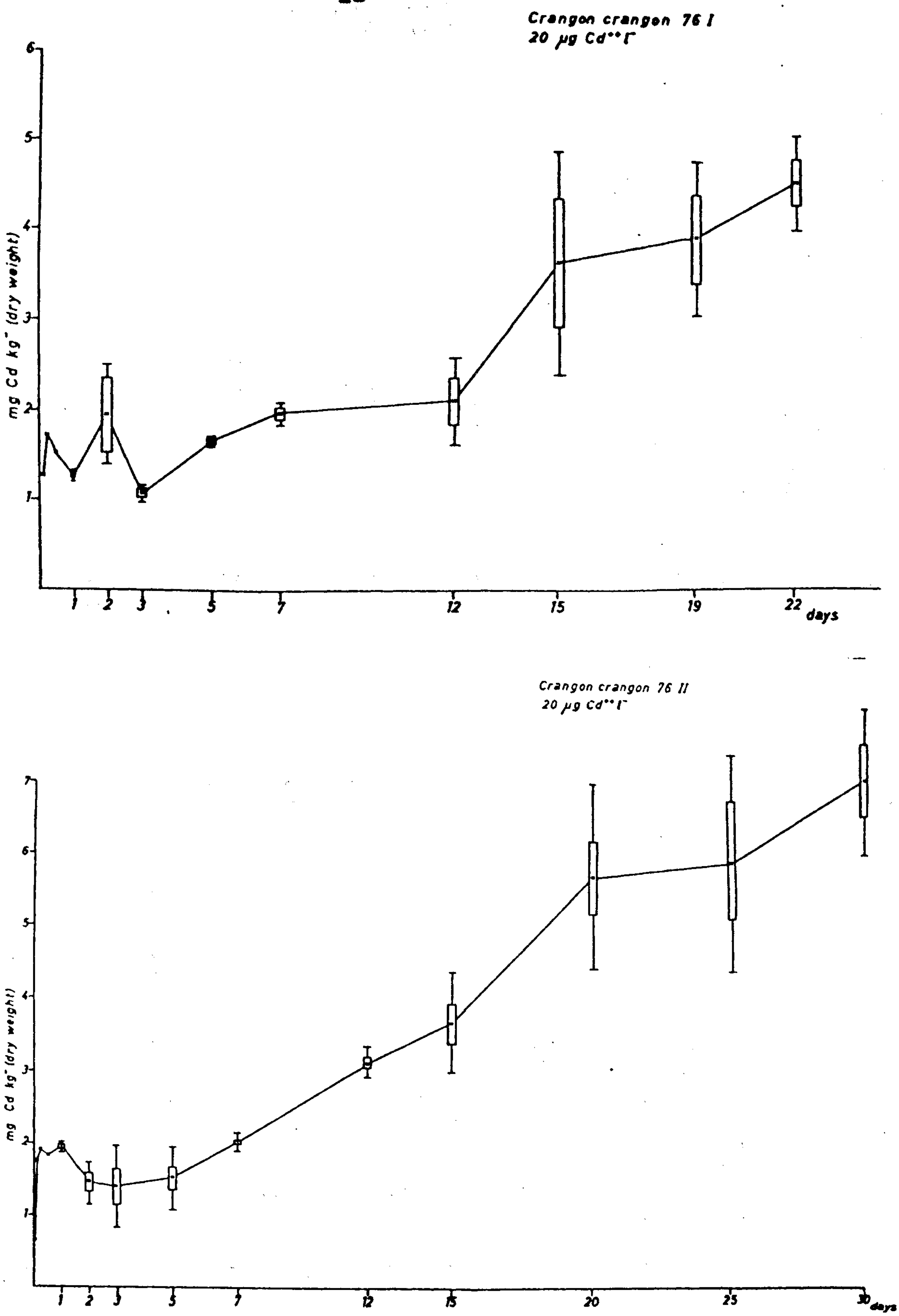


Fig. 5 Long term accumulation of Cd by <u>Crangon crangon</u> exposed to 20 μg Cd/l, mg/kg total body load, dry weight, means, standard deviations and standard errors.

