THE ROLE OF PREY CONTAMINATION EXPOSURE HISTORY IN CADMIUM TROPHIC TRANSFER

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Abstract

Through feeding experiments with the deposit feeding oligochaete *Limnodrilus hoffmeisteri* and the grass shrimp *Palaemontes pugio*, we tested the hypothesis that prey contamination exposure history controls Cd trophic transfer. Oligochaetes were exposed to three Cd concentrations (3ng, 50μg and 150μg l^{-1}; including ^{109}Cd as a tracer) for two exposure times (one and six weeks) and were fed to shrimp. Oligochaete subcellular ^{109}Cd distributions were also determined to aid in elucidating its control on Cd trophic transfer. Shrimp ^{109}Cd absorption efficiencies (AE) increased significantly when worm Cd exposure concentration as well as exposure duration increased. An exposure to 150μg l^{-1} for six weeks was lethal to worms. Increases in shrimp ^{109}Cd AE were directly related to an increase in worm protein-bound ^{109}Cd. Calculations of net Cd transfer from oligochaete to shrimp indicated the ecologically important result of a four-fold increase when worms were exposed for six weeks to 50μg l^{-1}, over the one week treatment. This net Cd transfer was also twice that for shrimp fed worms exposed for one week to 150μg $^{-1}$. Since all oligochaetes exposed to 150μg l^{-1} for six weeks died, it could be assumed that very little Cd would be transferred under high contamination conditions.

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Introduction

The hypothesis that prey contamination exposure history influences Cd trophic transfer to predators was investigated. Metallothioneins (MT) and metal-rich granules (MRG) are mechanisms which members from many phyla use to sequester and detoxify absorbed metals (Hamer 1986; Brown 1982). Detoxification mechanisms are inducible (Cherian and Goyer 1978; Kojima and Kagi 1978; Shaikh and Lucis 1970; Brown 1977; Brown 1978) and have been shown to play an important role in controlling metal trophic transfer (Wallace 1992; Nott and Nicolaidou 1989 and 1990).

Populations of the tubificid oligochaete, *Limnodrilus hoffmeisteri*, have been shown to evolve Cd resistance and this resistance was related to Cd exposure in the field (Klerks and Levinton 1989). The most Cd resistant oligochaetes were found to possess both MT-like proteins and MRG for storing and detoxifying the metal (Klerks 1987; Klerks and Bartholomew 1991). Through feeding experiments with the grass shrimp *Palaemonetes pugio*, oligochaete contamination exposure history was investigated for its influence on Cd trophic transfer.

The ability of aquatic organisms to tolerate metal pollutants such as Pb, Hg and Cd is of considerable advantage if they are to persist in contaminated environments. Biochemical mechanisms for the detoxification of absorbed metal

is one method which organisms use to endure polluted conditions (e.g., George 1982; Brown 1982; Roesijadi 1980, Cherian and Goyer 1978; Brouwer et al. 1986). Two important and widely distributed detoxification pathways involve internal sequestration of metal by metal-binding proteins such as MT, and precipitation of metal into MRG. These detoxification and storage systems however, result in high contaminant body burdens which may be transferred to predators.

MT and MT-like proteins are a group of cysteine-rich low molecular weight proteins. The cysteinyl residues in these proteins are actively involved in metal-binding and form mercaptide bonds with a ratio of three cysteines per metal atom (Kagi et al. 1974). Strong affinities for toxic metals such as Cd and Hg is a result of this high binding capability. This binding sequesters metal from the organism's vital systems, thereby detoxifying these potentially dangerous substances (Kagi and Kojima 1987; Hamer 1986).

MRG are electron dense concretions of concentrated metal and metal salts. Granules range in size from 0.5 to > 25 μm in diameter and function in storage and excretion of metals. This storage functionally detoxifies metal by rendering it biologically inactive (see reviews Brown 1982; George 1982).

Pollutant trophic transfer is controlled, in part, by factors such as the contaminant's chemical and physical form in water, sediment and food (Sunda et

al. 1978; Luoma 1989; Bryan 1979). Detoxification mechanisms which function by altering a metal's physical properties, by binding it to MT or sequestering it within MRG, may play an important role in controlling pollutant trophic transfer.

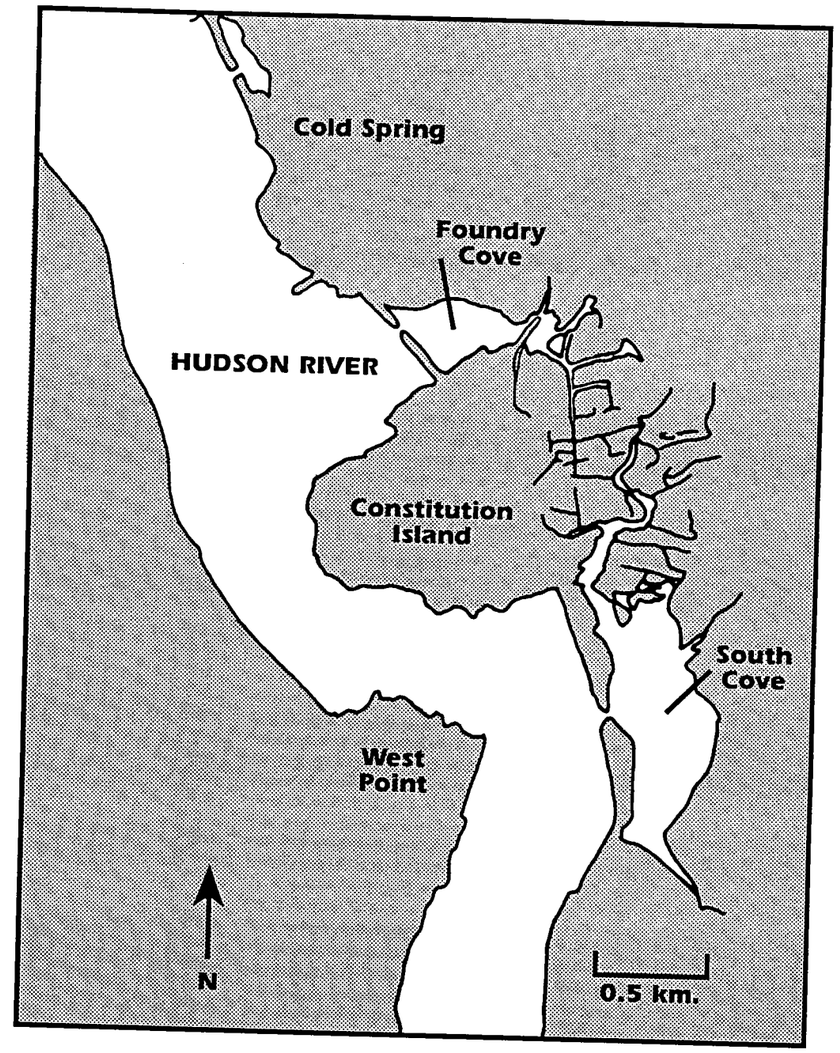
In previous research we demonstrated that grass shrimp absorb protein bound Cd with higher efficiencies than granular bound Cd (Wallace 1992). Also, Nott and Nicolaidou (1990) found that some types of MRG contained within prey pass through predators undigested.

