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Cadmium resistance in an oligochaete and its effect on cadmium trophic transfer to an omnivorous shrimp

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ABSTRACT: It has been demonstrated that the deposit-feeding oligochaete *Limnodrilus hoffmeisteri* inhabiting Foundry Cove (FC), a severely cadmium (Cd)-contaminated cove located on the Hudson River, New York, USA, has evolved resistance to Cd. In this study we investigate how this resistance influences Cd trophic transfer from this oligochaete to the grass shrimp *Palaemonetes pugio*. Cadmium-resistant worms collected from FC and nonresistant worms collected from an adjacent unpolluted site were investigated for differences in Cd tolerance, accumulation, subcellular distribution and bioavailability to shrimp. FC worms were more tolerant of Cd, surviving twice as long as worms from the unpolluted site during a toxicity bioassay. The 7 d concentration factor of Cd-resistant worms was 4 times greater than that of nonresistant worms (2020 vs 577). There were also differences between worm populations with respect to subcellular Cd distributions. Cd-resistant worms produced metallothionein-like proteins (MT) as well as metal-rich granules (MRG) for Cd storage and detoxification; nonresistant worms only produced MT. These differences in subcellular Cd distributions led to large differences in Cd bioavailability to shrimp; shrimp fed Cd-resistant worms absorbed 21% of the ingested Cd, while those fed nonresistant worms absorbed roughly 4 times that amount (~75%). These absorption efficiencies were in good agreement with the proportions of Cd bound to the worm's most biologically available subcellular fractions (i.e. the cytosol and organelles). Although Cd-resistant worms predominantly stored the toxic metal in biologically unavailable MRG, their increased accumulation of Cd would still result in substantial trophic transfer to shrimp because of the storage of Cd in the biologically available fractions. This work demonstrates that the evolution of Cd resistance can have profound implications for Cd bioavailability and cycling within aquatic ecosystems.

KEY WORDS: Cadmium resistance · Detoxification · Trophic transfer · Oligochaetes · Grass shrimp

INTRODUCTION

Over the past few decades evidence has been mounting that populations evolve resistance in response to metal pollutants (Bradshaw 1952, Antonovics et al. 1971, Klerks & Weis 1987). The impact of pollutant adaptations on ecosystems, in particular on processes such as pollutant trophic transfer, is not understood (Levinton 1980). The purpose of this and related studies was to determine how the evolution of cadmium (Cd) resistance in the aquatic oligochaete *Limnodrilus hoffmeisteri* influences Cd trophic transfer and toxicity to the benthic feeding grass shrimp *Palaemonetes pugio* (Klerks & Levinton 1989, Klerks & Bartholomew 1991, Wallace 1992, 1996, Wallace & Lopez 1996, 1997).

Cadmium-resistant *Limnodrilus hoffmeisteri*, inhabiting the metal (Cd, Cr, Ni)-contaminated Foundry Cove on the Hudson River, New York, USA, possess 2 means for detoxifying Cd: metallothionein-like proteins (MT) and metal-rich granules (MRG) (Klerks & Levinton 1989, Klerks & Bartholomew 1991). It is known that MT and MRG play important roles in the regulation, storage and detoxification of trace metals

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(Brown 1982, Roesijadi 1992). By using the detoxification of Cd by *L. hoffmeisteri* and *Palaemonetes pugio* as a predator, the aim of this research is to determine the importance of these divergent detoxification pathways in controlling metal trophic transfer.

The storage of metal via proteins (including MT) and MRG can control metal bioavailability to predators. Copepods and bivalve larvae fed phytoplankton, and grass shrimp fed oligochaetes absorbed all of the metal associated with cytoplasm and cytosol, while metal bound to cell walls, tissue and MRG was less available (Reinfelder & Fisher 1991, 1994, Wallace & Lopez 1996, 1997). Bioavailability to predators of Zn, Mn and Mg stored in granules of gastropods depends upon granule elemental composition; metal was less available in phosphate granules than in carbonate granules (Nott & Nicolaidou 1989, 1990, 1993).

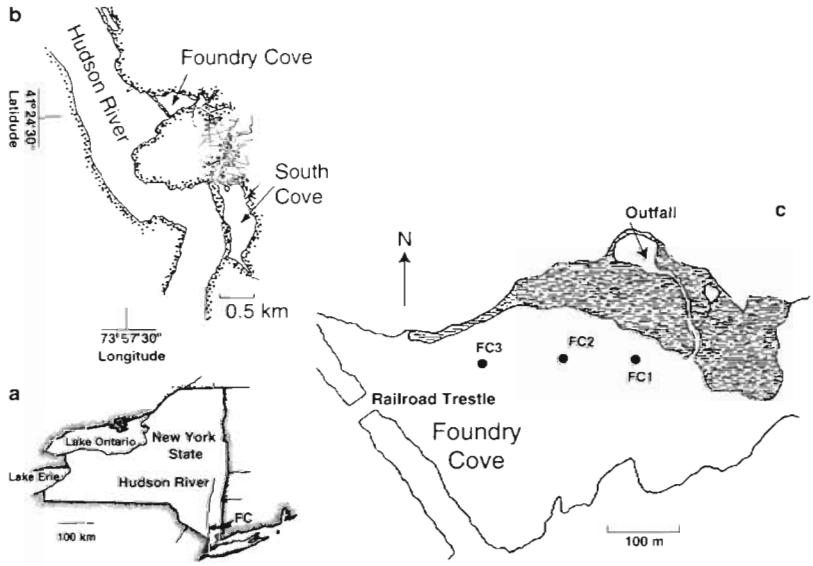

Fig. 1 Maps indicating geographic location of study sites (Foundry and South Coves) (a & b) and locations within Foundry Cove (FC 1, FC 2 and FC 3) where sediment and oligochaetes were collected for use in Cd accumulation and trophic transfer studies (c). Site of the former battery plant outfall is also indicated

Exposure conditions control metal uptake and storage, and chronic, long-term exposure can result in increased metal tolerance and even resistance (Klerks & Weis 1987, Roesijadi & Klerks 1989, Mason & Jenkins 1991, Brouwer et al. 1992, Canli & Furness 1995, Wallace & Lopez 1996). In some instances metal resistance results in the internal storage and detoxification of metal, and can lead to increased metal body burdens (Bryan & Hummerstone 1971, Brown 1977, Klerks & Bartholomew 1991). Increased metal body burdens and accompanying changes in subcellular metal distributions can therefore profoundly affect metal cycling within aquatic ecosystems.

The study site for this work, Foundry Cove, near Cold Spring, New York, is a fresh-to-brackish-water cove located on the Hudson River, and represents a unique opportunity for studying the effects of Cd in an aquatic ecosystem (Fig. 1). This cove was polluted with Cd, Ni and Co by effluent discharged from a Ni-Cd battery plant between 1953 and 1979, and until the fall of 1994 when the cove was dredged, Foundry Cove was one of the most severely Cd-contaminated sites in the world (Simpson 1981, Resource Engineering 1983). Much of the sediment contained up to 500 µg Cd g dry wt^{-1}, with the most polluted area, the outfall site, having concentrations as high as 225 000 µg Cd g dry wt^{-1} (22% Cd on a dry wt basis) (Knutson et al. 1987, Klerks & Levinton 1989). Cadmium in the sediment near the outfall was mostly present as a relatively insoluble Ca-Cd carbonate, while in the middle regions of the cove Cd was predominantly associated with organic material (Bower et al. 1978). Concentrations of Ni and Co in the cove were also very high, reaching as high as 21 100 and 437 µg g dry wt^{-1}, respectively (Knutson et al. 1987).

