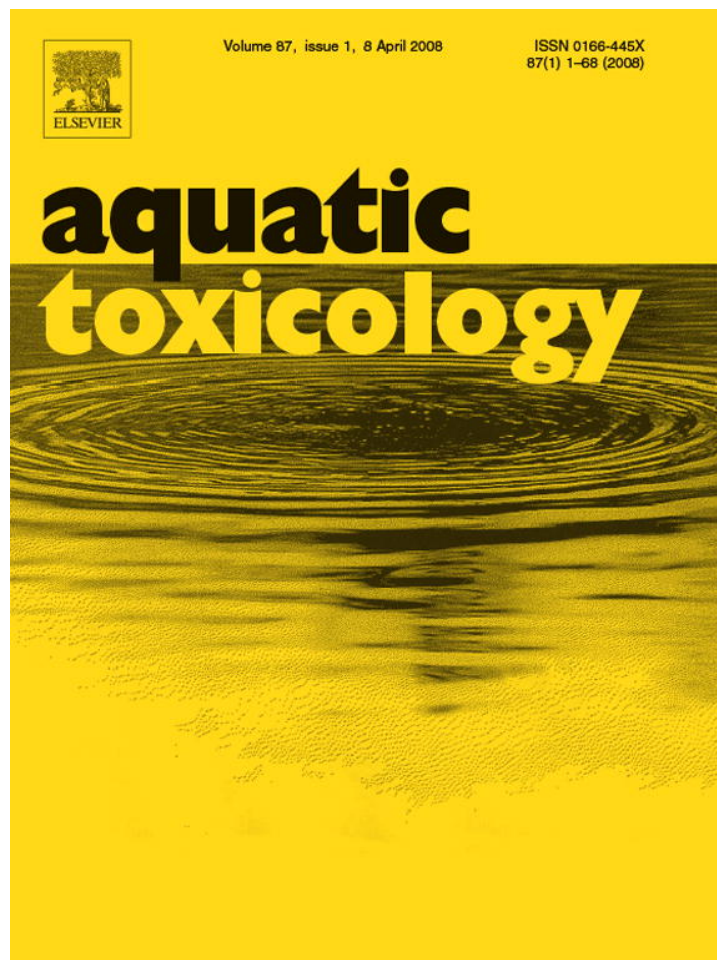


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Trophic transfer and dietary toxicity of Cd from the oligochaete to the rainbow trout

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Abstract

Dietary toxicity of metals on fish is often studied using commercial pellet food, and there is a lack of investigation on the toxicity of metals that are biologically incorporated into the natural food from the aquatic environment. In this study, we investigated the toxicity of dietborne Cd from the oligochaete *Lumbriculus variegatus* to the rainbow trout *Oncorhynchus mykiss*. The oligochaete worms were exposed to waterborne Cd (0.1, 5, 20, and 200 $\mu\text{g L}^{-1}$) for 1 week and the fish were fed this food exclusively (daily ration = 3.5% body wet weight) for 1 month. Cd concentrations in the worms averaged 0.1, 0.6, 2.2, and 30.3 $\mu\text{g g}^{-1}$ wet weight respectively, whereas the whole fish accumulated 0.002, 0.005, 0.019, and 0.387 $\mu\text{g Cd g}^{-1}$ wet weight respectively, after feeding upon control or Cd-contaminated worms for 4 weeks. Highest concentrations of Cd were retained in the gut, followed by the kidney and liver of the fish, with the latter two increasing over time; however, gut tissue accounted for >80% of whole body Cd burdens at all times. The trophic transfer efficiency of Cd was low (0.9–6.4%) although higher than in previous studies using Cd-spiked commercial diets, and was only weakly correlated to the internal Cd storage in the worms. The level of Cd in the contaminated worms did not affect Cd trophic transfer efficiency, but was reduced over the dietary exposure period. Dietborne Cd did not interfere with whole body Ca uptake from the water or alter plasma [Ca], but reduced growth by 50% in the trout exposed to the highest Cd dose. Cd stored in the metallothionein-like proteins of the fish gut tissue increased while that in the heat-denaturable proteins was reduced, suggesting detoxification over time. This study suggests a higher bioavailability and toxicity of Cd from the natural diets than from the commercial diets used in previous studies. © 2008 Elsevier B.V. All rights reserved.