Detoxification mechanisms, however, may not always be functioning properties of an organism's immediate biochemical capabilities. These mechanisms may have to develop through continued exposure to a pollutant by induction at the organismal level or through natural selection of the population. A selective advantage for an inducible gene to produce detoxifying proteins was suggested by Engel and Fowler (1979). And indeed, many studies have shown that sublethal metal exposure increases metal resistance by inducing MT production (Cherian and Goyer 1978; Kojima and Kagi 1978; Kagi and Shaffer 1988). Although not inducible in the short term, MRG formation has been shown to develop in chronically exposed populations with granular formation leading to increased metal resistance (Brown 1977; Brown 1978). MRG formation may also contribute to Cd resistance in a chronically exposed oligochaete population (Klerks and Bartholomew 1991).

Metal trophic transfer is influenced by prey detoxification mechanisms (Nott and Nicolidou 1990; Wallace 1992) and this detoxification is controlled by exposure conditions (Roesijadi and Klerks 1989; Brown 1977; Klerks 1987). This study was focused on determining whether the contamination exposure history of prey, in terms of the level and duration of exposure, influences Cd trophic transfer.

STUDY SITES. Foundry Cove near Cold Spring, New York is a fresh to brackish water cove located on the Hudson River, approximately 100 km upriver from the Verrazano Narrows (Fig. 1). This cove, the most severely Cd contaminated site in the world (Kniep et al. 1974, referenced in Simpson 1981), was polluted with Cd, Ni and Co by effluent discharged from a Ni-Cd battery plant between 1953 and 1971 (Resource Engineering 1983). Much of the sediment contains up to 500 μg Cd g^{-1} dry wt. (Klerks and Levinton 1989) with some sediment in the cove reaching concentrations as high as 225,000 μg Cd g^{-1} dry wt. (22% dry wt.) (Knutson et al. 1987). Although sedimentary Cd is mostly present as a relatively insoluble mixed Ca-Cd carbonate (Bondietti et al. 1974) resuspension by tidal action and activities of benthic organisms provide a continual source of Cd which is available to the cove's biota. Accumulation of Cd has been noted in Foundry Cove plants, blue crabs, killifish, and oligochaetes (Kneip and Hazen 1979; Hazen and Kneip 1980; Klerks and Bartholomew 1991).

Figure 1. Collection site for *Limnodrilus hofmeisteri* (South Cove).



South Cove, which is adjacent to Foundry Cove, did not receive the contaminated waste water, and is relatively uncontaminated.

TEST ORGANISMS. The deposit feeding oligochaete *Limnodrilus hoffmeisteri* is the most abundant macrofaunal organism inhabiting the sediments of both Foundry Cove and South Cove (Klerks 1987). Klerks and Levinton (1989) demonstrated that the Foundry Cove oligochaete population has evolved resistance to Cd, over that of South Cove worms, with adaptation resulting in high Cd body burdens. Binding of Cd to MT-like proteins and precipitation of Cd into MRG, believed to be CdS, were responsible for this elevated body burden (Klerks 1987; Klerks and Bartholomew 1991).

Lack of Cd tolerance in South Cove worms may be the result of a non-existent or poorly developed detoxification system. If detoxification differences do exist between these oligochaete populations, differences in Cd trophic transfer may occur.

The grass shrimp *Palaemonetes pugio* is an abundant benthic omnivore of marsh-cove ecosystems. This shrimp, which is distributed along the east and Gulf coasts of the United States (Knowlton 1973; Williams 1974), has a salinity tolerance of 2 to 35 ppt (Wood 1967) and includes oligochaetes (among other benthic invertebrates) in its diet (Bell and Coull 1978). *P. pugio* is a vital link in coastal water embayment food chains and is a favorite prey item of many

important commercial and recreational species (deSylva et al. 1962; Hoffman 1980; Nixon and Oviatt 1973).

Methods

COLLECTION AND RADIOLABELLING OF WORMS. Sediment was collected at low tide from various locations within South Cove in June, 1992. Adult *Limnodrilus hoffmeisteri* were sorted from a > 240 μm sediment fraction, cleaned of sediment and placed individually into multi-well culture plates containing 4 ml of GF/C filtered Hudson River water (0 ppt). After the depuration of gut contents (approx. 36 hr), worms were randomly grouped into 12 sets (about 20 worms per set), assigned to treatments and replicates, and were wet weighted in bulk. Approximate mean wet weights were determined by dividing bulk wet weights by the number of worms in each group.

Worms were exposed to Cd concentrations and exposure time as described by the 2x3 matrix design shown in Table 1. Exposure times were one and six weeks with Cd concentrations of 3ng l^{-1}, 50μg l^{-1} and 150μg l^{-1}. There were two replicates per treatment with roughly 20 worms per replicate. Individual worms were placed into 20 ml glass scintillation vials containing 5 ml of labelling solution. Labelling solutions were prepared by adding nominal levels of the gamma-emitting radioisotope ^{109}Cd (Amersham), as $CdCl_2$ in .1M Hcl, to 0.2 μm

Table 1. Cadmium versus time treatments for exposing *Limnodrilus hoffmeisteri* to stable and radioactive ^{109}Cd.

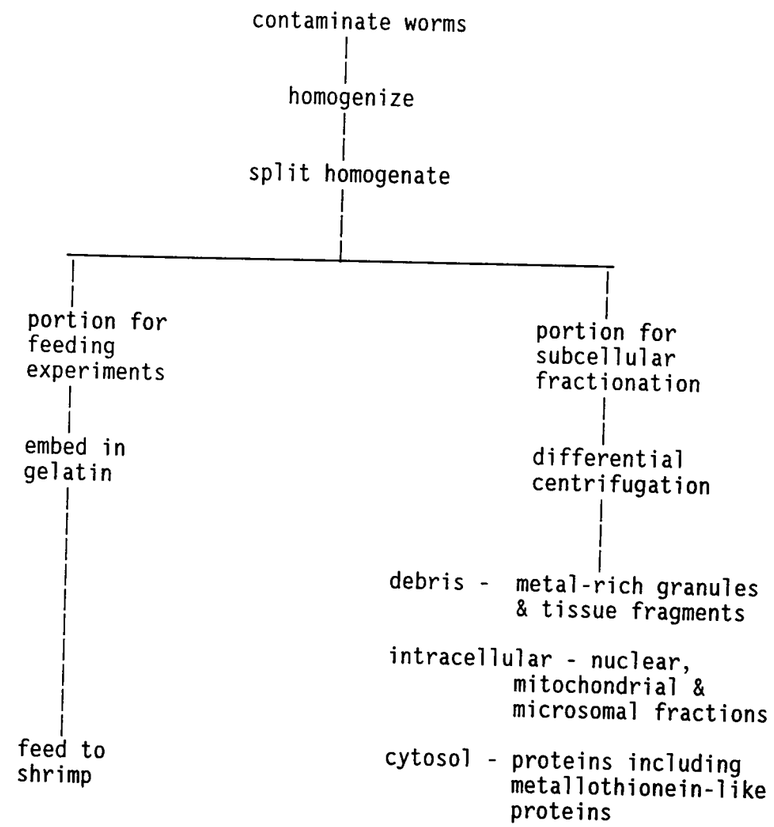
		Cadmium concentration		
		3ng l^{-1}	50μg l^{-1}	150μg l^{-1}
Exposure time (weeks)	1	A B	A B	A B
	6	A B	A B	A B

filtered Hudson River water (0 ppt) containing appropriate amounts of stable Cd, added as chloride salt.

