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Cadmium and Zinc Accumulation and Elimination by Freshwater Crayfish

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Abstract. A theoretical compartment model for the uptake of Cd and Zn by the freshwater crayfish, *Procambarus acutus acutus* (Girard), was constructed, and included a factorially designed experiment to study the relative importance of food and water as uptake vectors for ^{109}Cd and ^{65}Zn. Food and water were important pathways for Cd accumulation and the two uptake vectors were first order, independent, and additive. The rate of Cd elimination was not significantly different from zero during either the uptake or depuration phases. Rate constants for uptake from 5 and 10 μg Cd $\cdot$ L^{-1}, calculated on a concentration basis, were 0.84 $\pm$ 0.031 and 0.83 $\pm$ 0.029 day^{-1}, respectively. Although there was no measurable Cd elimination, crayfish reached a steady state concentration of about 12.5 μg Cd $\cdot$ g^{-1}, dry weight. A small amount of Zn was accumulated via food, relative to that accumulated from water; the former had a much longer retention time than the latter, which was lost rapidly. When Zn in food was the only source, steady state was reached rapidly; Zn accumulation from food and water was not additive. Zn accumulated from water, reached no steady state. Zn uptake was proportional to water concentrations within the fed treatment groups and those not fed Zn-contaminated food. Zn elimination was first order to Zn concentration in the crayfish. Rate constants for Zn accumulation from 50 and 100 μg Zn $\cdot$ L^{-1} when fed Zn-contaminated food were 1.34 $\pm$ 0.064 and 1.46 $\pm$ 0.073 day^{-1} (estimate $\pm$ 95% CI based on concentration), respectively, while rate constants for Zn elimination were 0.034 $\pm$ 0.0021 and 0.05 $\pm$ 0.0032, respectively.

Cadmium is a toxic, non-essential trace element which occurs naturally in the environment (Cheremisinoff and Habib 1972; Fassett 1974; Rosenthal and Sperling 1974; Riordan 1976). Zinc has the same outside shell electron configuration as Cd and should act the same chemically. Zinc is a required element, but toxic when present in greater than trace amounts (Bryan 1964; Hiatt and Huff 1975). The literature on the distribution and toxicity of Cd contains few studies which elucidate environmental pathways which allow the construction of mechanistic accumulation models (Skinner *et al.* 1978). Concentrations of re-

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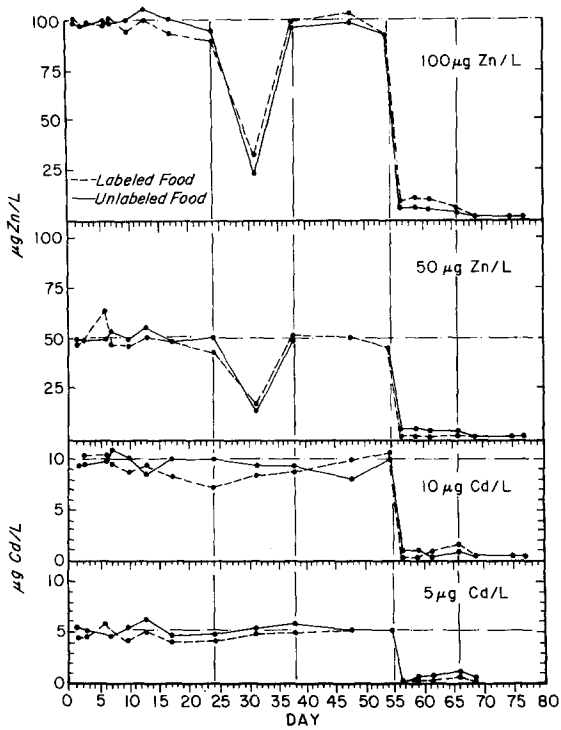

Fig. 1. Concentrations of Cd and Zn in experimental medium. Dashed horizontal line represents nominal concentration. Vertical lines represent water changes

quired elements, such as Zn in aquatic organisms, may be under more strict homeostatic control than non-essential elements, such as Cd; therefore, this study was conducted to (1) determine the relative importance of food and water as uptake vectors for Cd and Zn in crayfish, (2) construct descriptive models of Cd and Zn uptake and elimination dynamics, (3) determine uptake and elimination coefficients, and (4) to compare the derived models and coefficients to other crayfish species.

Methods and Materials

Crayfish, *Procambarus acutus acutus* (Girard), were collected from a small artesian well drainage on the Savannah River Plant near Aiken, SC., and ranged in length from 2.0 to 3.5 cm with a mean wet weight of 0.5 ± 0.1 g ($\bar{X}$ ± 95% CI). The wet/dry weight ratio was 11.1 ± 1.0 (n = 23). Organisms were acclimated for four months in individual containers before use. Crayfish (13 per tank) were exposed to Cd and Zn in water and/or food in six 12-L plexiglass tanks (29 × 42 × 10 cm, 8 L of water). Individual crayfish were held in plexiglass cylinders (5 cm I.D. × 8 cm in length), which were closed on one end with 0.6 cm mesh Nitex screen and suspended 2.5 cm above the tank bottom, to allow water circulation.