Cadmium accumulated in the biota of the cove, both animals and plants, but the consumption of contaminated blue crabs *Callinectes sapidus* appeared to be the only likely source of Cd exposure from aquatic food sources to human residents in the area (Kneip & Hazen 1979, Hazen & Kneip 1980, Klerks & Bartholomew 1991). The severity of the contamination was highlighted in 1981 when Foundry Cove was designated a Superfund site (i.e. being eligible for specific remediation funds) by the United States Environmental Protection Agency. To remove the estimated 12 metric tons of Cd remaining of the original 22 metric tons deposited in the cove, a major remediation project was initiated in the fall of 1993.

This dredging project commenced soon after the collection of samples that were used in portions of this study. Therefore, the research presented here was conducted with laboratory-cultured oligochaetes. South Cove, adjacent to and down river from Foundry Cove, was the control site for this study. This cove did not directly receive the waste effluent and is comparatively clean, though sediment Cd concentrations reach as high as 20 µg g dry wt^{-1}, demonstrating that Cd entering the Hudson River from Foundry Cove was being exported to other areas (Klerks 1987); uncontaminated sediments rarely have Cd concentrations exceeding 1 µg g dry wt^{-1} (Simpson 1981, Sadiq 1992).

The deposit-feeding oligochaete *Limnodrilus hoffmeisteri* is the one of the most abundant and widely distributed tubificid oligochaetes inhabiting the fresh to brackish waters of the Hudson River (Brinkhurst & Jamieson 1971), and it is the most abundant macrofaunal organism inhabiting Foundry Cove sediments (Klerks & Levinton 1989). In some areas of Foundry Cove *L. hoffmeisteri* have evolved resistance to Cd (Klerks & Levinton 1989). Adaptation has resulted in high Cd body burdens, resulting from Cd binding to MT-like proteins and precipitation into MRG, believed to be CdS (Klerks & Bartholomew 1991). Genetic studies have indicated that resistance evolved within 4 to 36 generations and is under the control of one gene (Klerks & Levinton 1989, Martinez & Levinton 1996). This resistance also varies with sediment Cd concentrations (Klerks & Levinton 1989). Foundry Cove *L. hoffmeisteri* was therefore an ideal prey species to investigate how chronic metal exposure and the evolution of metal resistance alters metal trophic transfer.

The predator used in these studies, the grass shrimp *Palaemonetes pugio*, is distributed along the Atlantic and Gulf Coasts of the United States, has a salinity tolerance of 2 to 35 psu and is an abundant benthic omnivore in marsh-cove ecosystems (Wood 1967, Knowlton 1973, Williams 1974, Welsh 1975). *P. pugio* feeds on benthic invertebrates, including oligochaetes, and is an important link in coastal food chains (Welsh 1975, Bell & Coull 1978). This shrimp is a favorite prey item of many ecologically and recreationally important species (i.e. *Fundulus heteroclitus*, *Callinectes sapidus*, *Pseudopleuronectes americanus*, *Pomatomus saltatrix*, *Anguilla rostrata*, *Roccus americanus*), and if exposed to a contaminated diet, may serve as a vector of pollutants to higher trophic levels (Nixon & Oviatt 1973, Hoffman 1980). *P. pugio* is abundant in Foundry and South Coves during periods of low river flow when salinities are ~5 psu (W. G. Wallace pers. obs.) and for these reasons was chosen as a representative predator for use in this study.

In this study we investigate how chronic Cd exposure and the evolution of Cd resistance in *Limnodrilus hoffmeisteri* influences Cd trophic transfer to *Palaemonetes pugio*. This was accomplished by determining the relationships between Cd resistance, accumulation and subcellular distributions in Cd-resistant and non-resistant oligochaetes and understanding how these differences influence Cd trophic transfer from oligochaetes to shrimp.

MATERIALS AND METHODS

Field sampling, sediment cores and worm cultures. In October 1993, 3 sediment cores and ~40 l of bulk sediment (wet) were collected from 3 stations (FC 1, FC 2 and FC 3) along the spatial gradient of Cd contamination in Foundry Cove (Fig. 1c). Based on previous studies (Kniep & Hazen 1979, Knutson et al. 1987, Klerks & Levinton 1989, R. R. Young unpubl.), study sites were chosen to encompass a gradient of sediment Cd levels ranging from 1500 µg g dry wt^{-1} and higher to less than 500 µg g dry wt^{-1}. Sediment cores and bulk sediment samples were returned to the laboratory in buckets containing overlying water collected in the cove, and were stored (10°C) with gentle aeration.

To obtain *Limnodrilus hoffmeisteri* for laboratory cultures, bulk sediment was sieved through a 500 µm screen and organisms were picked from the retained detrital material. Oligochaetes and sediment from the 3 Foundry Cove stations (FC 1, FC 2 and FC 3) and from South Cove (SC) were maintained in laboratory cultures according to Bonacina et al. (1989). The cultures consisted of plastic containers (23 × 40 cm) containing 2.5 l of cove water and a 2 cm layer of sediment. The sediment was wet sieved to the <240 µm fraction with Hudson River water. Ground commercial fish food (Tetramin®) was added weekly to the cultures. The cultures were maintained at 23°C under a 12 h light: 12 h dark photoperiod.

Sediment cores were processed under a nitrogen atmosphere in the following manner: the upper 6 cm of each core was extruded and sliced at 2 cm intervals (0–2, 2–4 and 4–6 cm); sections were homogenized and samples (3 ml; ~1 g of dry wt sediment) were taken for analysis of Cd and for the determination of wet weight/dry weight ratios. Sediment samples were also taken from laboratory worm cultures (see below) and were treated and analyzed in the same manner as those collected from cores.

Cd was extracted from wet sediment by digestion with 0.02 M HNO_3 (pH 2) and 30% H_2O_2 (85°C for 2 h). After cooling, 3.2 M NH_4OCH_3COOH in 3 M HNO_3 was added to prevent readsorption of extracted metal to the oxidized sediment (Tessier et al. 1979). Extractions were conducted in centrifuge tubes to minimize losses, and supernates were collected by centrifugation at 12000 × *g* for 30 min. Supernates were then filtered through 0.45 µm membrane filters and stored (4°C) until analysis by flame atomic absorption spectrophotometry (Perkin-Elmer Zeeman/5000 Atomic Absorption Spectrophotometer equipped with an HGA-500 graphite atomizer and an AS-40 autosampler). Recovery of reference material (MESS-1, National Research Council, Canada) approximated 100%. Wet wt/dry wt ratios were determined by dividing wet sample weights by dry sample weights (constant wt at 60°C). All materials used for Cd analysis were acid washed with 1.6 M HNO_3 and rinsed with distilled water.