After exposure, worms were rinsed in three separate distilled water baths, placed into gamma-counting tubes containing 1 ml of GF/C filtered Hudson River water (0 ppt), assayed for radioactivity and regrouped as per treatment and replicate. Also, 1 ml of labelling solution from each vial was assayed for final ^{109}Cd concentrations. Each group of worms, with a range in total wet weight of 10 to 50 mg, was homogenized by hand in 0.12 ml of distilled water in a glass tissue homogenizer. Homogenate was transferred to a cryogenic storage vial, assayed for total radioactivity, split into two equal portions (one portion for feeding experiments, the other for subcellular fractionation; see Fig. 2) and stored frozen (-20°C). The radioactivity of each portion was determined.

RADIOANALYSIS. All samples were analyzed for ^{109}Cd in a Pharmacia-

Figure 2. Protocol for feeding experiments and subcellular fractionation.



Wallac LKB automated gamma counter equipped with a NaI crystal. Counting times were three minutes unless noted otherwise and counting efficiency was 65%.

SUBCELLULAR FRACTIONATION. The portion used for subcellular fractionation was thawed and subjected to differential centrifugation as modified

from Klerks (1987) and Nash et al. (1981) (Fig. 2). Centrifugation resulted in a cellular debris fraction (300xg pellet, containing tissue fragments and MRG), an intracellular fraction (100,000xg pellet, containing nuclear, mitochondrial and microsomal fractions) and cytosol (100,000xg supernatant) which contained proteins. Fractions were assayed for radioactivity and stored frozen (-20°C).

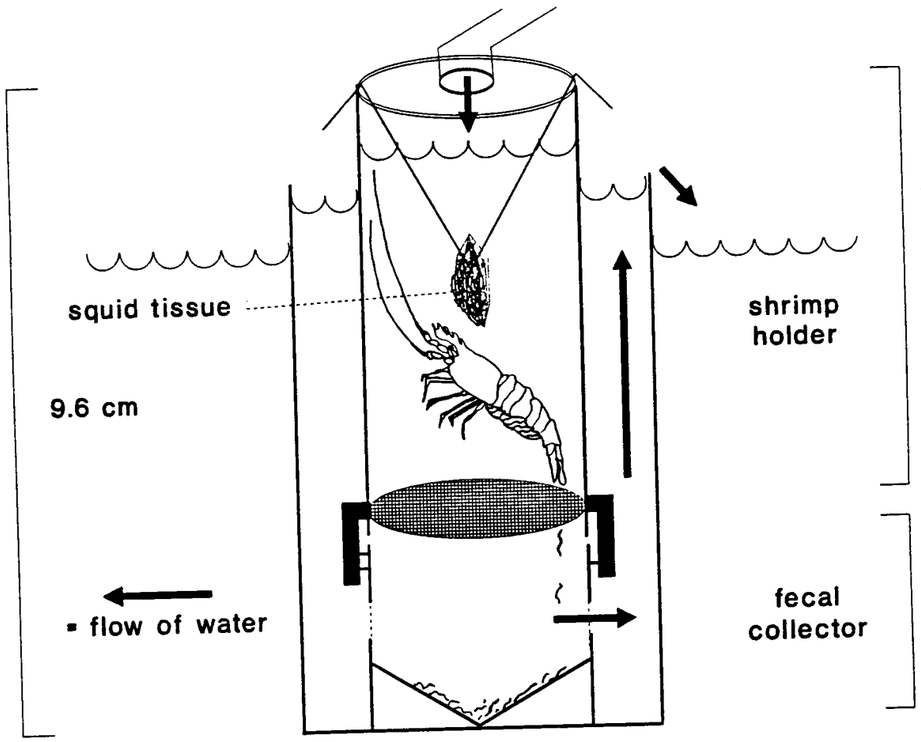
MEAL PREPARATION. The portion of worm homogenate used for feeding experiments was freeze-dried, resuspended in 0.05 ml distilled water and mixed with 0.2 ml of a gelatin solution. Six μl aliquots of the homogenate/gelatin slurry were pipetted onto pre-chilled (-20°C) 0.2 μm polycarbonate membrane filters. Filters were returned to the freezer (-20°C) and gelatin discs were allowed to set for 30 minutes. A random subset of five gelatin discs were assayed for radioactivity (counting time - 10 sec). All gelatin discs were stored frozen (-20°C) until needed (<24 hr). Gelatin discs were taken off of filters with a scalpel blade and were fed to shrimp (Fig. 2). This method, developed for our previous research, works well and does not alter Cd bioavailability (Wallace 1992).

All worm manipulations (i.e., homogenization, subcellular fractionation, and gelatin disc preparation) were performed on ice with sterile techniques. All materials used for worm manipulations (including distilled water and gelatin solution) were autoclaved for 15 minutes at 250°F and 15 psi.

SHRIMP PREPARATION. Grass shrimp, *Palaemonetes pugio*, were collected with a dip net from Great South Bay, on the south shore of Long Island, NY between June and August 1992. Adult males (20 to 23mm in length) were held in aquaria within individual mesh bottomed containers. Shrimp were monitored for molting, since crustacean feeding behavior as well as digestive enzymatic activity vary with this physiological process (O'Halloran and O'Dor 1988; Trellu and Ceccaldi 1977). Aquaria were maintained at room temperature (20-23°C), and over a two-week period shrimp were acclimated from an approximate field salinity of 25 ppt to the experimental salinity of 5 ppt. During this acclimation period, shrimp were fed daily on commercial dried fish food. Two days prior to feeding experiments, shrimp were starved, allowing for standardization of hunger levels.

FEEDING EXPERIMENTS. Two replicate feeding experiments per treatment (A and B) were run in parallel with roughly 10 shrimp per replicate. Shrimp were placed in 120 ml polypropylene specimen cups containing 65 ml of 5 ppt seawater. Radiolabelled meals were presented to shrimp for a 30-minute feeding period. Those shrimp which ate were removed from specimen cups and rinsed with distilled water. Shrimp were then placed individually into gamma counting tubes containing 5 ml of 5 ppt seawater and were assayed for radioactivity. Shrimp were kept in tubes for no more than 15 minutes.

Figure 3. Experimental chambers for monitoring the retention and egestion of ^{109}Cd from grass shrimp which have ingested Cd contaminated oligochaetes, homogenized and embedded in gelatin.