Two concentrations of Cd and Zn combined, plus a control, were prepared for exposure of crayfish to these elements from water alone. Nominal Cd and Zn concentrations were 5 μg Cd with 50 μg Zn · L^{-1} and 10 μg Cd with 100 μg Zn · L^{-1} (Figure 1). Chemical characteristics of the well water have been reported (Williams and Giesy 1978). Actual and nominal concentrations of the solutions were checked daily. Experimental solutions were removed and replaced with fresh solutions three times during the 55-day uptake period and twice during the 22-day depuration period, when crayfish were maintained in well water, to which no Cd or Zn was added (Figures 2, 3, and 4).

Crayfish were exposed to Cd and Zn with red earthworm (*Lumbricus rubellus*) feed contami-

Table 1. Cadmium and Zn concentrations and Cd/Zn ratios for worms fed to crayfish. Sample size (n) and 95% confidence intervals are indicated. Cadmium or Zn means not significantly different from one another (Scheffe's test, $\alpha = 0.05$) are denoted by the same letter (a or b)

Day	n	Cd	Zn	Cd/Zn
		$\mu g \cdot g^{-1}$ (wet weight)		
0	2[c]	2.4 ± 0.1	2.9 ± 0.1[a]	0.81 ± 0.01
6	1[c]	3.2	3.8	0.85
24	2[c]	4.4 ± 0.1	4.7 ± 0.3[b]	0.95 ± 0.03
27	9	7.4 ± 0.7[a]	3.1 ± 0.5[a]	2.5 ± 0.4
38	10	8.5 ± 3.6[a]	4.3 ± 1.7[ab]	2.1 ± 0.2
48	10	9.9 ± 1.7[a]	3.4 ± 0.7[a]	3.3 ± 1.0

[c] Each replicate of this mean represents a pooled sample of 10 organisms

nated with these metals. They were fed one worm weekly immediately following the first day of counting. Worms weighed 0.0106 ± 0.0013 (n = 78) g, wet weight. Worms were reared from capsules on aged compost and peat moss. Cadmium-109 and ^{65}Zn were incorporated into the worms with the culture medium moistened with stock solution once after all egg capsules had hatched. Cadmium and Zn were accumulated by the natural sorption processes of the organisms and the concentrations as well as proportions of isotope accumulated were directly the result of natural rate processes (Table 1).

Uptake and elimination of Cd and Zn were monitored in individual crayfish by measuring ^{109}Cd and ^{65}Zn activity and calculating changes in Cd and Zn concentrations from the specific activity. At each interval, individual crayfish were removed from the tanks, rinsed with well water, blotted dry, placed into a glass counting tube containing five ml of fresh well water and counted. The organisms were returned to the tanks and fed. Cadmium-109 and ^{65}Zn activities in water and crayfish were determined at each sampling interval with a 3 × 3 cm well-type NaI crystal interfaced to a 1024 multi-channel analyzer.

After the final sampling, surviving organisms were lyophilized, weighed, and wet ashed in fired porcelain crucibles with double distilled HNO_3 and 30% H_2O_2 (Thorp *et al.* 1979). Determinations of total Cd and Zn in whole crayfish were made by either flame or flameless atomic absorption spectroscopy, dependent upon the concentration. Matrix interferences and contamination were evaluated by standard additions, blanks, and measurements at an adjacent non-absorbing wavelength.

The experimental design was a 3 × 2 factorial for both Cd and Zn (3 water concentrations, contaminated and uncontaminated food). Each treatment combination consisted of 13 replicate animals. All statistical tests were conducted on population means with 95% confidence limits. Factorial main effects and interactions were examined statistically with a 2-way ANOVA and Type IV sums of squares. Because sample sizes per cell varied, due to differential mortality, multiple comparisons of means were made by the modified Tukey's HSD test (Stolinge 1978). Responses of each treatment were examined by profile analysis (Morrison 1967). Rates of accumulation for Cd and Zn were calculated, from data reported by others, as the slope of the least squares linear regression of the initial uptake. All calculations were made with the Statistical Analysis System (SAS) (Barr *et al.* 1976). Rate constants were calculated by assuming first order kinetics for uptake and depuration, respectively. All non-linear curve fitting was done by the Marquardt iterative least squares technique (procedure NLIN).

Results and Discussion

Cadmium accumulation by earthworms was continuous throughout the experiment, but Zn did not increase significantly (Scheffe's test, $\alpha = 0.05$) after an initial accumulation (Table 1). This resulted in an increase in the Cd/Zn

ratios during the experiment. Ireland and Wooton (1976) found that whole body Zn concentrations of the earthworm *Dendrobaena rubida* were not related to soil Zn concentration and suggested that Zn tissue concentrations are homeostatically regulated, a contention supported by our data, which shows increasing Cd concentrations while Zn concentrations reach a steady state rapidly. Gish and Christensen (1973) reported earthworms accumulated as much as 670 μg Zn $\cdot$ g^{-1}, dry weight from soil which is a greater accumulation than observed in the present study.

Blau *et al.* (1975) stated that the two major problems associated with the use of compartment models were the development of plausible models for a particular system and the determination of when data should be collected to decide which model was the most suitable to attain meaningful estimates of the model parameters. While many estimates of partitioning factors are available for metals, only a few estimates of uptake and elimination rate constants are available (Jorgensen 1979).

Most models of the flux of trace elements are descriptions of fluxes between pools in the organisms and environment (Willis and Jones 1976) and assume first order kinetics (Atkins 1969). Uptake and elimination are assumed to be a function of the size of the storage compartment and rate constants. The present study was not intended to model all of the possible compartments within the organism. Instead, the crayfish was considered as a unit in a larger ecological system. For this reason crayfish were treated as a single compartment.