Keywords: Cadmium; Dietborne; Toxicity; Trophic transfer; Oligochaete; Rainbow trout

1. Introduction

Dietary metal exposure is often ignored in environmental regulations for protecting aquatic species. However, recent research has raised significant concerns with respect to dietborne metals (Meyer et al., 2005). Total metal exposure (concentration multiplied by volume flow or mass flow of the exposure medium) may be similar in exposure via ingestion or via the gills (Clearwater et al., 2002). In addition, dietary metal exposure can be the dominant pathway of exposure for some aquatic organisms for example, Ag, Cd and Cu for the clams *Macoma balthica* (Griscom et al., 2002) and *Corbicula fluminea* (Croteau and Luoma, 2005), also Cu and Zn for some fish (Clearwater et al., 2002). Dietborne metals can also reduce the survival, growth and reproduction of aquatic invertebrates and fish (Meyer et al., 2005). Despite the important role of dietborne metals in the

aquatic ecosystem, the interaction between dietborne metals and the gastrointestinal tract of aquatic organisms is still unclear.

Many studies on dietborne metals have used commercial dry pellets enriched with metal salts. These have reported that 10–125 $\mu\text{g Cd g}^{-1}$ dry feed for 35 days to 4 months caused adverse physiological responses such as hypocalcaemia, increased enterocyte proliferation and apoptosis in the gut, decreased nutrient digestibility, and increased gill chloride cells in fish (Pratap et al., 1989; Pratap and Wendelaar-Bonga, 1993; Berntssen et al., 2001). Medium-term dietary exposure to Cd generally did not create toxic effects at a higher level of biological organization; for example, exposure to diets with 300–500 $\mu\text{g Cd g}^{-1}$ for 4 weeks did not affect the survival and growth of rainbow trout (Baldisserotto et al., 2005; Franklin et al., 2005) and 35 days exposure to 10 $\mu\text{g Cd g}^{-1}$ tropical fish flakes did not cause any mortality in the tilapia *Oreochromis mossambicus* (Pratap et al., 1989). Nevertheless, it is still possible that Cd-contaminated invertebrates may have negative impacts on fish growth and survival (Farag et al., 1999). Harrison and Curtis (1992) reported that the absorption efficiency of Cd

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from natural food by the rainbow trout was 5-fold higher than that from the commercial pellet food. These discrepancies are possibly caused by the differences in metal binding between the natural and artificial food, thus affecting the absorption or assimilation efficiency of metals by the predator (Harrison and Curtis, 1992). The metal-contaminated commercial food may not reflect the true biological incorporation and internal speciation of the metals as found under natural conditions.

Trophic transfer efficiency of metals can be primarily described by the kinetic parameters derived from the bioenergetic based kinetic model that predicts metal accumulation (Thomann, 1981; Reinfelder et al., 1998) – metal assimilation efficiency by the predator, weight specific ingestion rate of the predator and metal elimination rate from the predator. Metal assimilation efficiency by the predator is affected by the metal biochemistry in the prey/food and the physiology of the predator. Wallace and Lopez (1996) first demonstrated that the percentage of Cd in the cytosol of oligochaete worms had a 1:1 relationship with the percentage of Cd assimilated by grass shrimps, thereby suggesting that the subcellular distribution of metals in the prey may affect the metal bioavailability to the predator. Following this, several studies have shown a correlation between the subcellular metal distribution in invertebrate prey and metal assimilation by crustaceans (Seebaugh and Wallace, 2004; Rainbow et al., 2006; Seebaugh et al., 2006), gastropods (Cheung and Wang, 2005) and fish (Zhang and Wang, 2006b). Metals bound to cytosolic proteins of the prey appear to be more bioavailable to the predator than metals bound to the insoluble fractions. In general, metal subcellular partitioning in the prey plays a significant role in the trophic transfer of metals to the predators.