Once ingested ^{109}Cd activities were determined, shrimp were transferred to experimental chambers (Fig. 3) where they were allowed to feed *ad libitum* on squid tissue to purge radioactive gut contents. Experimental chambers consisted of two polypropylene centrifuge tubes (50 ml cap., 2.5 cm diameter) which were

cut in half and attached at the tops. Other modifications provided the shrimp holder, chamber's top half, with a false meshed bottom (mesh size - 4 mm) allowing fecal strands to fall into the bottom half of the chamber (fecal collector). This separated shrimp from their fecal strands and eliminated the possibility of coprophagy which has been noted in decapod crustaceans (Forster 1953). Also, fecal collectors had two water outflow ports covered with 150 μm mesh which allowed water to flow through the chamber but retained fecal strands. Fecal collectors could be detached from shrimp holders, allowing for fecal strand removal.

Chambers were maintained at room temperature (20-23°C) in a 75 liter glass aquarium containing 40 l of continuously aerated seawater (5 ppt salinity). Each chamber received a continuous flow of water (1.7 l hr^{-1}), supplied by a submersible pump, which flushed chambers of dissolved wastes as well as washed fecal strands into fecal collectors. Aquaria also had a closed circulating filtration system equipped with activated carbon which stripped ^{109}Cd from water.

At various times after ingesting the radiolabelled meals (i.e., 6 hr, 12 hr, 24 hr, etc.), shrimp were removed from chambers, assayed for radioactivity as described above and returned to aquarium in fresh chambers. Fecal material for all shrimp from each replicate was collected by filtering onto a single GF/C glass fiber filter. Filters with fecal material were subsequently assayed for

radioactivity. Monitoring of shrimp and fecal strand radioactivity was repeated until no further changes in shrimp ^{109}Cd retention and egestion occurred (i.e., after complete egestion of the radiolabelled meal) about 48 to 72 hr.

CONTROLS. At each sampling time, 5 ml samples of both the aquarium water and stock seawater (water used for gamma counting shrimp) were assayed for radioactivity. This was done to monitor levels of background radioactivity as well as levels of dissolved ^{109}Cd in aquarium water (i.e., ^{109}Cd leached from fecal strands or depurated by shrimp).

ABSORPTION EFFICIENCY CALCULATIONS AND STATISTICAL ANALYSIS. Shrimp ^{109}Cd absorption efficiency (AE) was calculated from initial and final whole body counts as modified from Lopez et al. (1989). Terms for AE calculations are as follows:

Initial Body-activity (S_{int}): Initial radioactivity in shrimp after ingestion of radiolabelled meal.

Final Body-activity (S_{fin}): Radioactivity remaining in shrimp after cessation of radiolabelled feces production.

Percent A.E.s were calculated as follows:

$$(S_{fin}/S_{int}) \times 100 = AE$$

Shrimp ^{109}Cd AE and oligochaete subcellular ^{109}Cd distributions were compared among treatments. For statistical analysis, data were arcsine transformed. AE was analyzed using two-level nested ANOVA for unequal sample sizes and mean squares were pooled according to Sokal and Rohlf (1981). AE was further investigated through multiple comparisons among pairs of means (Sokal and Rohlf 1981). Oligochaete subcellular ^{109}Cd distributions were investigated as per fraction (i.e., debris, intracellular and cytosol) with single classification ANOVA (Sokal and Rohlf 1981). Oligochaete subcellular ^{109}Cd distributions were further investigated through multiple comparisons among pairs of means (Sokal and Rohlf 1981) for separate fractions (i.e., debris, intracellular, and cytosol). Where appropriate, linear regression analysis was performed on untransformed data. Prior to analysis, assumptions of ANOVA were verified (Sokal and Rohlf 1981). All data transformations and analyses were conducted using the BIOM statistical programs package (Sokal and Rohlf 1981).

Results

ABSORPTION EFFICIENCY EXPERIMENTS. When fed oligochaetes exposed for one week to 3ng l^{-1} (hereafter treatments will be referred to by week and Cd concentration, i.e., 1 x 3ng), shrimp absorbed 36.0% (±2.5; n=9; a), and 48.8% (±6.1; n=12; b) (mean $\pm SE_{mean}$; n=sample size; replicate) of the

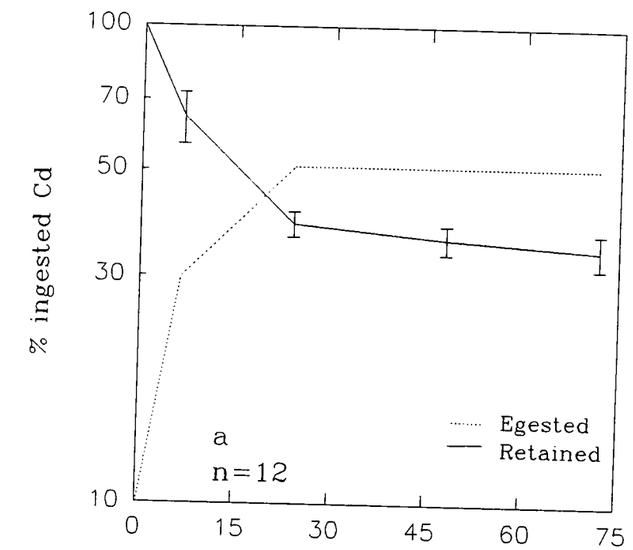
oligochaetes sequestered ^{109}Cd.

Mean ^{109}Cd retention and cumulative ^{109}Cd egestion curves for this treatment are shown in Figs. 4a and b. Two components describe Cd loss; an initial rapid loss phase and a gradual stabilization phase. Rapid Cd loss is attributed to production of radiolabelled feces and the stabilization phase is due to physiological turnover (Figs. 4a and b). This two phase ^{109}Cd loss is representative of shrimp from all treatments. Shrimp ^{109}Cd AE was calculated from retentions of ^{109}Cd at t=48 hr, as there was a 12-hour period following this time during which ^{109}Cd retention and egestion curves remained relatively stable.

Mean ^{109}Cd AE for shrimp fed worms exposed to Cd for one week are shown in Figure 5. Shrimp fed worms from 1 x 3ng and 1 x 50μg treatments had similar AE at 36.0% (±2.5; n=9; a) and 48.8% (±6.1; n=12; b) and 44.7% (±5.3; n=10; a) and 42.0% (±3.6; n=10; b) respectively. When fed worms from 1 x 150μg treatment, shrimp ^{109}Cd AE increased significantly ($P<0.01$) (Table 2) to 66.3% (±4.0; n=12; a) and 62.9% (±4.7; n=9; b).

Mean ^{109}Cd AE for shrimp fed worms exposed to Cd for six weeks are shown in Figure 6. AE for shrimp fed worms from 6 x 3ng and 6 x 50μg treatments were similar at 76.5% (±3.6; n=11; a) and 80.2% (±3.4; n=9; b) and 63.3% (±4.5; n=9; a) and 70.3% (±7.4; n=7; b) respectively. The six week AEs were significantly ($P<0.01$) higher than their one week

Figure 4. Retention (mean $\pm SE_{mean}$) and egestion (cumulative for group) of ^{109}Cd for grass shrimp which have ingested oligochaetes exposed for one week to 3ng Cd l^{-1}; replicate a (a) and replicate b (b).