An attempt was made to construct the most parsimonious descriptive model possible and supply empirical estimates of model parameters and develop a general model void of particular mechanisms. The accumulation of metals by crayfish can be represented by the model represented by the equation: $dQ_a/dt = Q_f \cdot K_{fa} + Q_w \cdot K_{wa} - Q_a \cdot K_{aw}$, where Q_f = mass of metal in food, Q_a = mass of metal in animal, Q_w = mass of metal in water, K_{fa} = coefficient of uptake from food, K_{wa} = coefficient of uptake from water, K_{aw} = coefficient of elimination from animal, (See Appendices I and II).

A simple case was examined in which crayfish were exposed to constant water concentrations of Cd and Zn. In field situations, exposures may be pulsed and will result in variable exposure concentrations, which will require knowledge of the duration of exposure to each concentration to construct an accurate predictive model. Elimination via the digestive tract was not suggested for either Cd or Zn and not included in the model for either metal.

Cadmium

The initial mean Cd concentration in crayfish was 1.3 $\pm$ 0.6 μg Cd $\cdot$ g^{-1} (dry weight, 95% CI, n = 10). Final Cd accumulation after 55 days was 5.7 μg Cd $\cdot$ g^{-1}, dry weight, when the only source of Cd was from food (Figure 2). Cadmium was accumulated from food and directly from the water (Figure 3). Accumulation from food and water was independent and additive (profile analysis; Morrison 1967, α = 0.05) during the periods the desired exposure concentrations were maintained.

The flux of Cd into crayfish from food was 0.008 $\pm$ 0.001 μg Cd $\cdot$ g^{-1} $\cdot$ day^{-1} (wet weight) during the first 24 days, while that from 5 μg Cd $\cdot$ L^{-1} in the

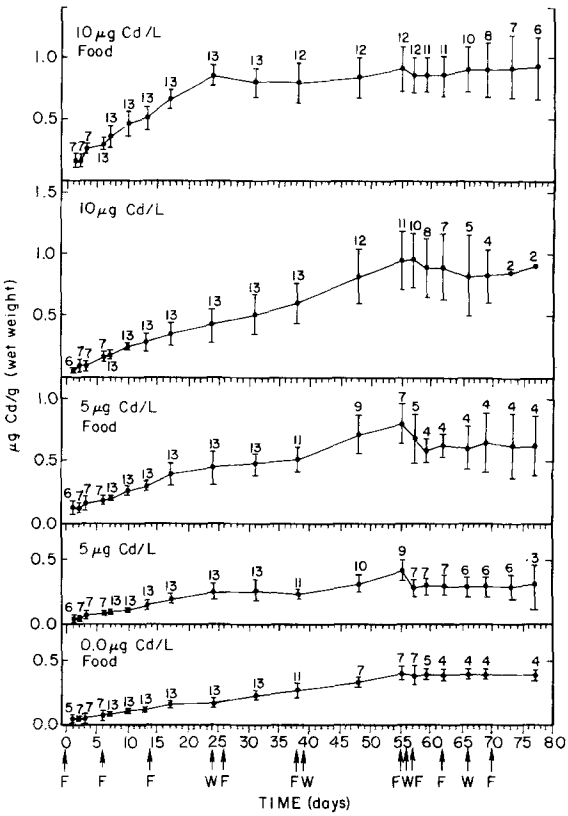

Fig. 2. Cadmium accumulation from 5 and 10 μg Cd · L^{-1} in water and from labeled worms. Food indicates that crayfish were exposed to Cd in earthworms as well as in water. Sample size (N) and 95% CI are indicated. F↑ indicates when crayfish were fed. W↑ indicates water changed

water was 0.010 ± 0.001 μg Cd · g^{-1} · day^{-1} (wet weight) during the first 24 days of the experiment (Table 2). When crayfish were exposed to Cd in both the water (5 μg · L^{-1}) and food, for 24 days the flux was 0.015 ± 0.002 μg Cd · g^{-1} · day^{-1}. The accumulation from food and water in combination was not significantly different (T-test, $\alpha = 0.05$) from the combined accumulations from food and water separately (Figure 3). Similar results were observed for organisms exposed to 10 μg Cd · L^{-1}. The flux into crayfish from 10 μg Cd · L^{-1} was greater; however, the accumulation rates from food and water were additive.

Accumulation of Cd by crayfish, from food and water was first order and independent (Equation 2, Appendix I; Figures 2 and 3). The first order rate constant for Cd accumulation from food was 0.24 day^{-1}, while that for Cd accumulation from water was 1×10^{-4} day^{-1}. The rate constants calculated from exposures to 5 and 10 μg Cd · L^{-1} were not significantly different (T-test, $P > 0.6$).