In this study, our aim was to investigate the toxicity and trophic transfer of dietary Cd from the oligochaete *Lumbriculus variegatus* to the rainbow trout *Oncorhynchus mykiss*. We also attempted to correlate the subcellular Cd distribution in the worms to the trophic transfer efficiency of Cd. We chose *L. variegatus* as the prey because it has similar nutrients compared to standard diets (for example, *Artemia nauplii*, frozen brine shrimps or trout chow) and its ease of culturing (Mount et al., 2006). The oligochaete worms were exposed to waterborne Cd for 1 week; the fish were fed this food exclusively for 4 weeks. During the 4-week period, we examined the toxic effects of diet-borne Cd on growth and Ca regulation of the fish every week. In addition, we investigated the tissue-specific distribution of Cd and its concentration in subcellular fractions of the fish so as to provide information on how fish deal with the toxicant. We also measured the subcellular distribution of Cd in sub-samples of worms that were used for feeding the fish.

2. Materials and methods

2.1. Exposure of oligochaete worms to Cd

Oligochaete worms *L. variegatus* were purchased in bulk from Aquatic Foods Ltd., California, USA and acclimated in aerated and dechlorinated Hamilton (ON, Canada) city tap water (12 ± 1 °C; $0.06 \mu\text{g L}^{-1}$ Cd; 1 mM Ca^{2+} ; 0.2 mM Mg^{2+}) in the

laboratory for 2 weeks before exposure. They were fed ground 0.5 point size commercial trout chow once each week.

About 52 g worms were put into an aquarium tank with 10 L aerated dechlorinated Hamilton tapwater (1 worm mL^{-1}) for the Cd exposure. The worms were exposed to 4 different aqueous Cd concentrations (Control, 5, 20, $200 \mu\text{g L}^{-1}$) for 7 days without food, with five replicate tanks for each concentration. Waterborne exposure is a relevant exposure to worms because they can be exposed to metals from overlying water, porewater and sediment simultaneously in the natural environment. The highest waterborne Cd concentration, $200 \mu\text{g L}^{-1}$ was chosen because it can provide information on how the worms store Cd and how much Cd can be transferred to the fish in an extreme scenario. Stable Cd dilution was made from the primary stock of 1 g L^{-1} Cd (analytical grade $\text{Cd}(\text{NO}_3)_2$). Besides stable Cd, ^{109}Cd was also added to the water in each tank at $0.2 \mu\text{Ci L}^{-1}$. After addition of the stable Cd and ^{109}Cd , the water was stirred and a 1 mL water sample was taken from each tank for ^{109}Cd analysis. The radioactivity of ^{109}Cd was used for checking the Cd concentration in the water during the exposure.

Since about 30% Cd was lost after 2 days, corresponding amounts of new ^{109}Cd ($0.6 \mu\text{Ci}$) and stable Cd (15, 60, $600 \mu\text{g}$ to Cd5, Cd20, Cd200, respectively) were added to each tank on day 2, 4 and 6, except only ^{109}Cd was added to the control tanks. Similarly a 1 mL water sample was taken for ^{109}Cd analysis after each addition. The water was not renewed during the exposure and the worms survived well in all treatments. On day 7, the worms were rinsed with clean water twice to remove the surface-adsorbed Cd, then 10 worms from each tank were weighed and put into vials for ^{109}Cd analysis. Through knowledge of the specific activity in the exposure water, this provided information on Cd accumulation per gram of worms. All worms were then stored at -20 °C for feeding or subcellular Cd analysis.

The exposure of worms to aqueous, radio-labeled Cd was run five times in order to accumulate enough worms for feeding the fish. The mean total Cd concentrations in the water of the five exposure runs were $0.1 \pm 0.002 \mu\text{g L}^{-1}$ (Control, not spiked with stable Cd); $5.0 \pm 0.09 \mu\text{g L}^{-1}$ (Cd5); $20.0 \pm 0.4 \mu\text{g L}^{-1}$ (Cd20); $200.6 \pm 3.8 \mu\text{g L}^{-1}$ (Cd200) when the specific activity of ^{109}Cd and background Cd concentration in the water were taken into account.