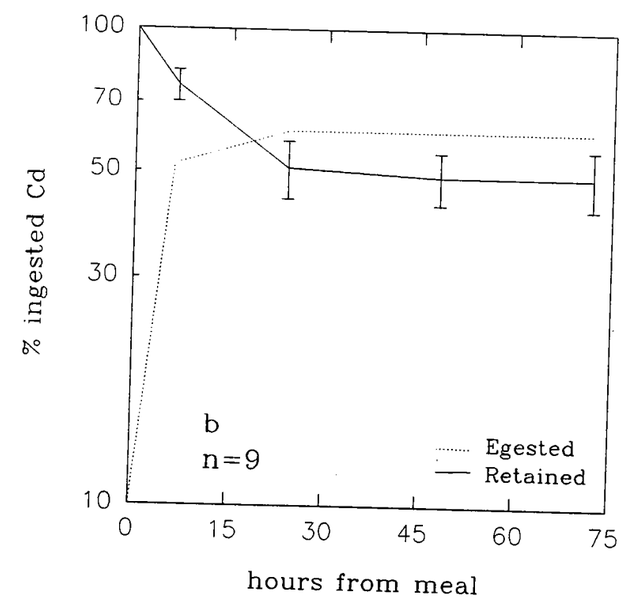
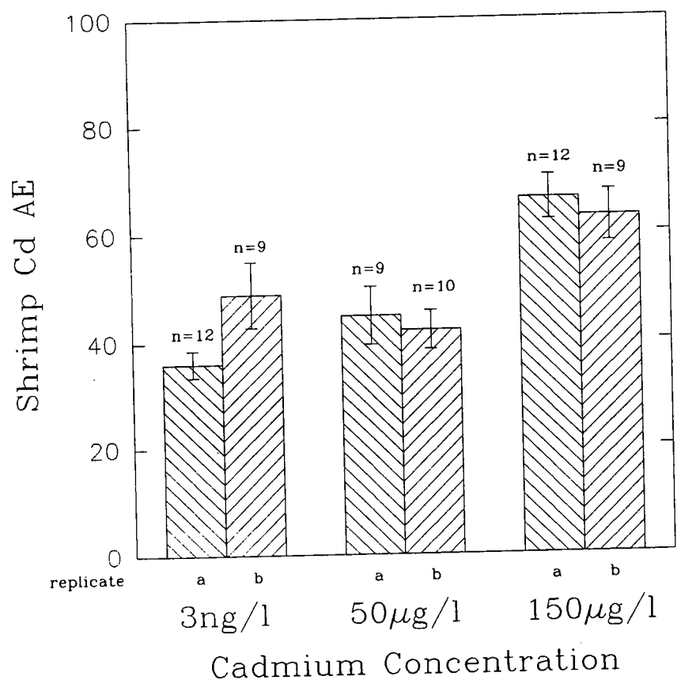


Figure 5. Absorption efficiencies (AE) (mean $\pm SE_{mean}$) for grass shrimp which have ingested oligochaetes exposed for one week to three different Cd concentrations.



counterparts (Table 2). Increasing worm Cd exposure duration to six weeks proved to be lethal for all worms in the 6 x 150μg treatment, therefore, feeding experiments using these worms could not be done. ANOVA reveals a significant ($P<0.001$) between group difference in shrimp ^{109}Cd AE (Table 3). Other results from multiple comparisons among pairs of means are shown in Table 2.

OLIGOCHAETE SUBCELLULAR ^{109}Cd DISTRIBUTIONS. Oligochaete

Table 2. Results from multiple comparisons among pairs of means for grass shrimp ^{109}Cd absorption efficiencies (AE) and oligochaete subcellular ^{109}Cd distributions.

week	[Cd]	1			6		
		3ng	50μg	150μg	3ng	50μg	150μg
week	[Cd]	-					
	3ng		-				
1	50μg			-			
	150μg	A			-		
	3ng	A	AD	A		-	
6	50μg	ADC	AD				-
	150μg	100% worm mortality					-

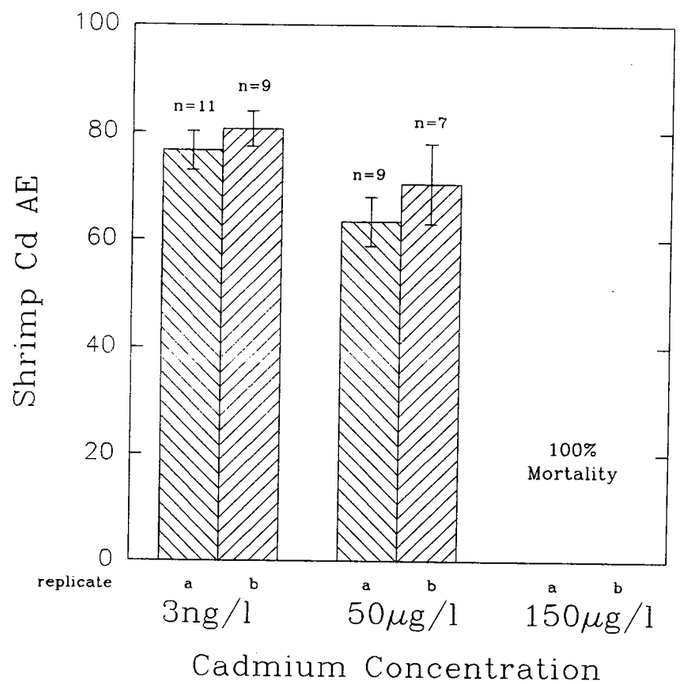
A - Pairs are significantly different at $P<0.01$ with respect to shrimp ^{109}Cd AE.

D - Pairs are significantly different at $P<0.05$ with respect to percentage of oligochaete subcellular ^{109}Cd in debris fractions.

C - Pairs are significantly different at $P<0.05$ with respect to percentage of oligochaete subcellular ^{109}Cd in cytosolic fractions.

subcellular ^{109}Cd distributions for worms exposed to Cd for one week are shown in Fig. 7. Worms from 1 x 3ng and 1 x 50μg treatments had similar subcellular ^{109}Cd distributions with 36.6% (±6.0) (mean ± SE_{mean}; n=2) and 37.3% (±2.2) of total ^{109}Cd being associated with respective debris fractions and 34.4% (±1.3) and 37.0% (±7.2) being found in the respective cytosolic fractions. When worm Cd exposure was increased to 1 x 150μg, there was a shift in worm subcellular ^{109}Cd

Figure 6. Absorption efficiencies (AE) (mean $\pm SE_{mean}$) for grass shrimp which have ingested oligochaetes exposed for six weeks to three different Cd concentrations.



distribution with debris ^{109}Cd decreasing to 22.7% (±2.7) and cytosol increasing to 51.4% (±2.2). The intracellular fractions for worms from all one week Cd exposure treatments were unaffected by exposure condition, remaining virtually unchanged at 18.9% (±4.7), 15.7% (±4.3), and 17.8% (±2.4) for the 1 x 3ng, 1 x 50μg and 1 x 150μg treatments respectively.