Cadmium resistance determinations. Differences in Cd tolerance among worm populations were determined by conducting toxicity tests in which worms were exposed to 8.9 µM Cd (~1 ppm) (added as $CdCl_2$) in soft reconstituted fresh water (ASTM 1980, Klerks 1987). This exposure concentration, although unrealistically high, was chosen because previous work has shown that survival times are long enough to detect differences in Cd resistance yet short enough to avoid stresses due to living out of sediment (Klerks 1987). As a comparison, the 96 h LC_{50} for *Limnodrilus hoffmeisteri* is 170 µg l^{-1} (Chapman et al. 1982). Toxicity tests were only conducted with worms from FC 1 and SC because the cultures of FC 2 and FC 3 worms did not thrive under the laboratory culture conditions; the dredging of Foundry Cove negated the possibility of reestablishing these cultures.

Worms were removed from the cultures, allowed to evacuate gut contents in soft reconstituted fresh water (~24 h) and placed individually in well dishes (2.2 cm diameter) containing 4 ml of the test solution. Well dishes were covered to prevent evaporation, and worms (96 from each site) were checked hourly for survivors. A worm was scored as dead when it did not respond to a gentle disturbance with a pipet (Klerks 1987). Worms were monitored until 50% from each population had died. Differences in Cd tolerance were determined by pair-wise comparisons of survivorship curves using Gehan's Generalized Wilcoxon Test (Lee 1980). This test compares 2 survivorship curves and is appropriate for use with partial data sets.

Cadmium accumulation by oligochaetes. Cadmium accumulation by FC 1, FC 2, FC 3 and SC *Limnodrilus hoffmeisteri* was investigated by exposing worms to ^{109}Cd for 7 d, then calculating weight-normalized concentration factors. Worms were removed from the cultures, allowed to evacuate gut contents in Hudson River water (~24 h), then placed in flasks containing 150 ml of the labeling solution. There were 6 replicates per station with 8 worms per replicate. The labeling solution was prepared by adding a trace amount of ^{109}Cd (0.7 ng l^{-1}; 24 kBq l^{-1}), added as $^{109}CdCl_2$ in 0.1 M HCl, to 0.2 µm (Nuclepore®) filtered Hudson River water (0 psu). The acidification of the labeling solution caused by the addition of the label ($^{109}CdCl_2$ in 0.1 M HCl) was offset by the addition of 1 M NaOH. The Hudson River water was estimated as having a background Cd concentration of approximately 0.5 µg Cd l^{-1} (Wallace & Lopez 1996). After the 7 d exposure to ^{109}Cd, worms were removed from solution, rinsed with distilled water, individually weighed and assayed for ^{109}Cd. Worms were then stored frozen (–80°C) for use in subsequent subcellular fractionations. Samples of the labeling solution were also assayed for ^{109}Cd. Concentration factors were calculated by dividing the ^{109}Cd concentration of the worm (^{109}Cd g wet wt^{-1}) by the ^{109}Cd concentration of the water (^{109}Cd ml^{-1}).

Subcellular cadmium distributions. Differences in oligochaete subcellular ^{109}Cd distributions were investigated by homogenizing the worms from the aforementioned Cd accumulation experiment and subjecting the homogenate to differential centrifugation and tissue digestion procedures. Some of the laboratory cultured worms (FC 1 and FC 3) were also investigated for subcellular distributions of stable Cd.

Worms from the accumulation experiment were thawed and homogenized in 0.5 ml distilled water with a glass tissue homogenizer; there were 4 samples per treatment, each consisting of roughly 8 worms. After homogenization, the homogenates from one FC 1 replicate and one SC replicate were split; one portion from each was reserved (–80°C) for use in feeding experiments; the other portions as well as the unseparated SC, FC 1, FC 2 and FC 3 samples were fractionated according to the procedure in Fig. 2. Homogenized worms were first fractionated by centrifugation at 300 × *g* (15 min at 4°C). This produced a pellet containing tissue fragments and other cellular debris (i.e. membranes, setae and MRG). MRG were isolated from tissue fragments by resuspending the pellet in 0.5 ml distilled water and heating at 100°C for 2 min. An equal volume of 1 N NaOH was then added, followed by heating at 60 to 70°C for 1 h. This procedure dissolved the tissue, and MRG were then collected by

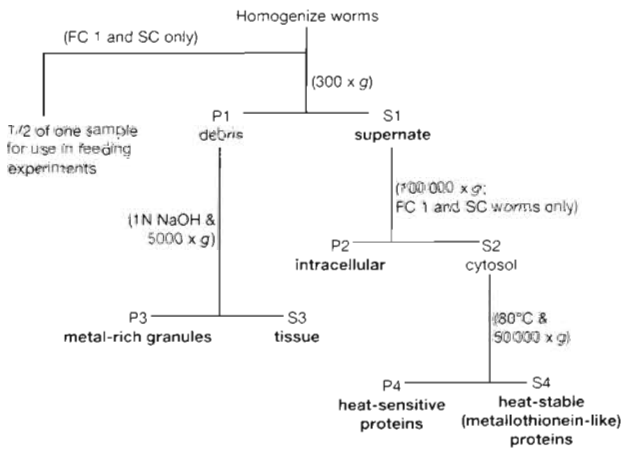

Fig. 2. Procedure for obtaining subcellular ^{109}Cd distributions of *Limnodrilus hoffmeisteri*. Worms were homogenized, and differential centrifugation and tissue digestion procedures were used to obtain the following subcellular fractions: metal-rich granules (MRG), tissue, supernate (intracellular/cytosol). The supernates of South Cove (SC) and Foundry Cove (FC 1) worms were further fractionated into intracellular, and heat-sensitive and heat-stable (MT-like) proteins. Prior to centrifugation half of the homogenate from one sample of FC 1 and one sample of SC worms were reserved for use in feeding experiments

centrifugation at 5000 × *g* (10 min at 20°C). The pellet was resuspended and rinsed several times with 1 N NaOH (Silverman et al. 1983). Isolated granules were then examined via compound microscopy.

The 300 × *g* supernates were either left unfractionated (FC 2 and FC 3) or were further centrifuged (FC 1 and SC) at 100 000 × *g* (1 h at 4°C) to produce an intracellular pellet containing nuclear, mitochondrial and microsomal fractions; the 100 000 × *g* supernate contained the cytosol and included proteins (Fig. 2). The cytosol of FC 1 and SC worms was fractionated into heat-stable (MT-like) proteins and heat-sensitive proteins. Preliminary work showed that heat-sensitive proteins could not be collected from a homogenate of 8 worms (pellets were too small); therefore, all of the cytosolic samples obtained from FC 1 worms were combined prior to heat treatment. The cytosolic samples of SC worms were also combined. Composite samples were heat denatured at 80°C for 10 min. Samples were then cooled on ice for 1 h. Heat-sensitive proteins were separated from the heat-stable fraction by centrifugation at 50 000 × *g* (10 min at 4°C) (Bebianno & Langston 1992). Percentage subcellular ^{109}Cd distributions were estimated based on total recovered radioactivities; previous work has shown that recovery of initial Cd is high (~90%) (Wallace & Lopez 1996, 1997).