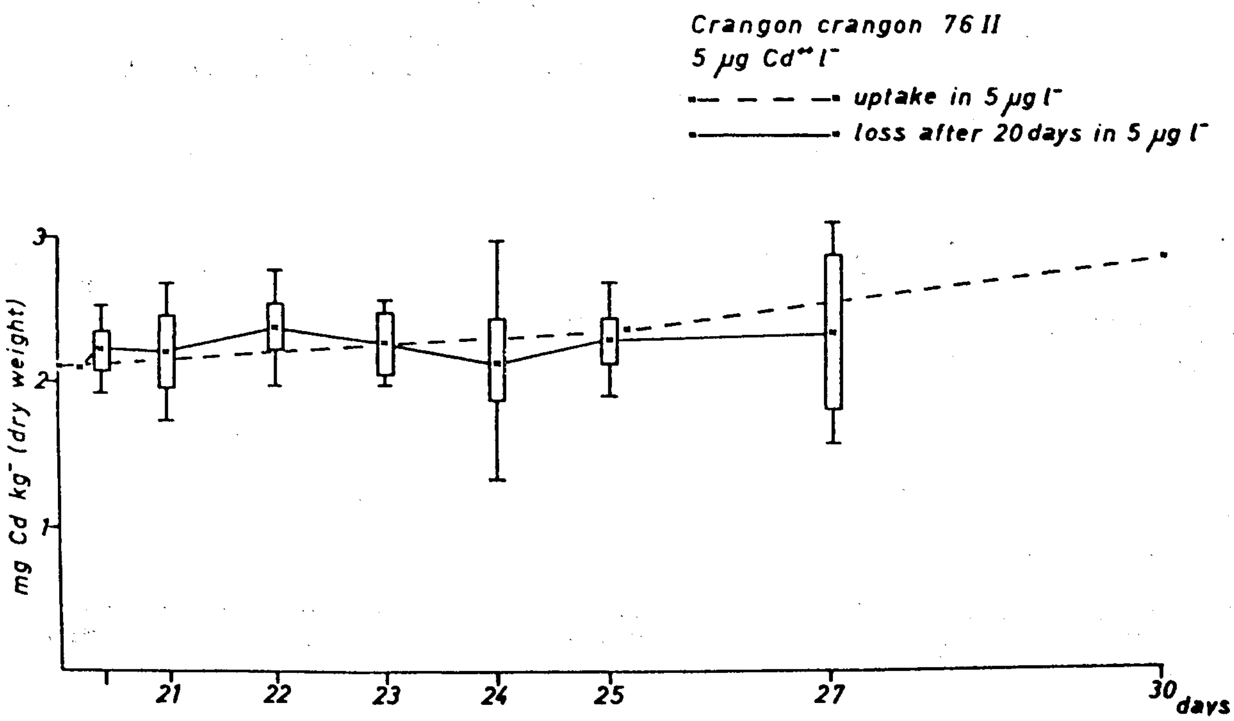


Fig. 6 Loss and retention of Cd by <u>Crangon crangon</u> after 20 days of exposure to 5 µg Cd/l, mg/kg total body load, dry weight. Dashed line represents uptake as obtained in long term accumulation experiments; means, standard deviations and standard errors.

