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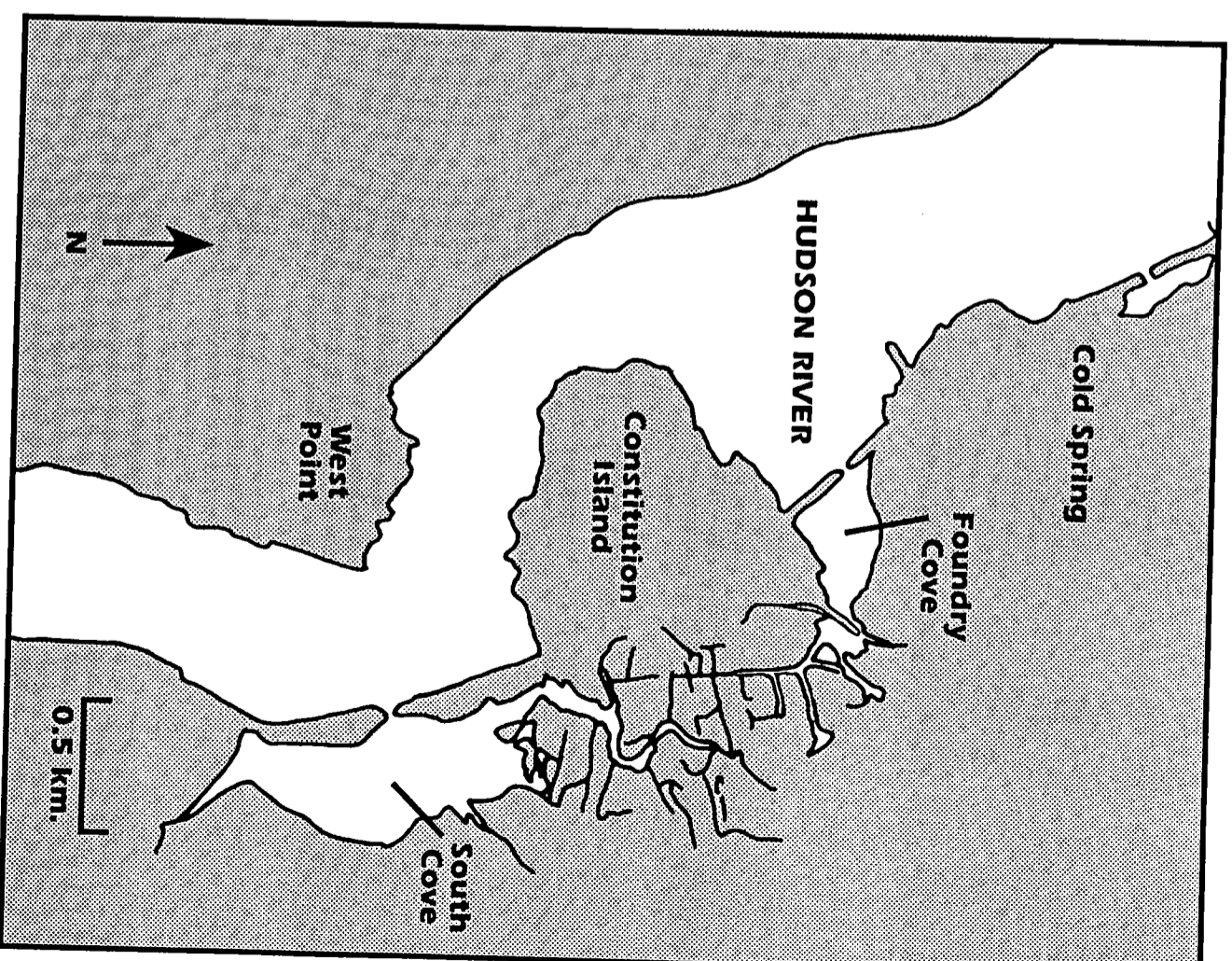
Figure 1. Collection site for *Limnodrilus hoffmeisteri* (South Cove).