It is difficult to compare rate constants between studies, because of the inability to invoke the mass balance equation when total volumes and weights are unknown. Masses Q_w or Q_A can be converted to concentrations (C_w or C_A) by dividing by the total volume (V) or animal weight (W). Rate constants calculated with concentrations rather than masses have the units g^{-1} · L^{-1} · day^{-1} instead of day^{-1}. Rate constants obtained by the use of animal concentrations ($\mu g \cdot g^{-1}$, wet weight) and water concentrations ($\mu g \cdot ml^{-1}$) can be

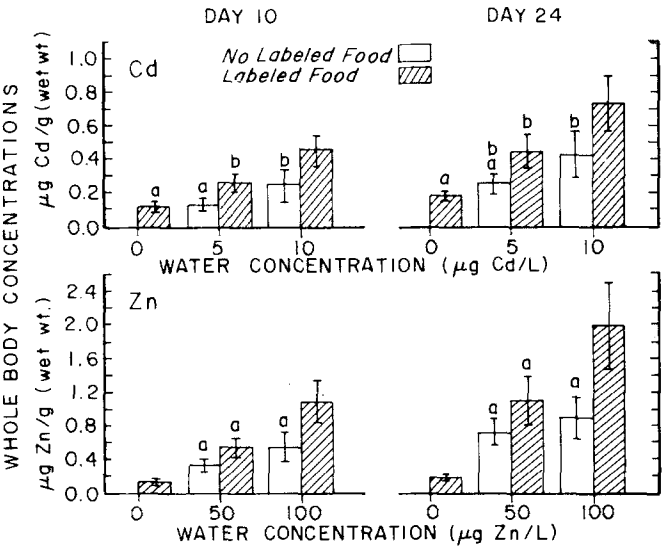

Fig. 3. Mean whole body Cd and Zn accumulation by crayfish after 10 and 24 days exposure in food, water, or both. Ninety-five percent confidence limits are indicated. Mean concentrations of Cd or Zn which are not significantly different from one another (modified Tukey's HSD test, $\alpha = 0.05$) within a sampling are denoted by the same superscripts. N = 13 for all means

compared to uptake rate constants obtained in this study by multiplying the rate constants obtained by using masses by approximately 16 (see Appendix I, Equation 4).

The results in this study are similar to those obtained for Cd accumulation by mosquitofish (Giesy *et al.* 1979), where uptake rate constants 5.9×10^{-3} and 4.8×10^{-3} L · g^{-1} · day^{-1} uptake from 5 and 10 μg Cd · L^{-1}, respectively were obtained. The coefficient for Cd uptake from food by *G. affinis* was 0.307 · day^{-1}.

Using the regression of Gillespie *et al.* (1977) for the crayfish *Orconectes propinquus propinquus,* an uptake coefficient of 0.019 L · g^{-1} · day^{-1} was determined at a water concentration of 10 μg Cd · L^{-1}, which is approximately a factor of 10 greater than the uptake coefficient observed in the present study. The coefficients for uptake from 100 and 1000 μg Cd · 1^{-1} were 0.013 and 0.046 L · g^{-1} · day^{-1} respectively (Gillespie *et al.* 1977). The fact that this study was conducted with a different species of crayfish and different water quality than that of Gillespie *et al.* (1977) may have been responsible for some of the differences observed in the accumulation rate. The rate constant for Cd accumulation by brown shrimp exposed to 5 μg Cd · L^{-1} in seawater, 0.0036 L · g^{-1} · day^{-1} (calculated from data of Dethlefsen 1978), was smaller than that observed for Cd uptake from 5 μg Cd · L^{-1} in this study. The smaller Cd accumulation may be due to a reduced available Cd pool because of Cd complexation by Cl^{-}.

Steady state Cd concentrations in aquatic macroinvertebrates are related to Cd concentration in the water up to approximately 100 μg Cd · L^{-1} (Spehar *et al.* 1978). However, the relationship is not linear, which indicates that uptake coefficients are concentration dependent and decrease with increasing Cd exposure. Uptake coefficients in this study were statistically independent of exposure concentration; however, there was a slight decrease in coefficients with increasing exposure concentration. Much higher Cd concentrations may result

in different coefficients. Gillespie *et al.* (1977) reported an effect of Cd concentration on the observed Cd uptake coefficient. These results indicate that coefficients determined at one exposure concentration may be of limited value when predicting accumulation from exposure to different concentrations; Renfro *et al.* (1975) stated that, "In laboratory accumulation studies, it is important to present contaminants to organisms in the same proportion to which they would be exposed in nature". This problem can be addressed by fitting the kinetic data to an isotherm which describes the changes in rate constant as a function of concentration to which an organism is exposed. If rate constants are concentration-dependent, they are conditional rate constants. When this phenomenon is observed, the model chosen may not adequately describe the system. One possible explanation is that the overall rate constant for uptake may be the result of several processes each of which may be the overall rate of accumulation under variable conditions.

The assumption that Cd concentration to which crayfish were exposed via food was constant was satisfied during the period of day 0 to 24, for which accumulation fluxes were studied and is not unreasonable for most environmental situations. The relationship was simplified by assuming that the rate of Cd excretion from the crayfish was zero or much smaller than the rates of uptake. This assumption was verified by the relatively low rate of excretion observed when Cd exposure via food and/or water was terminated (Figure 2, Table 2). The small, rapid loss of Cd when exposure was terminated and crayfish transferred to fresh water and fed uncontaminated food was not statistically significant (T-test, $p > 0.61$). The model was written as a single crayfish component, because the initial rapid uptake (Figure 2), assumed to be surface adsorption, was relatively small.