To verify that ^{109}Cd followed the distribution of stable Cd, the subcellular distribution of stable Cd within oligochaetes was determined as follows. Approximately 100 worms from FC 1 and FC 3 were removed from the cultures and were allowed to depurate gut contents. Worms were then wet weighed in bulk (~125 mg per group) and homogenized in 1.75 ml distilled water. Homogenates were then centrifuged at 300 × *g* (15 min at 4°C). Supernates (cytosolic and intracellular fractions) and pellets (MRG and tissue fractions) were prepared for Cd analysis by adding 8 M HNO_3, refluxing at 95°C for 10 min, in glass beakers, after which 16 M HNO_3 was added and samples were refluxed for an additional 30 min. After refluxing, 30% H_2O_2 was added and the samples were heated a second time. Digested samples were then filtered and analyzed for Cd by flame atomic absorption spectrophotometry. The Cd concentration in each fraction was calculated on a wet weight basis and percentage Cd distributions were estimated based on the total recovered Cd. All materials and utensils used for Cd analysis were washed with 1.6 M HNO_3 and rinsed with distilled water.

Bioavailability of cadmium sequestered by oligochaetes. The portions of the homogenates from FC 1 and SC worms reserved prior to subcellular fractionation (see above) were freeze dried and mixed with 0.05 ml distilled water. Fractions were then mixed (1:4) with a gelatin solution prepared from 0.6 g gelatin crystals (Knox®) and 10.5 ml distilled water (Wallace & Lopez 1996, 1997). Aliquots (6 µl) of the homogenate/gelatin slurry were pipetted onto pre-chilled 0.2 µm polycarbonate membrane filters and were stored frozen (–20°C). The 'gelatin discs' on these filters were then fed to shrimp. Previous work has shown that this method does not alter Cd bioavailability; shrimp fed whole worms or homogenized worms mixed with gelatin absorb similar amounts of ingested Cd (Wallace & Lopez 1996, 1997).

Grass shrimp *Palaemonetes pugio* and water collected from Great South Bay, Long Island, New York, were returned to the laboratory and placed in glass aquaria. Over the course of 1 wk shrimp were slowly acclimated from a field salinity of ~20 psu to the experimental salinity of 5 psu. A salinity of 5 psu was chosen because it is the highest noted in Foundry Cove and is within the salinity tolerance of the shrimp (Wood 1967, Bower et al. 1978). After acclimation shrimp were held for an additional 7 d period (20 to 23°C). Shrimp were fed daily on Tetramin® fish food, but 2 d prior to the feeding experiment food was withheld. Because our previous work has shown that the feeding activity of these shrimp is extremely variable, even after a period of food deprivation, this feeding experiment was initiated by placing 25 shrimp in a large glass petri dish (20 cm) containing a 3 cm layer of seawater with a subsequent addition of only 14 gelatin discs. The shrimp that were most eager to feed would grab one of the gelatin discs and rapidly consume it (~5 to 10 min); only those shrimp ingesting a majority (>90%) of a gelatin disc were used for subsequent ^{109}Cd analysis.

After ingesting the worm/gelatin mix, shrimp (10 per treatment) were rinsed with distilled water, placed into gamma counting tubes containing 5 ml seawater, and assayed for ^{109}Cd. Shrimp were then transferred to holding chambers, consisting of a mesh-bottomed cylinder and a fecal collector, where they were allowed to feed ad libitum on squid tissue (Wallace & Lopez 1997). Chambers were maintained in a glass aquarium containing 40 l of seawater that was continuously aerated and passed through an aquarium filter containing activated carbon and filter media. Over the next 6 d shrimp were periodically assayed for ^{109}Cd. The amount of egested ^{109}Cd (^{109}Cd in fecal material) was determined by periodically filtering the contents of the chamber's fecal collector onto GF/C glass fiber filter and assaying the filter for ^{109}Cd.

Radioanalysis. All samples were placed in 11.5 ml polypropylene test tubes and analyzed for ^{109}Cd by determining photon emissions at 88 keV in a Pharmacia-Wallac LKB automated gamma counter equipped with an NaI crystal. Counting times were usually 5 min and propagated counting errors were kept below 10%.

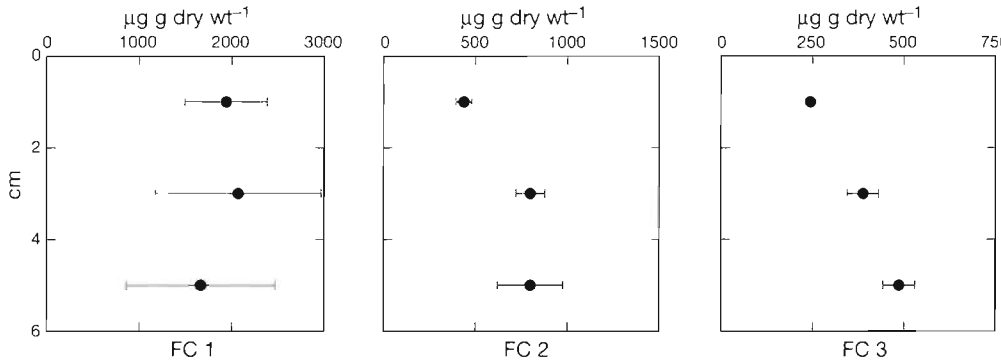

Fig. 3. Cd depth profiles (µg g dry wet wt^{-1}, n = 3; mean ± SE from 3 Foundry Cove stations (FC 1, FC 2, and FC 3). Sediment cores were sliced at 2 cm intervals and Cd concentrations were determined by sediment digestion procedures and atomic absorption spectrophotometry. Note difference in scales among the 3 profiles

RESULTS

Sediment cores and worm cultures

Profiles of sedimentary-bound Cd for the 3 Foundry Cove stations (FC 1, FC 2 and FC 3) are shown in Fig. 3. FC 1 had the highest Cd concentration at ~2000 µg g dry wt^{-1}, and concentrations were relatively uniform over the sampling depth. Cadmium concentrations at stations FC 2 and FC 3 were much lower and increased with depth from ~400 µg g dry wt^{-1} in the surface layer (0 to 2 cm) to ~800 µg g dry wt^{-1} in deeper sediments (2 to 6 cm) for FC 2 and from ~250 to ~500 µg g dry wt^{-1} for similar depth intervals for FC 3. Sediment Cd concentrations from the 3 worm cultures (FC 1, FC 2 and FC 3) were, respectively, 386, 358 and 231 µg g dry wt^{-1}, and with the exception of FC 1, were similar to those found in the surface sediments (0 to 2 cm) from the respective stations.