Oligochaete subcellular ^{109}Cd distributions for six week Cd exposure

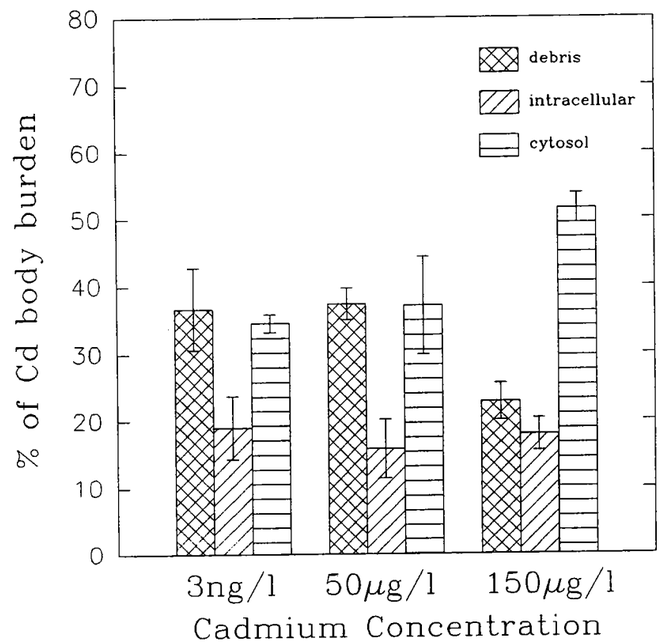
Table 3. Results from ANOVA examining shrimp ^{109}Cd absorption efficiencies.

	df	SS	MS	F_s
Among groups	4	7271.73	1817.93	19.38*** (20.28)[a]
Among subgroups within groups	5	468.89	93.77	1.04[b]
Within subgroups	88	7867.56	89.40	

***Results are significant at $P<0.001$.
[a] F_s resulting from pooling of mean squares.
[b] Not significant so mean squares were pooled according to Sokal and Rohlf (1981).

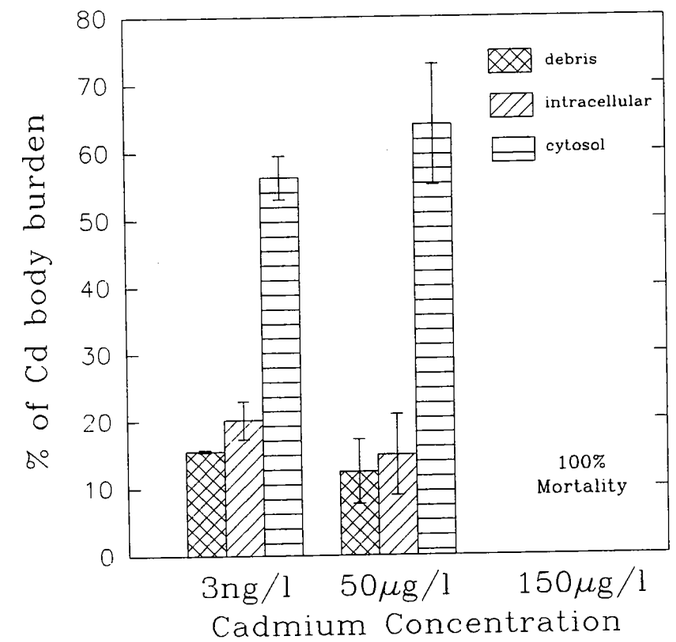
treatments are shown in Fig. 8. As noted above, all worms from 6 x 150μg treatment died prior to experimentation. When worm Cd exposure duration was increased to six weeks, there was a drastic change in oligochaete subcellular ^{109}Cd distribution. Worms from both 6 x 3ng and 6 x 50μg treatments had similar subcellular ^{109}Cd distributions with 15.5% (±.19) and 12.4% (±4.7) being found in respective debris fractions and 56.2% (±3.2) and 64.0% (±8.9) associated with respective cytosol. Changes in oligochaete subcellular ^{109}Cd distribution with increasing Cd exposure time were significant ($P<0.05$) for the 6 x 50μg treatment when compared to its one week counterpart (Table 2). Statistically significant mean pair comparisons are shown in Table 2. The intracellular fractions for both 6 x 3ng and 6 x 50μg treatments again remained relatively unchanged at 20.0% (±2.8) and 14.9% (±6.0) respectively.

Figure 7. Subcellular ^{109}Cd distributions for oligochaetes which were exposed for one week to three different Cd concentrations. Bars represent means ($\pm SE_{mean}$; n=2).



Results from ANOVA examining oligochaete subcellular ^{109}Cd distributions (Table 4a-c) showed a significant ($P<0.05$) between group difference for the debris fractions (a), no significant difference between intracellular fractions (b), and although not significantly different at the standard P of 0.05 ($F_{.05\,[4,5]} = 5.19$), results for cytosolic fractions are suspect at an F_s of 5.14 (c). This F_s is extremely close to a significance at P of 0.05 and is the result of only one of ten

Figure 8. Subcellular ^{109}Cd distributions for oligochaetes which were exposed for six weeks to three different Cd concentrations. Bars represent means ($\pm SE_{mean}$; n=2).



possible mean pair comparisons being statistically different ($P<0.05$) (Table 2).

<u>RELATIONSHIP BETWEEN SHRIMP ^{109}Cd AE AND OLIGOCHAETE SUBCELLULAR ^{109}Cd DISTRIBUTION</u>. Changes in shrimp ^{109}Cd AE with increasing worm Cd exposure to lethal concentrations, as was the case for 1 x 150μg treatment, or continuing worm Cd exposure for six weeks at sublethal levels, such as the 6 x 3ng and 6 x 50μg treatments, can be explained more

Table 4. Results from ANOVAs examining oligochaete subcellular ^{109}Cd distributions; (a) debris, (b) intracellular and (c) cytosol fractions.