Cadmium accumulation was linear up to approximately 1.0 μg Cd $\cdot$ g^{-1}, wet weight (Figure 2). The Cd concentrations in crayfish exposed to Cd in food alone, food plus 5 μg Cd $\cdot$ L^{-1} or 10 μg Cd $\cdot$ L^{-1} alone did not reach steady state after 55 days exposure. Crayfish exposed to Cd in food as well as 10 μg Cd $\cdot$ L^{-1} in water reached a steady state concentration of approximately 1.0 μg Cd $\cdot$ g^{-1}, wet weight. Crayfish exposed to 10 μg Cd $\cdot$ L^{-1} without Cd contaminated food almost reached 1.0 μg Cd $\cdot$ g^{-1}, wet weight, after 55 days. The fact that only a small amount of Cd was lost from crayfish when they were transferred to uncontaminated water suggested that a constant infusion model of the form $Q_a = Q_{ss} (1 - e^{-Kaw \cdot t})$ was not appropriate. This model would generate a curvilinear function, which would approach a steady state asymptotically. It may be difficult to distinguish between linear uptake to a maximum and curvilinear approach to a dynamic steady state due to site saturation or elimination which is dependent on the body burden of Cd. Also, there is no lag phase or inflection point in Cd uptake by crayfish. Thus, accumulation of Cd is not self-damping and logistic expressions of accumulation are not appropriate. If the elimination constant is small, as in this study, the linear uptake model with boundary conditions is accurate. We postulate that this maximum applies mainly to the outside surface of the organism. The rate of accumulation from food was slow enough that it may have continued after accumulation of water ceased in the treatment where crayfish were exposed to food as well as 10 μg Cd $\cdot$ L^{-1}.

An alternative explanation to saturation of external sites could be induc-

Table 2. Linear regression estimates of rates of whole body Cd accumulation ($\mu g\ Cd \cdot g^{-1}$ wet weight$\cdot day^{-1}$) estimate (m) ± standard error (SE) of estimate with coefficient of determination (r^2) total degrees of freedom (Tdf) and probability > F for regression (P > F)

Time segment (Days)	Water $\mu g \cdot L^{-1}$ labeled food	0 Yes	5 No	5 Yes	10 No	10 Yes
	m	0.0079	0.0097	0.015	0.016	0.025
	SE	±0.0010	±0.0010	±0.0019	±0.0024	±0.0029
0–24	r^2	0.44	0.47	0.43	0.32	0.47
	Tdf	73	90	88	90	90
	P F	[a]	[a]	[a]	[a]	[a]
	m	—	0.00004	0.011	0.013	0.0038
	SE		±0.0035	±0.0012	±0.0083	±0.0080
25–38	r^2		<0.00	0.44	0.06	0.00
	Tdf		36	112	38	37
	P F		NS	[a]	NS	NS
	m	—	0.0050	0.020	0.028	0.0045
	SE		±0.0042	±0.010	±0.016	±0.012
39–55	r^2		0.07	0.17	0.12	0.00
	Tdf		20	19	24	28
	P F		NS	[b]	NS	NS
	m	0.0004	0.0018	0.00042	−0.0015	0.0041
	SE	±0.000003	±0.0024	±0.00008	±0.0004	±0.0055
57–77	r^2	0.067	0.01	0.00	0.05	0.01
	Tdf	97	41	28	37	64
	P F	[a]	NS	NS	NS	NS

[a] P > F, 0.0001; [b] P > F, 0.05; NS = P < F, 0.05

tion of Cd excretion. Cearley and Coleman (1974) suggested that fish accumulate Cd until a threshold concentration in the kidney is reached, at which time excretion is induced; a mechanism not supported by this study, since no excretion was observed once exposure had ended.

Fassett (1974) indicated that Cd is accumulated continuously and that organisms exposed to a continuous supply of Cd would not reach equilibrium. However, in this study, crayfish were apparently saturated with Cd. When exposed to 5 and 10 $\mu g\ Cd \cdot L^{-1}$ for five months in soft water, the freshwater crayfish *Cambarus latimanus* accumulated 5.0 and 7.3 $\mu g\ Cd \cdot g^{-1}$, wet weight, respectively (Thorp *et al.* 1979). The maximum Cd concentrations in this crayfish study may be less, because they were exposed for a much shorter time period. It is also possible that maximum Cd concentrations attained may be species specific. Also, the organisms in this study did not molt as in the study of Thorp *et al.* (1979).

Many animals have a non-specific metal-binding protein, metallothionein, and may have evolved a strategy of toxic metal sequestration rather than excretion. This mechanism would result in the very long biological half-lives of Cd in organisms as suggested by the very slow depuration rates observed in this study. No Cd was lost from brown shrimp (*Crangon crangon*) after Cd-exposed animals were transferred to Cd-free water (Dethlefsen 1978).