Cadmium resistance determinations

Upon exposure to 8.9 µM Cd, FC 1 worms survived significantly longer ($p < 0.001$) than SC worms (Fig. 4). SC worms started dying after 7 h of exposure, after which there was a constant loss of individuals (~3.6% h^{-1}). FC 1 worms did not start to succumb to the Cd exposure until after 16 h and there was only a loss of approximately 10% in the next 9 h (~1.1% h^{-1}). After 25 h, the mortality rate for FC 1 worms increased dramatically (~8% h^{-1}). The median survival times for FC 1 and SC worms were 30 and 21 h, respectively.

Cadmium accumulation by oligochaetes

Accumulation of ^{109}Cd by oligochaetes depended upon site of collection and increased with sediment Cd exposure (Fig. 5). Foundry Cove worms came from sediment having large differences in Cd contamination, but there were no differences in mean 7 d concentration factors among worms from the 3 sites. Worms from all Foundry Cove sites (FC 1, FC 2 and FC 3) exhibited similar mean concentration factors, (2020, 2180, 2253, respectively) that were significantly higher ($p < 0.001$) than the concentration factor of 577 for SC worms. This lack of a difference among concentration factors of Foundry Cove worms can be explained by a few high values for FC 2 and FC 3 worms; this is apparent from the large difference between the means and medians for these 2 stations. Further examination of concentration factors among worms from the 3 Foundry Cove stations reveals that there is a significant ($p < 0.05$; Jonckheere's ordered alternatives test) increase in Cd accumulation along the gradient.

Subcellular cadmium distributions

Oligochaetes from all Foundry Cove stations had similar distributions of subcellular ^{109}Cd with ~30 to

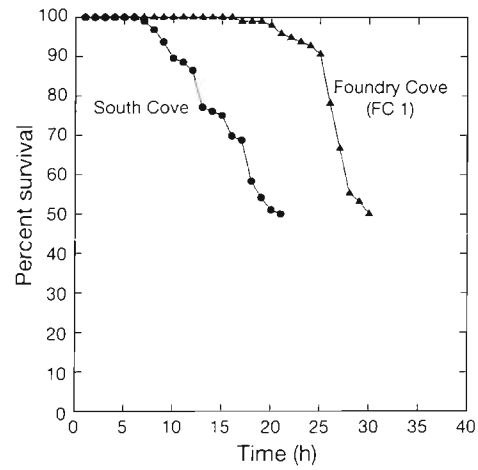

Fig. 4. *Limnodrilus hoffmeisteri*. Survivorship curves (percentage) for South Cove (●) and Foundry Cove (FC 1) (▲) worms exposed to 8.9 µM Cd in soft reconstituted fresh water. Worms were checked hourly for survivors, and the toxicity test was terminated after worms from each station experienced 50% mortality

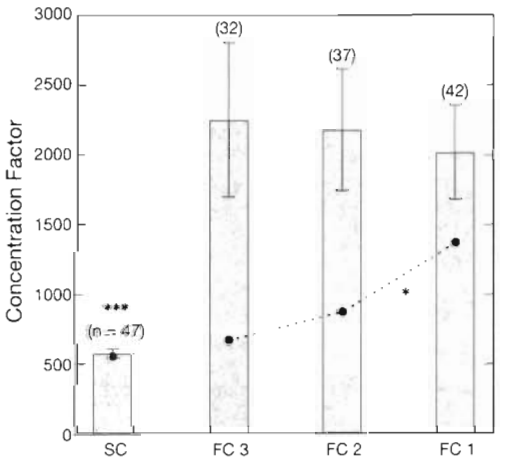

Fig. 5. *Limnodrilus hoffmeisteri*. Accumulation of ^{109}Cd by worms collected from South Cove (SC) and 3 Foundry Cove stations (FC 3, FC 2 and FC 1). Worms were exposed for 7 d to filtered Hudson River water containing a spike (24 kBq l^{-1}) of ^{109}Cd. Seven day concentration factors (bars are means ± SE; dots are medians) were calculated by dividing the concentration of ^{109}Cd in worms by the concentration of ^{109}Cd in the water. The concentration factor for SC worms was significantly (***$p < 0.001$) lower than that for worms from the other stations. The dashed line connecting the median concentration factors of Foundry Cove worms represents a significant (*$p < 0.05$; Jonckheere's ordered alternatives test) increase in Cd accumulation along the contamination gradient

40% being distributed among each of the 3 main subcellular fractions (MRG, tissue and intracellular/cytosolic [i.e. supernate of 300 × *g*]) (Fig. 6). The subcellular ^{109}Cd distribution of SC worms was in marked contrast to this with 72% of accumulated ^{109}Cd being in the intracellular/cytosolic fraction and only ~2% in MRG. Further fractionation of both SC and FC 1 worms revealed that 57 and 11% of total ^{109}Cd was bound in the respective cytosols, while worms from both sites had ~15% associated with the intracellular fraction (Fig. 7a). SC worms had ~34% of the accumulated ^{109}Cd bound in the heat-stable (MT-like) protein fraction, while FC 1 worms had ~8% in this fraction. SC and FC 1 worms had similar proportions (~65%) of cytosolic bound ^{109}Cd associated with the heat-stable (MT-like) protein fraction. On a concentration basis, it is clear that the MRG fraction of FC 1 worms is an important site for Cd storage; this fraction contains nearly as much ^{109}Cd as was accumulated by SC worms (Fig. 7b).

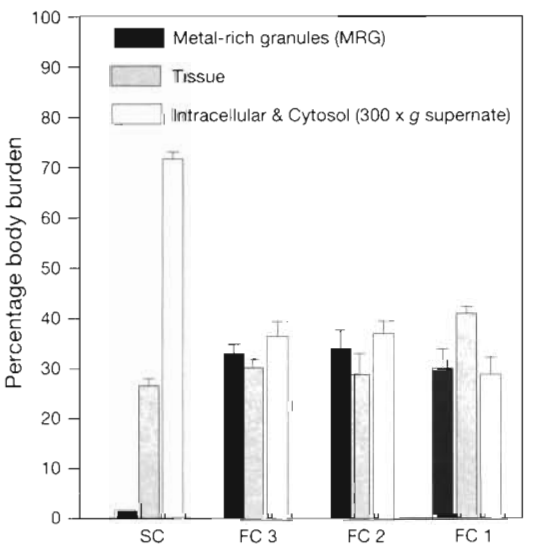

Fig. 6. *Limnodrilus hoffmeisteri*. Percentage subcellular ^{109}Cd distributions (n = 4; mean ± SE) of South Cove (SC) and Foundry Cove (FC 3, FC 2 and FC 1) worms exposed for 7 d to Hudson River water containing a spike of ^{109}Cd. Subcellular fractions (metal-rich granules [MRG], tissue and supernates [intracellular/cytosol]) were obtained through homogenization, differential centrifugation and tissue digestion procedures

The MRG fractions isolated from FC 1 and SC worms were examined with a compound microscope and numerous granules of varying sizes (10 to 30 µm in diameter) were found in the MRG fraction of FC 1 worms. One of the larger granules is shown in Fig. 8. This granule is approximately 30 µm in diameter and is composed of many smaller granules (~1 µm). These isolated granules were similar in size and appearance to those found via electron microprobe analysis in other Foundry Cove *Limnodrilus hoffmeisteri* (Klerks & Bartholomew 1991). No granules were found in the corresponding fraction of SC worms.