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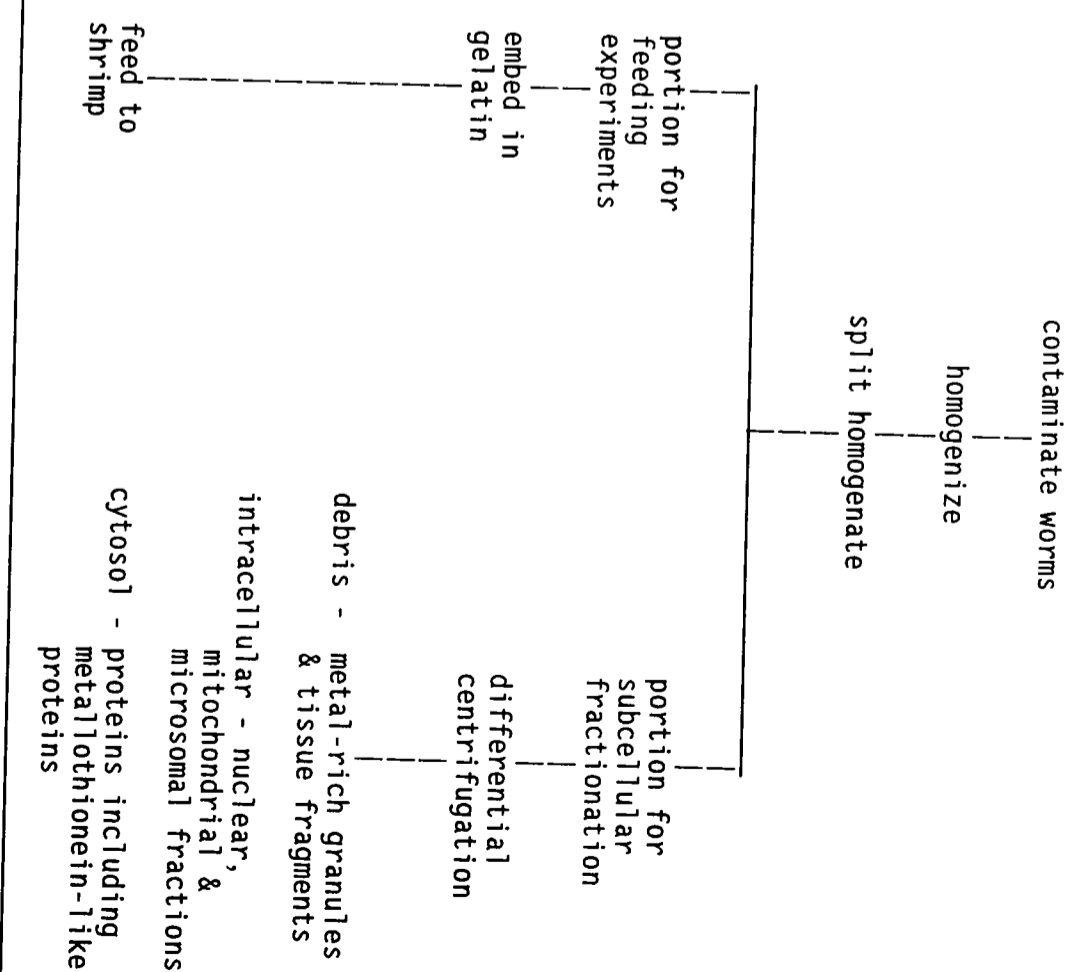
Exposure time (weeks)		Cadmium concentration		
		3ng l^{-1}	50µg l^{-1}	150µg l^{-1}
1	A B	A B	A B	A B
6	A B	A B	A B	A B

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-

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Figure 2. Protocol for feeding experiments and subcellular fractionation.


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                          contaminate worms
                                 |
                             homogenize
                                 |
                          split homogenate
                      _____|_____
                     |                     |
              portion for            portion for
              feeding                subcellular
              experiments            fractionation
                  |                        |
              embed in                differential
              gelatin                 centrifugation
                  |             _____|_____
              feed to          |              |                      |
              shrimp        debris -      intracellular -        cytosol -
                            metal-rich    nuclear,               proteins including
                            granules      mitochondrial &        metallothionein-like
                            & tissue      microsomal             proteins
                            fragments     fractions
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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.