(a) debris	df	SS	MS	F_s
Among groups	4	503.23	125.83	8.64*
Within groups	5	72.77	14.55	

(b) intracellular	df	SS	MS	F_s
Among groups	4	24.64	6.16	.27
Within groups	5	11.87	22.37	

(c) cytosolic	df	SS	MS	F_s
Among groups	4	436.38	109.09	5.15[a]
Within groups	5	105.75	21.15	

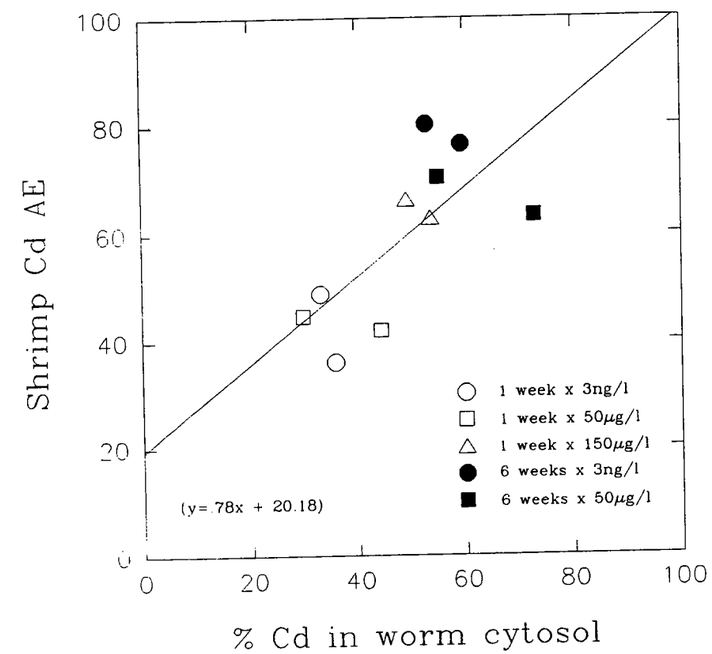
* Results are significant at $P<0.05$.
[a] Results are significant at $P<0.1$ but are very close to being significant at $P<0.05$ ($F_{.05[4,5]} = 5.19$).

clearly by Fig. 9. As the percentage of ^{109}Cd associated with oligochaete cytosol increases, there is a corresponding increase in shrimp ^{109}Cd AE ($y = .78x + 20.18$; slope not statistically different from 1).

To further clarify the effects of contamination exposure history on Cd trophic transfer, net Cd transfer from oligochaete to shrimp was investigated. Net Cd transfer was calculated with the following equation:

$$N_{tr} = W_{Cd} * AE$$

Figure 9. Relationship between oligochaete cytoplasmic ^{109}Cd distribution and grass shrimp ^{109}Cd absorption efficiency (AE).



Where N_{tr} is equal to the net Cd transfer from worm to shrimp (gram Cd per gram worm wet weight; gCd gwwt.$^{-1}$), W_{Cd} is the total Cd concentration in worm (ngCd or μgCd gwwt.$^{-1}$) and AE is shrimp Cd AE.

Table 5 shows values used to determine net Cd trophic transfer from worm to shrimp. Oligochaete concentration factor (CF) was obtained from oligochaete wet weights and final ^{109}Cd water concentrations. It was assumed

Table 5. Values used for estimating net Cd transfer from oligochaetes to grass shrimp.

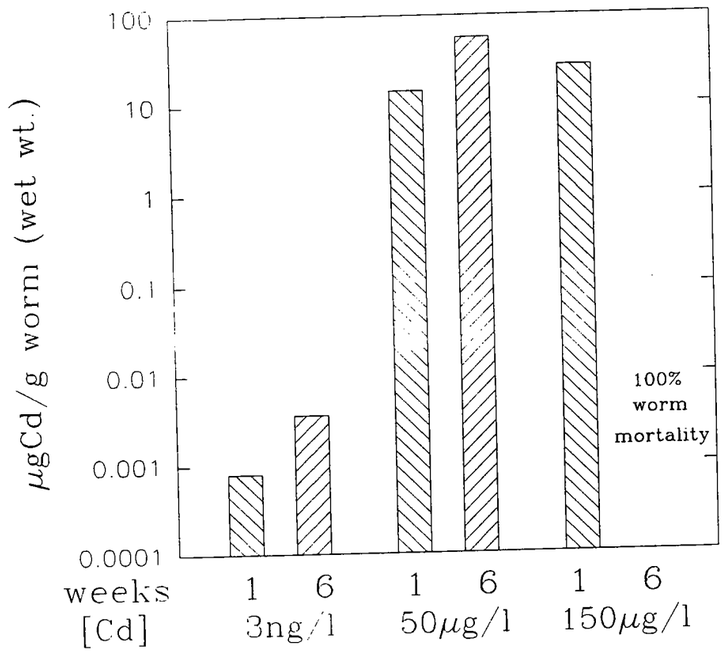
week x [Cd]	worm[a] CF	worm[b] [Cd]	shrimp[c] %AE	Net Cd transfer
1 x 3ng	664	1.9ng g^{-1}	43.3	.8ng g^{-1}
1 x 50μg	656	32.8μg g^{-1}	43.3	14.2μg g^{-1}
1 x 150μg	276	41.4μg g^{-1}	64.8	26.7μg g^{-1}
6 x 3ng	1586	4.7ng g^{-1}	78.1	3.6ng g^{-1}
6 x 50μg	1677	83.8μg g^{-1}	66.4	55.9μg g^{-1}
6 x 150μg	------ 100% worm mortality ------			

[a]Estimated from radioactivity in worm, mean worm wet weight and final water [^{109}Cd] (cpm gwwt.$^{-1}$/cpm ml^{-1}).
[b]Estimated from total [Cd] in water (Gcd ml^{-1}) and worm concentration factor (CF).
[c]Replicate shrimp ^{109}Cd absorption efficiencies were pooled and averaged.

that there was no isotopic effect, so CF for radioactive Cd was applied to total dissolved Cd. Worm Cd concentrations (W_{cd}) were calculated from estimated worm concentrations factors (CF) multiplied by total dissolved Cd concentrations.

Net Cd trophic transfer from oligochaete to shrimp for different worm exposure conditions are listed in the final column in Table 5 and shown in Fig. 10. When fed worms from the 1 x 3ng, 1 x 50μg and 1 x 150μg treatments, shrimp absorbed an estimated 0.8ng gwwt.$^{-1}$, 14.2μg gwwt.$^{-1}$ and 26.7μg gwwt.$^{-1}$, of

Figure 10. Estimations of Cd trophic transfer from oligochaetes to grass shrimp.



the worms respective sequestered Cd. When fed worms from the 6 x 3ng and 6 x 50μg treatments, shrimp absorbed 3.6ng gwwt.$^{-1}$ and 55.9μg gwwt.$^{-1}$, of the worms respective sequestered Cd.

Discussion

Marine and especially estuarine sediments can act as a sink as well as

source of pollutants from and to the water column. Sedimentary flux of pollutants can be caused by deposition and resuspension of sediments by tidal action as well as changing current regimes. Another important process in the redistribution of sediment bound pollutants is bioturbation (Boddington et al. 1979; Bothner 1973; Jernelov 1970; Karickoff and Morris 1985). Benthic organisms, especially deposit feeders, play an important role in redistributing pollutants by transporting sediment bound contaminants at depth to the sediment water interface where resuspension and oxidation can cause their release. This process can also work in reverse whereby "clean" sediment deposited at the surface buries surface sedimentary bound pollutants. Also, benthic organisms can further the release of sediment bound pollutants into the water column and interstitial water simply by activities such as burrowing (Nicholas and Thomas 1978).

These processes, among others, cause temporal and spatial changes in metal concentrations of sediment, porewater, and overlying water which can be dramatic. Metal concentrations to which benthic organisms are exposed therefore change as they burrow in the sediment. This continuously changing contaminant exposure can influence metal trophic transfer if benthic organisms can alter their detoxification capabilities in accordance with their environment.