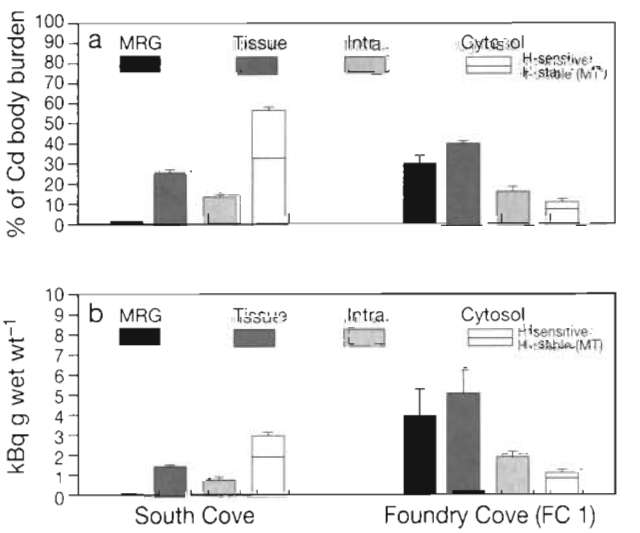

Fig. 7. *Limnodrilus hoffmeisteri*. (a) Percentage and (b) amount (kBq g wet wt^{-1}) of ^{109}Cd (n = 4; mean ± SE) among subcellular fractions (metal-rich granules [MRG], tissue, intracellular [Intra.], and heat-sensitive and heat-stable [MT-like] proteins) of South Cove (SC) and Foundry Cove (FC 1) worms exposed for 7 d to Hudson River water containing a spike of ^{109}Cd. Subcellular fractions were obtained through homogenization, differential contrifugation and tissue digestion procedures

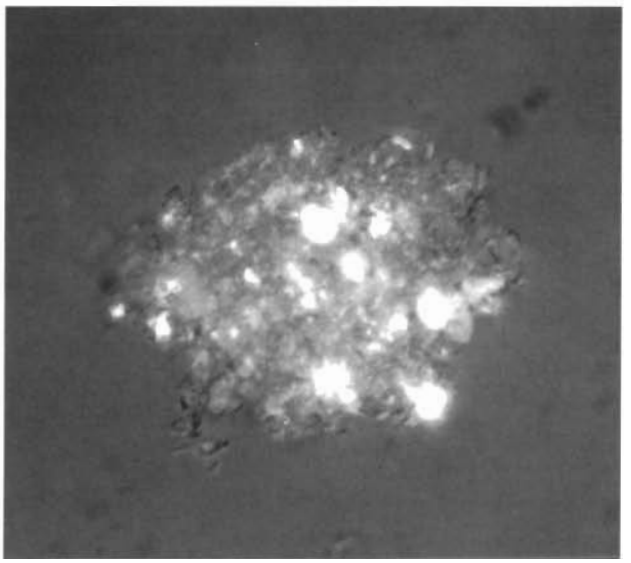

Fig. 8. *Limnodrilus hoffmeisteri*. Metal-rich granule (MRG) isolated from the debris fraction of Foundry Cove (FC 1) worms. This granule is approximately 30 µm in diameter and is composed of many small granules

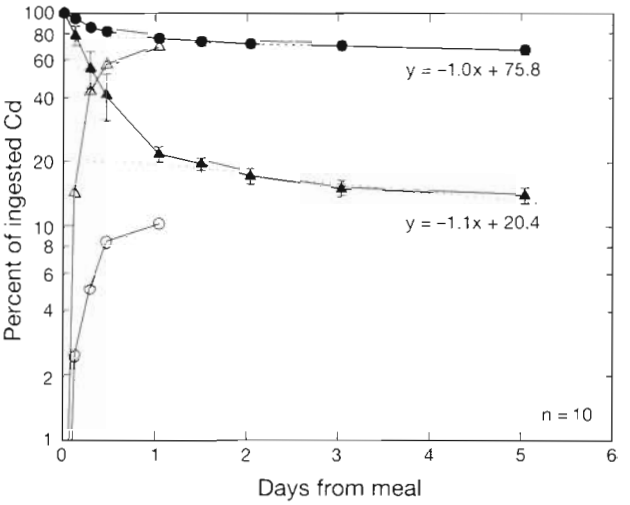

Fig. 9. Percentage retention (filled symbols; mean ± SE) and egestion (open symbols) of ^{109}Cd by *Palaemontes pugio* fed ^{109}Cd-labeled South Cove (●, ○) or Foundry Cove (FC 1) (▲, △) *Limnodrilus hoffmeisteri*. Linear regressions (dashed lines) were fit to the physiological loss components of each retention curve (i.e. time > 24 h) and *y*-intercepts give an estimate of ^{109}Cd absorption efficiency. Egestion curves were calculated by dividing the cumulative ^{109}Cd egested by all shrimp in the sample by the total ^{109}Cd ingested by these shrimp. Egestion curves are plotted only up until 24 h; no ^{109}Cd-labeled fecal material was produced beyond this point

There was good agreement between the subcellular distributions of both ^{109}Cd and stable Cd for FC 1 and FC 3 worms. The percentages of stable Cd in the unfractionated intracellular/cytosolic fractions (i.e. 300 × *g* supernatant) of FC 1 and FC 3 worms were 38 and 24%, respectively. The Cd concentrations of these fractions were 214 µg g wet wt^{-1} for FC 1 worms and 80 µg g wet wt^{-1} for FC 3 worms. The Cd concentration of the unfractionated tissue and MRG fractions (i.e. 300 × *g* pellet) were 354 and 264 µg Cd g wet wt^{-1}, respectively. Combining the Cd concentrations of these fractions yield total Cd body burdens of 568 µg g wet wt^{-1} for worms from FC 1 and 344 µg g wet wt^{-1} for those from FC 3. As a comparison, in related work worms from South Cove have been shown to have a Cd body burden of ~1 µg g wet wt^{-1} with approximately 0.44 µg g wet wt^{-1} being in the biologically available, intracellular and cytosolic fractions (Wallace 1996).

Bioavailability of cadmium sequestered by oligochaetes

Loss of ^{109}Cd from shrimp fed FC 1 and SC worms was in 2 components; an initial rapid loss (production of ^{109}Cd-labeled feces) followed by a gradual loss (metabolic depuration of absorbed ^{109}Cd) (Fig. 9). Following the ingestion of FC 1 and SC worms, shrimp produced radiolabeled feces for the first 12 h. Physiological depuration of absorbed Cd was responsible for the loss of ^{109}Cd after 24 h. Cd absorption efficiencies were therefore determined by fitting linear regressions to the physiological loss components of each curve (36 to 121 h). The *y*-intercept gives an estimate of the percentage of ingested ^{109}Cd initially absorbed by shrimp (Benayoun et al. 1974). It is this initial pool of absorbed Cd which is the source of the physiologically depurated ^{109}Cd. This method of calculating absorption efficiency assumes a constant rate of depuration from this initial pool of absorbed metal. Shrimp fed FC 1 worms absorbed an estimated 20.4% of the ingested ^{109}Cd, while shrimp fed SC worms absorbed 75.8%. These absorption efficiencies were significantly different ($p < 0.001$). Regardless of Cd dietary source, shrimp depurated the absorbed ^{109}Cd at similar rates (~1% d^{-1}).