squid tissue

9.6 cm

→ flow of water

shrimp holder

fecal collector
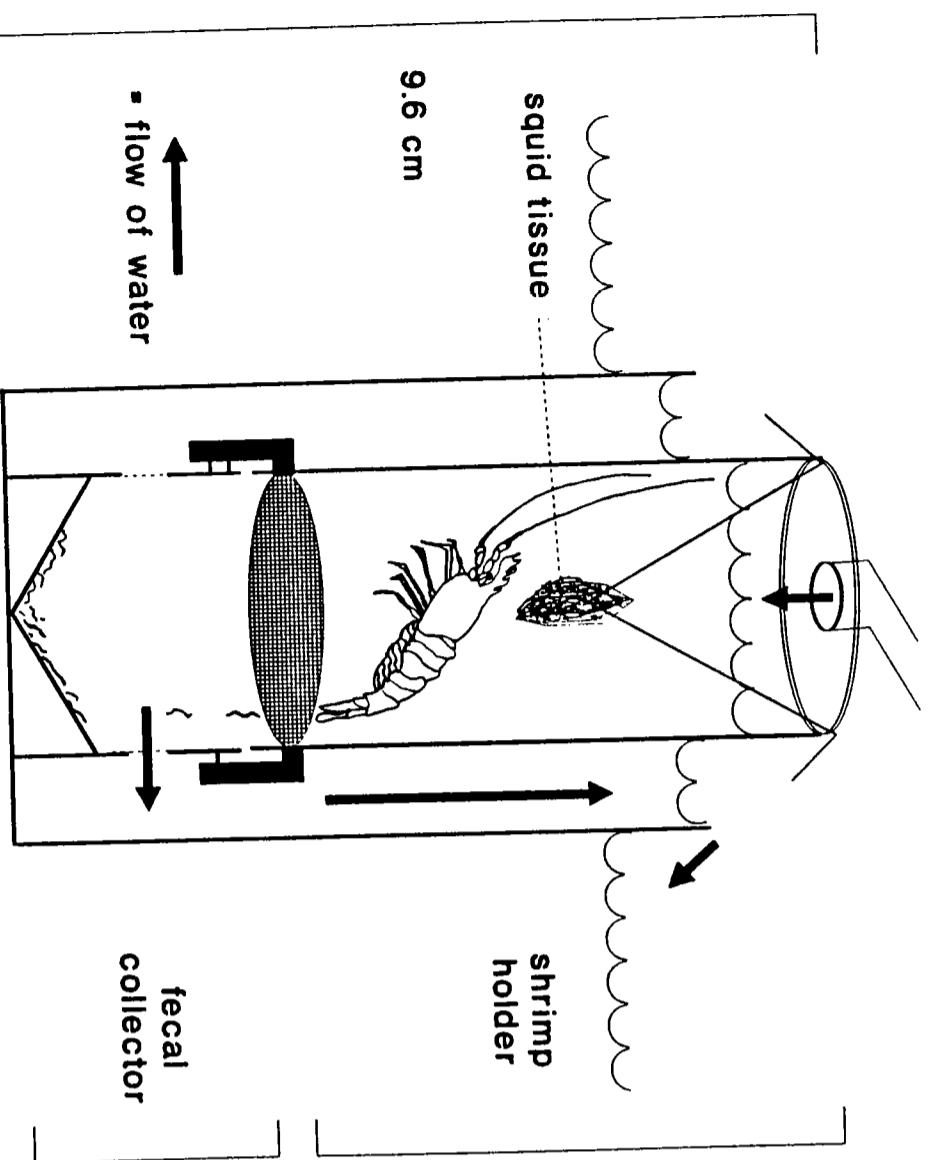
Once ingested ^{109}Cd activities were determined, shrimp were transferred to experimental chambers (Fig. 3) where they were allowed to feed *ad libitum* on squid tissue to purge radioactive gut contents. Experimental chambers consisted of two polypropylene centrifuge tubes (50 ml cap., 2.5 cm diameter) 