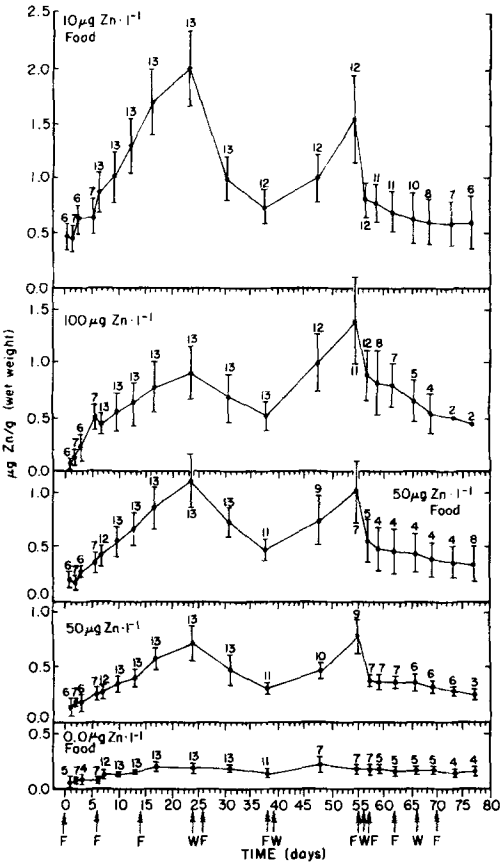

Fig. 4. Zinc accumulation from 50 and 100 μg Zn · L^{-1} in water and from labeled food. Food indicates that crayfish were exposed to Zn in earthworms as well as in water. Sample size and 95% CI are indicated. F↑ indicates when crayfish were fed. W↑ indicates water changed

The Cd accumulation curves by crayfish (*Orconectes propinquus propinquus*) obtained by Gillespie *et al.* (1977) were similar to those reported herein. However, steady state was not attained within eight days exposure to as much as 1000 μg Cd · L^{-1}. When exposed to Cd concentrations between 5 and 100 μg Cd · L^{-1}, brown shrimp *Crangon crangon* did not reach a steady state concentration during a 20-day exposure (Dethlefsen 1978).

Zinc

The initial Zn concentration of crayfish was 86.5 ± 9.9 μg Zn · g^{-1}, dry weight ($\bar{X}$ ± 95% CI, n = 10). Crayfish exposed to Zn in food alone accumulated 1.8 μg Zn · g^{-1}, dry weight after 55 days and achieved steady state after 17 days (Figure 4). There was significant (profile analysis, $\alpha = 0.05$) accumulation of Zn from both food and water (Figure 3). Approximately 22 μg Zn · g^{-1}, dry weight were accumulated from the greatest exposure, which was approximately 25% of the initial body burden. Greater steady state concentrations were observed when crayfish were exposed to 100 μg Cd · L^{-1} than when exposed to 50 μg Cd · L^{-1} (Figure 4 and Table 3).

Table 3. Steady state Zn concentrations (C_{ss}) and coefficients of uptake (K_{wa}) and elimination (K_{aw}) during exposure period (days 0–24). Estimate and asymptotic 95% confidence interval determined by Marquardt iterative non-linear least squares. N = 13. See Figure 6 and Appendix II.

Water Concentration of Zn (μg/L)	Labeled food	C_{ss} $\mu g\ Zn \cdot g^{-1}$ (wet weight)	K_{wa}[a]	K_{wa}[b]	K_{aw}
			day^{-1}		
50	no	1.5 ± 1.3	0.84 ± 0.031	$4.0 \pm 0.3 \times 10^{-5}$	0.029 ± 0.0014
50	yes	2.0 ± 1.7	1.34 ± 0.064	$6.2 \pm 0.3 \times 10^{-5}$	0.034 ± 0.0021
100	no	1.0 ± 0.4	0.83 ± 0.029	$1.2 \pm 0.4 \times 10^{-3}$	0.080 ± 0.0033
100	yes	2.9 ± 1.5	1.46 ± 0.073	$1.0 \pm 0.3 \times 10^{-3}$	0.051 ± 0.0032

[a] Based on water concentration ($\mu g\ Zn \cdot ml^{-1}$) and animal concentration ($\mu g\ Zn \cdot g^{-1}$, dry weight) assume density of water = $1\ g \cdot ml^{-1}$

[b] Based on total mass (μg Zn) in water and animal

Assuming a constant water concentration, supported by monitoring water concentrations, the accumulation model is reduced to a constant infusion model to estimate steady state masses (Q_{ss}) and coefficients of elimination (K_{aw}) during the uptake period between days 0 and 24 (Appendix II, Equations 6-8). The rate constants for elimination calculated for the depuration phase of the experiment (Appendix II, equations 9-11) were not significantly different from those determined during the uptake phase of the experiment. Good agreement between observed and predicted values was obtained with models of this form, which indicated that Zn accumulated by crayfish was self damping. Zinc-65 uptake is more linear than curvilinear; however, the exponential loss when exposure was terminated (Figure 4) suggested first order mechanisms.

Zinc accumulation from food and water was not independent (profile analysis, $\alpha = 0.05$, Figure 3). Although this mechanism is not fully understood, it may be the result of an increased gradient for excretion from the gut. Uptake from food alone was not great, but Zn-contaminated food may have reduced excretion via the gut. For modeling purposes, it is important to note that the uptake coefficients were similar between water concentrations within food treatments and independent of water concentration (Table 3). Thus, if it is assumed that crayfish exposed to Zn in water will also be exposed to Zn in food, the larger rate constants can be used. These results indicate that where animals are not fed labeled food care must be exercised in extrapolating laboratory-determined rate constants to natural situations. The variation in estimates of K_{aw} and K_{wa} is quite large, even though the Zn accumulation information is not as variable as that observed in some natural environments. Thus, predictive models with these rate constants will have low precision.