DISCUSSION

This study, in conjunction with previous work, directly relates evolution of Cd resistance in prey to alterations in metal trophic transfer to a predator (Klerks & Levinton 1989, Klerks & Bartholomew 1991, Wallace & Lopez 1997). Our goal in this work was to determine how chronic Cd exposure and the evolution of Cd resistance in the aquatic oligochaete *Limnodrilus hoffmeisteri* influences Cd trophic transfer to the grass shrimp *Palaemonetes pugio*. This goal was realized by determining that oligochaetes inhabiting a metal con-

taminated cove and those from an adjacent unpolluted cove differ with respect to Cd tolerances, accumulation, subcellular distributions and bioavailability to predators. This work was made possible by the extreme contamination of Foundry Cove with Cd by discharge from a Ni-Cd battery plant. The range in sediment Cd concentrations found along the transect in this present study (~200 to ~2000 µg g dry wt^{-1}) was in good agreement with, though lower than, those found in previous studies (Kneip & Hazen 1979, Knutson et al. 1987). The highest sediment Cd concentration found in this study, although extreme by 'normal' standards was roughly 2 orders of magnitude lower than the highest ever found in the cove (225 000 µg Cd g dry wt^{-1}) (Knutson et al. 1987).

Klerks & Levinton (1989) demonstrated that *Limnodrilus hoffmeisteri* in Foundry Cove evolved resistance to Cd in as few as 4 generations. While we did not conduct experiments to assess the inheritance of Cd resistance, FC 1 worms were more tolerant of Cd than SC worms, and survivorship was similar to genetically resistant worms studied by Klerks (1987). The type of mortality exhibited by FC 1 and SC worms supports the assertion of Klerks & Levinton (1989) that Cd resistance in Foundry Cove *L. hoffmeisteri* was possible because of high genetic heterogeneity in the 'pre-exposed' population. The gradual and consistent loss of SC worms once they start to succumb to the Cd exposure gives some indication as to the extent of this variability in Cd tolerance.

The enhanced accumulation of Cd by Foundry Cove worms over South Cove worms is indicative of internal storage and detoxification of Cd by MT-like proteins and MRG (Roesijadi 1980, Brown 1982, Klerks & Bartholomew 1991). The magnitude of ^{109}Cd accumulation by Foundry Cove oligochaetes in this study is similar to that obtained by Klerks (1987) for other Foundry Cove oligochaetes. The similarity in Cd accumulation among Foundry Cove worms (FC 1, FC 2 and FC 3) also suggests a uniform mechanism for Cd uptake. Other metal-resistant organisms exhibit increased metal accumulation. Copper-resistant populations of the polychaete *Nereis diversicolor* and the isopod *Asellus meridians* accumulate more metal than nonresistant conspecifics and both species stored the metal in granular form (Bryan & Hummerstone 1971, Brown 1977). Additionally, the difference between the mean and median concentration factors for FC 2 and FC 3 worms further suggests great variability in the physiological mechanisms of Cd accumulation within these 2 populations. It could be speculated that these worms exhibiting such a high accumulation of Cd might already possess the ability to successfully detoxify Cd and that they would produce the offspring most capable of coping with the contamination.

The most obvious differences between the subcellular ^{109}Cd distributions of Foundry Cove (FC 1, FC 2 and FC 3) and SC worms are the lack of MRG, as well as the greater importance of the intracellular/cytosol fraction for ^{109}Cd storage in SC worms. The similarity in the proportions of cytosolic ^{109}Cd bound to the MT-like protein fraction of FC 1 and SC worms is not surprising. In other studies, after a 6 d exposure to the extremely high concentration of 8.9 µM, no discernible difference could be found between nonresistant South Cove and Cd-resistant Foundry Cove worms with regard to the proportion of cytosolic Cd bound to MT-like proteins (Klerks & Bartholomew 1991). The formation of MRG was therefore cited as a likely reason for the resistance (Klerks & Bartholomew 1991). In the present study both FC 1 and SC worms produced heat-stable MT-like proteins, but only FC 1 worms produced MRG; it was these worms that were more tolerant of Cd. The relation between MRG production and increased metal tolerance has been noted elsewhere. The isopod *Asellus meridians* from a Cu-polluted environment had increased Cu-tolerance and produced Cu-rich granules (Brown 1977); a Cu-resistant strain of the fungus *Saccharomyces ellipsoideus* contained Cu-rich granules (Ashida 1965); and a Cu-resistant population of the polychaete *Nereis diversicolor* produced Cu-rich granules (Bryan & Hummerstone 1971).

Cadmium binding to MT-like proteins in *Limnodrilus hoffmeisteri*, however, may have a role in conferring resistance. MT plays a key role in metal transport, and the relationship between MT and transport of metal to sites of MRG production is likely to be important. Copper, Cd, Hg and Zn bound to MT in the kidney of the mussel *Mytilus edulis* become incorporated into granules (George 1983). Metal bound to MT in the digestive gland of *Mytilus galloprovincialis* becomes incorporated into lysosomes and is transformed into insoluble thionein polymers, a likely precursor of MRG (Viarengo et al. 1987). Metal binding to MT and subsequent transport to the endoplasmic reticulum and Golgi system, common sites for MRG production, has been described (Simkiss 1981, Brown 1982, George 1982).

Cadmium bound to the tissue fraction, which was composed of epidermis and intestinal epithelium, accounted for similar proportions of the total Cd absorbed by Foundry Cove and South Cove worms. This could be reflective of a surface-area relationship or a limited number of binding sites (Hare 1992, Wallace & Lopez 1996). However, because of the undifferentiated nature of this fraction it is difficult to completely understand its importance in Cd storage. The similar percentages of ^{109}Cd in the intracellular fractions of FC 1 and SC worms suggests that this fraction also has a limited number of binding sites. The rela-

tively low proportion of ^{109}Cd in this fraction is consistent with previous studies demonstrating the negligible role of the intracellular fraction in Cd storage (Jenkins & Mason 1988, Klerks & Bartholomew 1991, Wallace & Lopez 1996, 1997).

The similarity among subcellular distributions of Foundry Cove worms is consistent with similarities in Cd accumulation. Even though there was an order of magnitude difference in sediment Cd levels between FC 1 and the other 2 stations (FC 2 and FC 3) there were no strong differences among Foundry Cove worms with respect to subcellular Cd distribution or Cd accumulation. What might explain this absence of a concentration dependence on metal uptake and storage is a 'threshold' mediated change from the nonresistant to the resistant state. This change might then be accompanied by the changes in metal uptake and subcellular storage; in all other respects, FC 1 Cd-resistant worms were physiologically similar to FC 2 and FC 3 worms.