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 ±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 ±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).
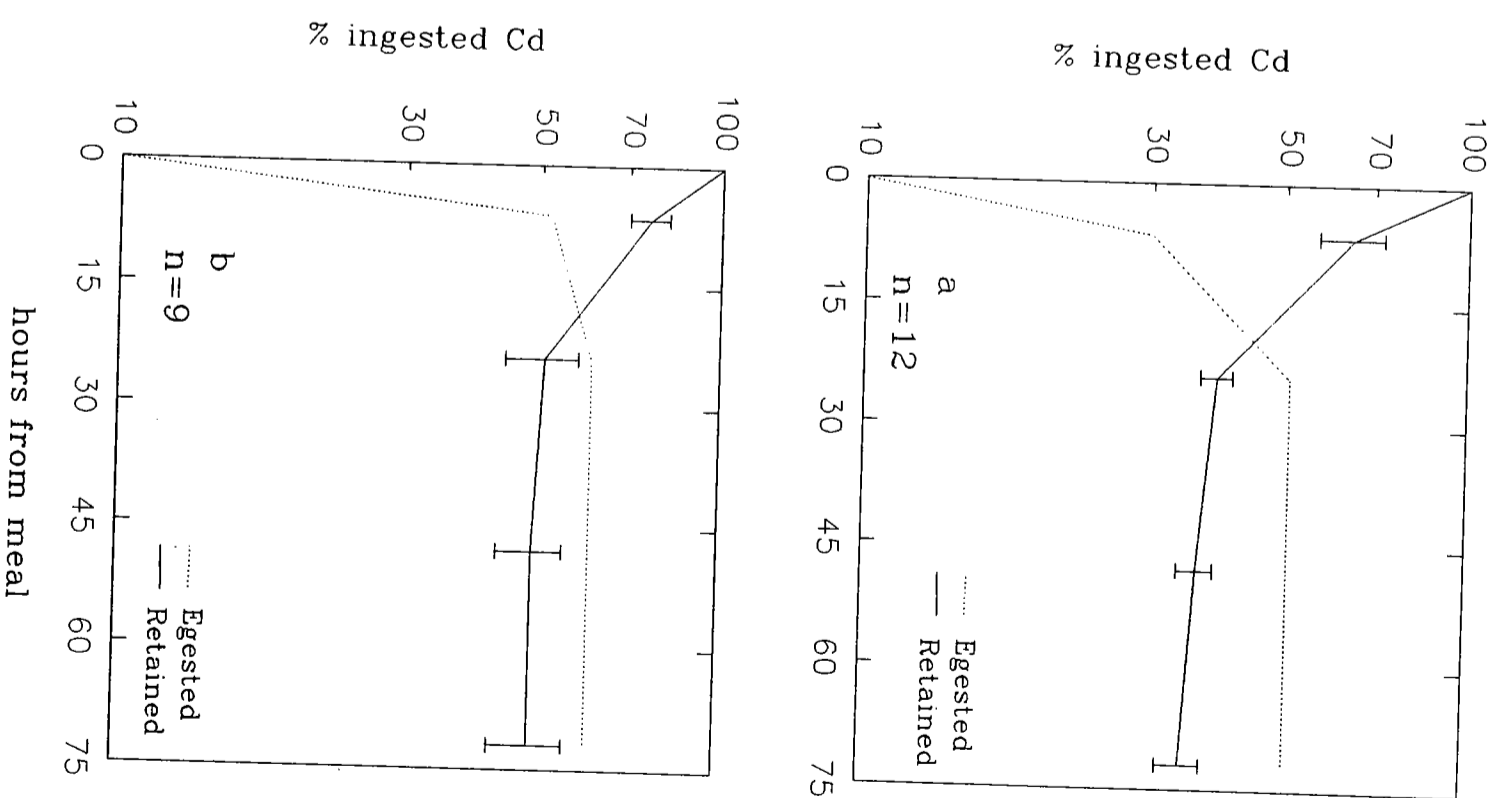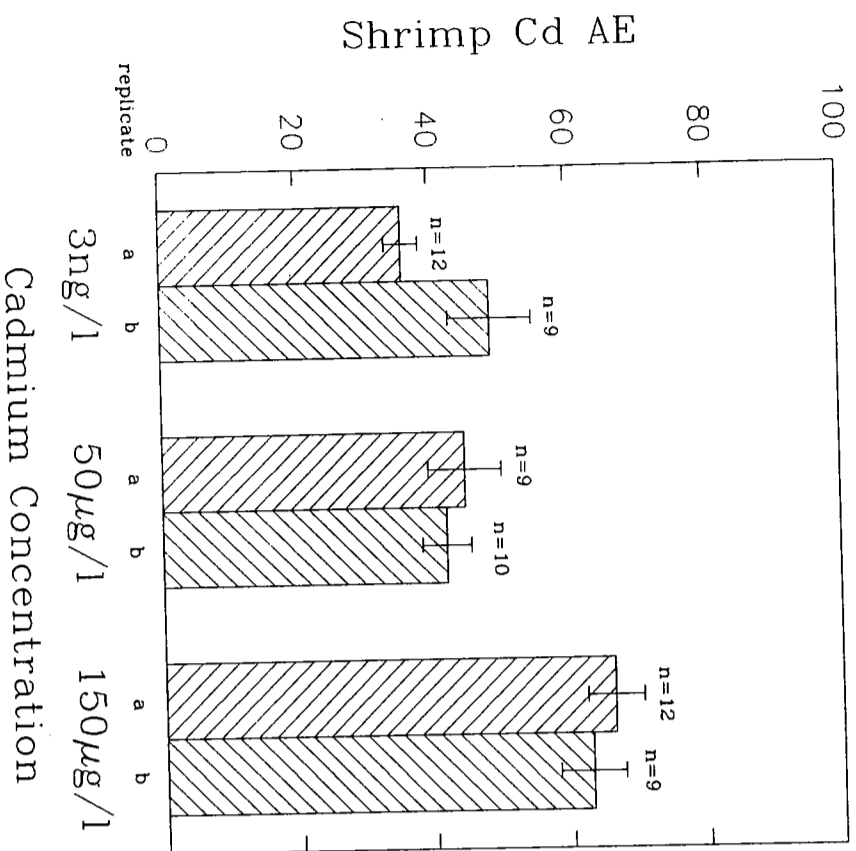
Figure 5. Absorption efficiencies (AE) (mean $\pm SE_{mean}$) for grass shrimp which have ingested oligochaetes exposed for one week to three different Cd concentrations.