This present research has shown that prey contamination exposure history

plays a key role in determining pollutant trophic transfer. Increasing worm Cd exposure in the short term (one week) from trace amounts, 3ng l^{-1}, to mid contamination, 50μg l^{-1}, causes no significant change in shrimp ^{109}Cd AE. A significant increase in shrimp ^{109}Cd AE however, is obtained when worm Cd exposure is increased to an extremely high level, 150μg l^{-1}. Continuation of worm Cd exposure for six weeks causes a significant increase in shrimp ^{109}Cd AE for both the 3ng l^{-1} and 50μg l^{-1} treatments. However, long term exposure at 150μg l^{-1} results in complete mortality of the worm population. This high mortality however, was not unexpected since the 96-hr LC_{50} for *Limnodrilus hoffmeisteri* is 170μg l^{-1} (Chapman et al. 1982).

Oligochaete subcellular ^{109}Cd distribution is not significantly changed when worm Cd exposure is increased in the short term (one week) from 3ng l^{-1} to 50μg l^{-1}. A significant change however, is observed when worm Cd exposure is increased to 150μg l^{-1}. A more dramatic shift in oligochaete subcellular ^{109}Cd distribution is achieved when exposure continues for six weeks at Cd concentrations of 3ng l^{-1} and 50μg l^{-1}.

Changes in oligochaete subcellular ^{109}Cd distribution can be caused by a number of factors. It has been well documented that exposure to toxic metals such as Cd causes the induction of MT and MT-like proteins in invertebrates (see review by Roesijadi 1981). This MT induction is most likely the causative

agent for the change in oligochaete subcellular ^{109}Cd distribution from that of the 1 x 3ng and 1 x 50μg treatments to that of the 1 x 150μg treatments distribution. In this case, there is an increase in the percentage of worm ^{109}Cd associated with cytosol, which is indicative of MT production (Rosesijadi 1982; Roesijadi and Klerks 1989).

Reasons for changes in oligochaete subcellular ^{109}Cd distribution from the 1 x 3ng and 1 x 50μg distributions to that of their 6 week counterparts however, are not as clear. MT-like proteins have been induced in organisms (i.e., the oyster *Crassostrea virginica*) at a Cd concentration of 50μg l^{-1} as soon as 4 days after initial exposure (Roesijadi and Klerks 1989). MT induction may therefore be responsible for the 6 x 50μg treatments shift in subcellular ^{109}Cd distribution. It is unlikely however, that MT production was induced in worms from the 6 x 3ng treatment.

A more reasonable explanation for the increase in percentage of ^{109}Cd associated with oligochaete cytosol for the 6 x 3ng and perhaps the 6 x 50μg treatment may involve preferred binding sites within *Limnodrilus hoffmeisteri* for Cd. In both the 1 x 3ng and 1 x 50μg treatments, ^{109}Cd was equally distributed between debris and cytosolic fractions. It may be that as preferred binding sites in the debris fraction become occupied with Cd, spillover of Cd into the cytosol occurs. This spillover of toxic metal into an organisms cytoplasm was found by

Winge et al. (1973) for rats exposed to Cd, by Brown and Parsons (1978) for salmon and zooplankton exposed to Hg and by Roesijadi (1982) for oysters exposed to Cd. Presently, it is unclear what role the intracellular fraction has in the redistribution of subcellular ^{109}Cd. There may be mechanisms, which are responsible for transporting Cd between the debris fraction and the cytosolic components, within this fraction which are continuously saturated with Cd. However, this should be investigated further.

Increases in shrimp ^{109}Cd AE with increases in percentage of ^{109}Cd associated with oligochaete cytosol (Fig. 10) suggests that all protein bound ^{109}Cd was absorbed by shrimp with ^{109}Cd from other pools (i.e., debris and intracellular) being available to lesser degrees. A similar result was found by Reinfelder and Fisher (1991) for a number of trace metals with copepods feeding on phytoplankton.

Through our previous research, we have obtained ^{109}Cd AE for shrimp feeding on isolated oligochaete subcellular fractions (e.g., debris; containing MRG, intracellular and cytosol; containing proteins). Shrimp ^{109}Cd AE were as follows: debris (48%), intracellular (72%) and cytosol (85-100%) (Wallace 1992). Although these ^{109}Cd AE were obtained for shrimp feeding on subcellular fractions isolated from Foundry Cove worms (Cd resistant population) and may not be directly applicable to the present study, it is clear that they provide some

insight as to the determining factors controlling shrimp ^{109}Cd AE.

Prey contamination exposure history has a profound and dramatic effect on Cd trophic transfer. In the short term (one week), net Cd transfer from worm to shrimp increases with increasing worm Cd exposure. When worm Cd exposure duration is increased to six weeks, net Cd transfer is increased by a factor of four for both the 3ng l^{-1} and 50μg l^{-1} treatments. The four-fold increase in net Cd transfer for the 3ng l^{-1} treatment however, is not ecologically significant since these are levels comparable to background concentrations. A four-fold increase in net Cd transfer at the 50μg l^{-1} level however, is more important ecologically since μg concentrations are of much greater concern. Increasing worm Cd exposure to lethal concentrations, 150μg l^{-1}, for six weeks results in a worm/shrimp net Cd transfer of zero. Although it is unlikely that no Cd would be available to higher trophic levels in an environment with such high contamination, it does indicate that bioavailability of Cd from lower trophic levels would be greatly reduced due to lethal effects of the contamination. However, this is by no means an advocation of increasing environmental pollution.

Conclusions

Laboratory investigations of pollutant trophic transfer that do not give

proper attention to the contamination exposure history of prey can be very misleading. Many organisms have detoxification mechanisms which can be "turned on" in very short periods of time while others can develop through natural selection. If studies on pollutant trophic transfer use test organisms from naive populations, recommendations made and conclusions drawn from such studies may be inappropriate or inaccurate when considering the effects of pollutants on resistant populations.

The subcellular distribution of pollutants within prey has an important role in determining pollutant trophic transfer. Any studies investigating factors controlling subcellular pollutant distribution will aid in our understanding of how these potentially dangerous substances impact food chains and communities.

This present study has shown that prey contamination exposure history has a key role in determining the subcellular distribution of pollutants and that this subcellular pollutant distribution controls pollutant trophic transfer. Presently it is unclear whether increases in the percentage of Cd associated with oligochaete cytosol were due to MT production or to metal spillover into cytoplasm. To gain a complete understanding of how prey contamination exposure history controls pollutant trophic transfer this process should be investigated further.

It has been established that prey detoxification capabilities control pollutant trophic transfer (Wallace 1992; Nott and Nicolaidou 1990).

Detoxification mechanisms not only play an important role in controlling pollutant trophic transfer, but may also have a role in controlling the toxicity of pollutants ingested by predators. The role which prey detoxification mechanisms play in controlling toxicity of pollutants ingested by predators would be a logical next step in furthering our understanding of how anthropogenically induced increases in environmental pollutants impact food chains in aquatic systems.

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