This threshold may be simply related to sediment Cd concentration and would lie between the Cd concentration of South Cove sediment (20 µg g dry wt^{-1}) and that of FC 3 sediment (250 µg g dry wt^{-1}). However, it is more likely related to the amount of Cd which is biologically available for uptake in the sediment or pore water; this biologically available metal however is difficult to quantify (Luoma 1989). Alternatively, there could be a saturating process, whereby, irrespective of the total sediment Cd, there is a rate-limiting step for metal uptake into the worm, such as binding of Cd within one fraction of the organism, or even entry into the dissolved phase in the porewater. The exact details of this process still remain to be elucidated.

The trophic transfer of Cd is clearly influenced by how Cd is stored within the body of prey organisms. The difference between ^{109}Cd absorption by shrimp fed FC 1 and SC worms is caused by differences in the oligochaetes' subcellular ^{109}Cd distributions. The intracellular/cytosolic fraction of oligochaetes is the bioavailable fraction for shrimp, and the percentages of Cd in these combined fractions agree very well with respective absorption efficiencies: ~76% for SC worms and ~30% for FC 1 worms (Wallace & Lopez 1996, 1997). Cadmium bound in the MRG is unavailable because these granules are insoluble in the gut of the shrimp (Mason & Nott 1981, Simkiss 1981, Nott & Nicolaidou 1993, Wallace & Lopez 1997). *Limnodrilus hoffmeisteri* possess 2 types of granules: high density granules, possibly CdS, as well as low density granules consisting of a mixed Ca-Cd-Fe phosphate (Klerks & Bartholomew 1991). Recent work indicates that the gut pH of *Palaemonetes pugio* is approximately 5.10 (M. Ahrens), a value which is comparable with similar crustaceans (DeGiusti et al. 1962, Van Weel 1970). CdS would not dissolve at these pHs (Gong et al. 1977). Cd in the mixed Ca-Cd-Fe phosphate granules should also be unavailable because similar granules isolated from bivalves are insoluble (Simkiss 1981). Metals in some granules are bioavailable; magnesium/calcium carbonate granules in herbivorous gastropods were leached of component metals during digestion by carnivorous gastropods, but phosphate granules were relatively unaffected (Nott & Nicolaidou 1990). Additionally, it is likely that tissue-bound Cd is unavailable because oligochaete cuticle is not fully hydrolyzed during digestion (Krasnoper 1989).

The possibility of a threshold in Cd concentration required to induce the mechanism(s) of Cd resistance in oligochaetes could have profound implications for Cd trophic transfer. Nonresistant oligochaetes store a majority of their absorbed Cd in the readily bioavailable intracellular and cytosolic fractions, while resistant worms sequester Cd within biologically unavailable MRG. The interplay between sediment Cd levels, Cd accumulation by oligochaetes and differences in subcellular Cd distributions could result in nonresistant worms having a biologically available Cd concentration equal to or greater than that of Cd-resistant worms. This situation would arise if nonresistant worms had a total Cd body burden only 3 times lower than that of FC 3 worms (108 vs 344 µg g wet wt^{-1}). The concentration of Cd in the biologically available fractions, the intracellular/cytosolic fractions, of these Cd-resistant and nonresistant would be the same (~80 µg g wet wt^{-1}).

Even though Foundry Cove was recently dredged sediment Cd levels are still on the order of 80 µg g dry wt^{-1} and Cd is still being exported to the Hudson River (Wallace & Levinton unpubl., L. Suatoni unpubl.). Additionally, most of the outer portion of Foundry Cove has Cd concentrations greater than 20 µg g dry wt^{-1} (South Cove sediment) but less than 250 µg g dry wt^{-1} (FC 3 sediment) (Bower et al. 1978). The sediment Cd concentrations of Foundry Cove are therefore still within this hypothetical threshold region. In order to fully understand the impact of the dredging of Foundry Cove on its inhabitants it would be useful to determine Cd resistance and concentrations of biologically available Cd in *Limnodrilus hoffmeisteri* presently inhabiting the cove. This could indicate whether the dredging of Foundry Cove reduced or increased the potential for Cd trophic transfer. The former would result if sediment Cd levels are low enough that the accumulation of Cd in the oligochaetes biologically available fraction is substantially lower than in previous populations. The latter would result if only enough contaminated sediment was removed from the cove so that the oligochaetes were released from the pressures selecting for Cd resistance. This loss in resistance might also

result in the loss of the ability to sequester Cd into insoluble, biologically unavailable MRG hence leading to proportionally more metal being stored in the biologically available intracellular/cytosolic fractions. Recent studies indicate that *L. hoffmeisteri* presently inhabiting Foundry Cove are slightly less tolerant than previous populations and have Cd body burden on the order of 13 to 52 µg g dry wt^{-1} (~2 to 6 µg g wet wt^{-1}) (L. Suatoni unpubl.). It is presently unknown if there have also been changes in the worms' subcellular Cd distributions and how these changes might impact the trophic transfer of Cd in this ecosystem.

The amount of Cd bound to the biologically available fraction of Foundry Cove oligochaetes ranged from 80 to 210 µg g wet wt^{-1} (FC 3 and FC 1). Even though this biologically available Cd only constitutes roughly 24 to 38% of the worm's total Cd, the body burden available for trophic transfer is still extremely high. The question of whether these Cd body burdens of Foundry Cove *Limnodrilus hoffmeisteri* are high enough to cause Cd toxicity in *Palaemonetes pugio* is the focus of a related study (Wallace 1996). That work indicates that in response to a Cd-contaminated diet of Foundry Cove oligochaetes, *P. pugio* exhibits sublethal toxicity as evidenced by the production of Cd-binding MT proteins and reductions in prey capture (Wallace 1996).

CONCLUSIONS

This work has shown that evolved Cd resistance alters Cd accumulation, which, in turn, influences the subcellular Cd distribution within *Limnodrilus hoffmeisteri*. These changes in the subcellular Cd distribution of *L. hoffmeisteri* result in drastic alterations in Cd bioavailability to *Palaemonetes pugio*. Increased Cd accumulation by resistant worms is linked to the storage of Cd into insoluble MRG. Although both nonresistant and resistant worms use heat-stable MT-like proteins for the storage of Cd, nonresistant worms do not store absorbed Cd in MRG. The absence of MRG in nonresistant worms leads to a high proportion of accumulated Cd being stored in the cytosol, probably resulting from the induction of MT Because protein bound metal is readily available to predators, this leads to the efficient transfer of Cd to shrimp. In contrast, the storage of Cd by resistant worms into MRG alters Cd trophic transfer by reducing the bioavailability of this toxic metal. The production of MRG by Cd-resistant worms, however, is not sufficient enough to prevent substantial Cd trophic transfer to shrimp, because a portion of the Cd accumulated by these worms is also stored in the biologically available cytosolic and intracellular fractions. The concentration of this biologically available Cd still turns out to be far greater than that found in the nonresistant, South Cove worms. Determining the toxicity to predators of this biologically sequestered metal and understanding effects on ecosystem function are the logical next steps in this series of studies linking the impacts of a metal polluant at multiple levels of biological organization.

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