Shrimp Cd AE

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		
week [Cd]	3ng	50µg	150µg	3ng	50µg	150µg
1 3ng	-					
50µg	-	-				
150µg	A	-	-			
6 3ng	A	AD	A	-		
50µg	ADC	AD	A	-	-	
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 repective 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 ±SE_{mean}) for grass shrimp which have ingested oligochaetes exposed for six weeks to three different Cd concentrations.

Shrimp Cd AE

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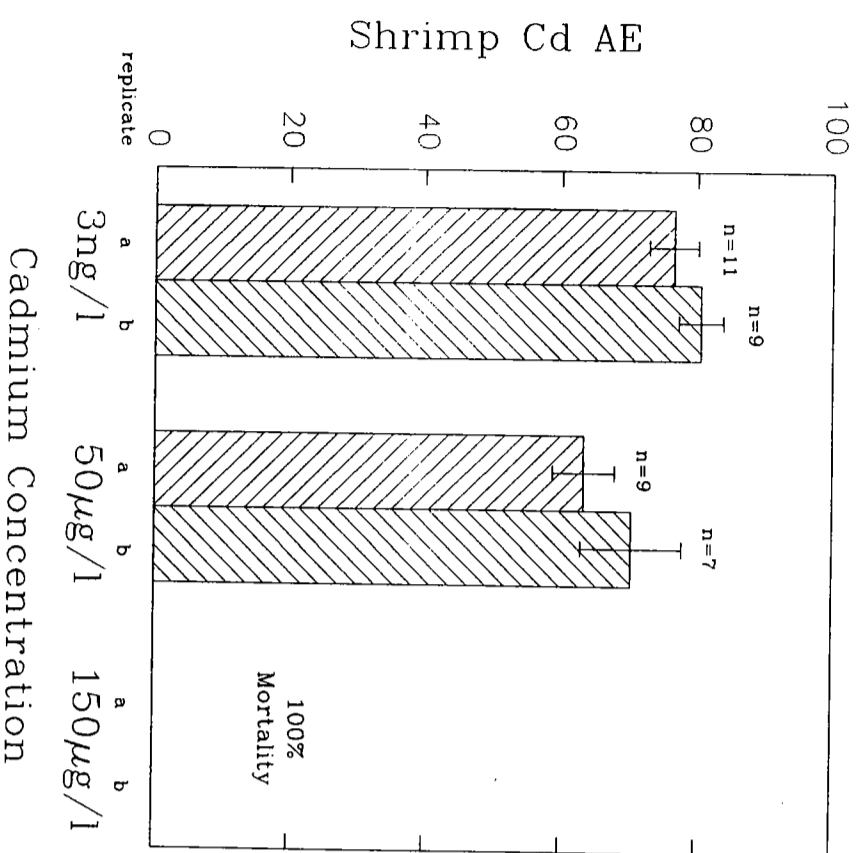
distribution with debris ^{109}Cd decreasing to 22.7% (±2.7) and cytosol increasing to 51.4% (±2.2). The intracellular fractions for worms from all one week Cd exposure treatments were unaffected by exposure condition, remaining virtually unchanged at 18.9% (±4.7), 15.7% (±4.3), and 17.8% (±2.4) for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