Bryan (1976) concluded that food was a much more important source of Zn than direct absorption from water, while Renfro *et al.* (1975) found that marine crabs and shrimp did not accumulate a significantly greater amount of ^{65}Zn from food and water in combination than from water alone. Accumulation of Zn through contaminated food alone resulted in a very slow rate of elimination upon termination of exposure (Figure 4). Bryan (1967) observed little Zn accumulation via the gastrointestinal tract of a freshwater crayfish but, as in this study, Zn accumulated in this manner had a long half-time for elimination.

Between days 24 and 38 of this experiment, the crayfish were exposed to reduced Zn concentrations due to loss of Zn from the stock solution (Figure 1). The reduction of Zn in the water caused a rapid reduction in total body burden of Zn (Table 3, Figure 4). The desired nominal Zn concentrations were restored on day 38 and resulted in a rapid increase in whole body Zn concentration, which supported the contention of Bryan (1967) that tissue-bound Zn is not absorbed or lost through the exterior of freshwater crayfish but is due to surface sorption and easily removed.

Unlike Cd, Zn concentrations in the body are homeostatically regulated, which results in upper and lower limits around the optimal Zn concentration.

Uptake coefficients are influenced by many environmental parameters, such as suspended solids, pH, inorganic and organic ligand concentrations, and temperature (Bryan 1976; Kinkade and Erdman 1975). Thus, cadmium and Zn accumulations should be predicted from free Cd^{++} and Zn^{++} concentrations instead of total concentrations. Water quality information is not available for the Gillespie *et al.* (1977) study, with which we compared our uptake coefficients, but the water used in our study was very soft (hardness = 10 $\mu g \cdot L^{-1}$ $CaCO_3$). It would seem that differences in hardness would not have accounted for the differences observed, since uptake was greater in the Gillespie *et al.* (1977) study.

Anderson and Brower (1978) observed no differences in Cd accumulation by *Orconectes virilis* (Hagen) due to size or sex; however, other factors, such as species, age, and reproductive state may influence metal uptake. Molt cycle is of importance to many crustacean physiological processes (Dethlefsen 1978), and must be considered as a potential complicating variable in predictive models, since susceptibility to trace metal accumulation increases immediately after molting in most crustaceans, and large proportions of the body burden of trace metals such as Zn can be lost at molting (Bertine and Goldberg 1973; Renfro *et al.* 1975). The organisms molted during the acclimation period but not during the experiment. The variability introduced by molting cycle will tend to reduce predictability of simulation models. If, in fact, there are enough differences in Cd uptake, due to organismal and environmental variation, the power of simulation models would be greatly reduced because of the lack of precise predictions without vast amounts of information on the organisms and environments of interest, as well as possible interactions.

The two elements Zn and Cd have the same electron configuration, and of all the possible elemental couplets, these elements might behave similarly. Unfortunately, for the purposes of predictive modeling, they do not behave similarly and a generalized model of metal dynamics in crayfish is not appropriate. The basic differences in the biological cycling of Cd and Zn are due to more than simple differences in rate constants. The actual mechanisms of uptake are beyond the scope of this study, but the differences should be considered when conceptualizing predictive models of the biological fates of trace elements.

The maximum Zn accumulated from the 100 μg Zn $\cdot$ L^{-1} medium resulted in a 25% increase over the initial whole body Zn concentration. Whole-body Cd concentration was increased by 830% over the initial concentration which indicated that Zn concentration was under homeostatic control while that of Cd was not. This result is more pronounced when one examines body concentrations after 77 days, when Cd and Zn exposures had been terminated for 20 days, the maximum Cd and Zn enrichments over pre-treatment values were

762% and 8%, respectively. The small loss of ^{65}Zn accumulated from food when crayfish were transferred to water and fed food containing no ^{65}Zn indicated that it was not due to faster Zn turnover rates but restricted Zn uptake.

This general pattern of Cd accumulation and Zn regulation seems to apply to other invertebrate species (Bryan and Hummerstone 1973). Anderson and Brower (1978) found that Cd concentrations in crayfish correlated with environmental concentrations, while those of Zn did not, and attributed this to the essentiality of Zn which is probably homeostatically regulated. Zinc concentrations in the crayfish *Austropotamobius pallipes pallipes* (Lereboullet) (Bryan 1967) and lobster *Homarus vulgaris* (Bryan 1964) were very constant and little affected by external concentrations. The behavior of required elements such as Zn will tend to decouple tissue or whole body concentrations of these elements from the concentrations to which organisms are exposed, thus negating the value of aquatic crustaceans as biomonitors for these elements.

It was not intended to determine the effects of Cd or Zn uptake on metabolism or *vice versa*. Other researchers have indicated that the metals are structurally similar and can compete for binding sites in metallothionein and that Cd can interfere with Zn metabolism in higher vertebrates. Neither Cd nor Zn interfered with the uptake of the other in this study or others (Merlini *et al.* 1971; Spehar *et al.* 1978). However, Thorp and Lake (1974) found that the acute toxicities of Zn and Cd to freshwater invertebrates were not additive but synergistic.

Thomann *et al.* (1974) used a trophic level model to simulate Cd distributions in Lake Erie and did not consider direct uptake of Cd from water by zooplankton and fish. The present study suggests that such a model would not be appropriate for the crayfish component of ecosystems and that pilot studies should be conducted before a theoretical model is conceptualized.