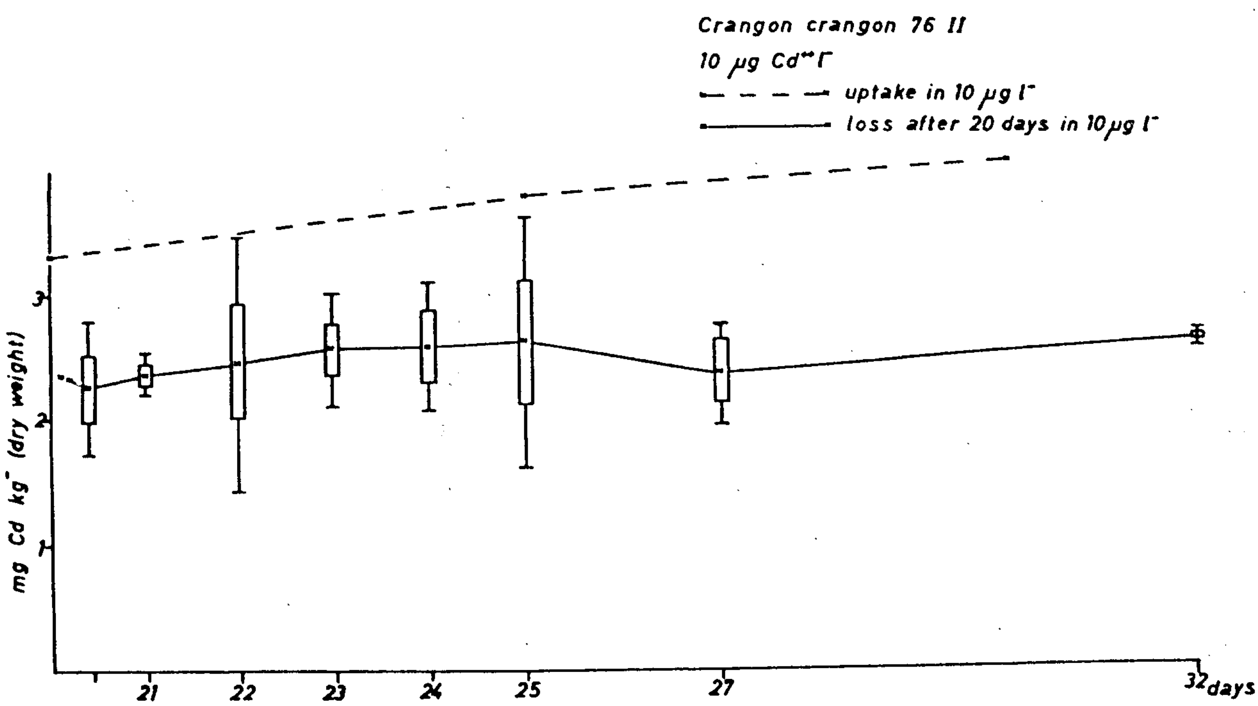


Fig. 7 Loss and retention of Cd by Crangon crangon after 20 days of exposure to 10 ug Cd/l, mg/kg total body load, dry weight. Dashed line represents uptake as obtained in long term accumulation experiments; means, standard deviations and standard errors.

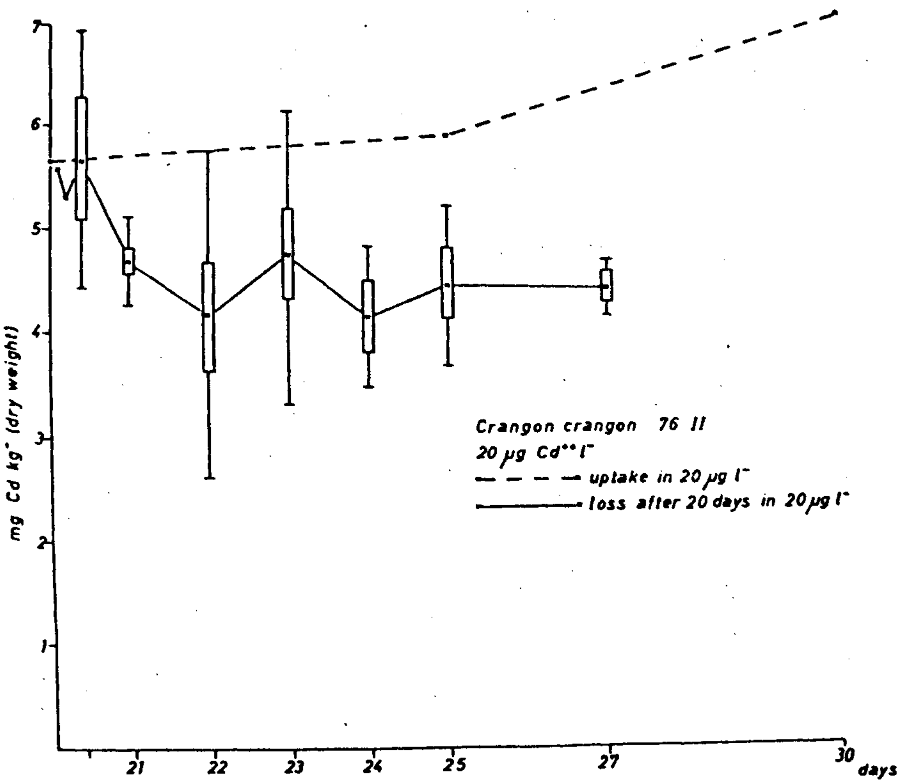


Fig. 8 Loss and retention of Cd by Crangon crangon after 20 days of exposure to 20 ug Cd/l, mg/kg total body load, dry weight. Dashed line represents uptake as obtained in long term accumulation experiments; means, standard deviations and standard errors.