The variability in estimates of rate constants and variability between this study and those of other investigators indicate predictive models of Cd and Zn kinetics of low accuracy and generality. Sullivan *et al.* (1977) found that Cd accumulation in laboratory studies was different than under field conditions. Laboratory studies are useful for determining the relative importance of mechanisms but coefficients determined in this way are highly conditional.

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Appendix I

The relationships used to calculate rate constants for Cd accumulation and depuration, given below, are based on simplifying assumptions applied to equation 1 (see Text). Because no elimination of Cd was observed, equation 1 was simplified to equations 2 and 3.

$$J = \frac{dQ_a}{dt} = Q_w \cdot K_{wa} + Q_f \cdot K_{fa} \quad (2)$$

$$Q_a = Q_w \cdot K_{wa} \cdot t + Q_f \cdot K_{fa} \cdot t \tag{3}$$

where t = time and J = flux of Cd into crayfish. The flux (J) can be estimated by linear regression of the initial uptake with units of μg Cd $\cdot$ g^{-1} $\cdot$ day^{-1} (see Table 2).

Concentration (C_A) is related to mass (Q_A) by equation 4.

$$C_A = \frac{Q_A}{W_A} \tag{4}$$

where C_A = concentration of Cd in crayfish and W_A = weight of crayfish. A similar relationship exists between the masses and concentrations of Cd in food and water.

Appendix II

Equations for determining Zn uptake and depuration rates and steady state concentrations.

$$\frac{dQ_a}{dt} = K_{wa} \cdot Q_w - K_{aw} \cdot Q_a \tag{5}$$

Assuming a constant water concentration or relatively small change with respect to the increase in Q_a, the elimination coefficient (K_{aw}) and steady state can be calculated during a period of accumulation with equations 6-8.

$$Q_a = Q_{ss}\,(1 - e^{-(K_{aw} \cdot t)}) \tag{6}$$

where Q_{ss} = steady state mass of Zn in crayfish with partial derivatives

$$\frac{dQ_a}{dQ_{ss}} = (1 - e^{-(K_{aw} \cdot t)}) \tag{7}$$

$$\frac{dQ_a}{dK_{aw}} = Q_{ss} \cdot t \cdot e^{-(K_{aw} \cdot t)} \tag{8}$$

The elimination coefficient and minimum steady state can be calculated from periods of depuration to clean water with equations 9-11.

$$Q_a = Q_I \cdot e^{-(K_{aw} \cdot t)} \tag{9}$$

$$\frac{dQ_a}{dQ_{ss}} = e^{-(K_{aw} \cdot t)} \tag{10}$$

$$\frac{dQ_a}{dK_{aw}} = Q_I \cdot t \cdot e^{-(K_{aw} \cdot t)} \tag{11}$$

Q_I = initial mass of metal in organism.

If the assumption of constant water concentration is violated in static laboratory studies due to accumulation of Zn by the organisms the mass balance equation 12 can be used to solve a two-compartment model for the appropriate rate constants with equations 13-16.

$$A_o = Q_w + Q_a \tag{12}$$

where A_o = total mass of Zn in system

$$Q_a = \frac{K_{wa} \cdot A_o}{(K_{wa} + K_{aw})}\,(1 - e^{-(K_{wa} + K_{aw})t}) \tag{13}$$

$$Q_w = \frac{-K_{wa} \cdot A_o + A_o\,(1 + K_{wa} \cdot e^{-(K_{wa} + K_{aw})t})}{(K_{wa} + K_{aw})} \tag{14}$$

$$\frac{dQ_a}{dK_{wa}} = \frac{A_o \cdot K_{aw} - (A_o(K_{wa} + K_{aw})e^{-(K_{wa} + K_{aw})t}(1 - K_{wa} \cdot t)) + (A_o \cdot K_{wa} \cdot e^{-(K_{wa} + K_{aw})t})}{1\,(K_{wa} + K_{aw})^2} \tag{15}$$

$$\frac{dQ_a}{dK_{aw}} = \frac{A_o \cdot K_{wa}\,(e^{-(K_{wa} + K_{aw})t}\,((K_{wa} + K_{aw})\,t + 1) - 1}{(K_{wa} + K_{aw})^2} \tag{16}$$

An additional relationship (equation 17) is useful for predicting animal concentrations from water concentrations.

$$C_a = \left(\frac{K_{wa}}{K_{aw}}\right) C_w \left(1 - e^{-(K_{aw} \cdot t)}\right) \tag{17}$$

where: C_a = concentration in animal and C_w = concentration in water. At steady state conditions, where $t \rightarrow \alpha$, C_{ss}, this expression reduces to

$$\frac{C_a}{C_w} = \frac{K_{wa}}{K_{aw}} \tag{18}$$

C_a/C_w, which is the bioconcentration factor, can be calculated from the rate constants and it is not necessary to conduct experiments to steady state. Also, the steady state concentration in the animal (C_{ss}) can be calculated with equation 19.

$$C_{ss} = \left(\frac{K_{wa}}{K_{aw}}\right) C_w \tag{19}$$

The rate constants for depuration will have the same units (day^{-1}) regardless of which equation is used (equations 6, 9, 13, 14 or 17). The rate constant for accumulation, however, will be different, dependent on whether masses or concentrations are used. The rate constants calculated by using masses can be converted to those based on concentrations by multiplying the former by V/W, where V = volume of water and W = weight of animal.

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