2.2. Feeding to fish

Juvenile rainbow trout, *O. mykiss* (10–12 g) were purchased from Humber Springs Fish Hatchery, Orangeville, ON, Canada. They were acclimated in 200 L flow-through tanks supplied with dechlorinated tapwater (12 ± 1 °C) for 2 months in the laboratory and fed commercial dry trout chow at a ration of 1% body weight daily. Approximately 1 month before the experiment, fish were anaesthetized with tricaine methane sulphonate and randomly chosen for individual tagging. The tagging was completed by implanting a 12 mm passive integrated transponder (PIT) (TX1400L-125 KHz, Destron Fearing, St. Paul, MN, USA) into the peritoneal cavity. When activated by a radio wave, the PIT tags send a unique identification code to a transponder, allowing tracking of individual fish over time. Since a lim-

ited number of PIT tags were available, only 60% of the fish were tagged. Tagged fish were randomly and evenly distributed amongst exposure tanks and were monitored for individual specific growth rate. Clean worms were used to feed the fish (daily ration = 3.5% fish wet weight) for 1 week before the experiment so as to acclimate the fish to the worm diet. In addition, the fish were transferred to four 200 L tanks that were divided into two partitions. The divider had perforations that were covered with a mesh, thereby allowing free movement of the water while retaining the fish and the diet within the designated compartment. Each divided half tank had separate aeration, and fish in each half tank represented a duplicate from a different treatment. This tank allocation was especially designed so as to place fish fed control worms and Cd-contaminated worms side-by-side, so that fish from both halves could be exposed to any Cd which leached from the contaminated worms. In total, there were 8 half tanks (each 60 fish) prepared for 4 treatments – fish fed control worms, Cd5 worms, Cd20 worms and Cd200 worms. The paired tanks were Control versus Cd200; Cd5 versus Cd20; Control versus Cd5; Cd20 versus Cd200. We expected the greatest leaching effect from the Cd200 worms on the control fish; therefore, the control fish were paired with fish fed Cd200 worms (a high leaching effect) and fish fed Cd5 worms (a low leaching effect). The other treatments were paired randomly. About 24 h before the start of the experiment, fish were starved and the weight of individual tagged fish, as well as the bulk mass of untagged fish, was measured for estimating the amount of worms used for feeding.

On the day of dietary exposure, worms were thawed and weighed, then fed to the fish at a measured ration of 3.5% wet weight. Since Cd may potentially be released from the worms, the feces, or the fish mucus to the water, the standpipes of the tanks were removed every day to drain the fecal material and food residue that settled at the bottom of the tanks. In addition, water was regularly checked for ^{109}Cd . In preliminary trials, significant leaching of Cd from the worms to the water was observed during feeding. Therefore, 70% of the water was replaced immediately after the feeding (<5 min), in order to reduce the fish exposure to any aqueous Cd that leached from the worms. Water samples were taken regularly for ^{109}Cd analysis after water replacement in order to monitor the exposure of aqueous Cd exposure to the fish; waterborne Cd was only detectable up to 4 h post-feeding (see Section 3). Fish were fed the Cd diet once daily for 4 weeks. The daily dose of Cd to the Control fish, and fish fed Cd5, Cd20 and Cd200 worms was 3.8 ± 0.2 , 21.3 ± 2.5 , 75.0 ± 15.0 and $1011.9 \pm 134.1 \text{ ng g}^{-1} \text{ day}^{-1}$ respectively. After each week of dietary exposure, the weight of individual tagged fish and the bulk mass of untagged fish were measured for re-calculating the weight of worms for feeding next week. A subset of 20 fish from each treatment was also transferred to a flow-through 20 L tank for gut purging. Clean worms (non-Cd spiked) were fed to these fish at a ration of 3.5% body weight each day continuously for 5 days to ensure gut-cleansing. There is a possible Cd loss from the fish, as opposed to just loss of gastro-intestinal contents, during this 5-day gut purging period. In general loss is often rapid in the first week due to gastro-intestinal clearance, and becomes slower

in the later weeks (Wang, 2002). In our study, it was desirable to measure the Cd concentration in the fish without contamination from gastro-intestinal contents, and when a steady-state of Cd loss had been reached. Five days provided the best compromise to achieve this goal.

2.3. Growth and mortality of fish

During the dietary exposure period, mortality of fish was recorded for all tanks. Weight of tagged fish was individually measured after each week of exposure. Individual specific growth rate (%) of fish between the weeks of exposure was calculated as described by Franklin et al. (2005).

2.4. Tissue specific Cd accumulation in the fish

After each week of exposure and 5-day gut purging, 10 fish were sampled from the 20 L tank, euthanized by MS-222 overdose, and weighed. The gill, liver, gut, kidney and carcass (remains of body) were dissected, weighed and counted for ^{109}Cd . The gut was then stored at -20°C for subcellular Cd analysis since it had the highest counts among the organs. Total Cd in the whole fish was calculated by multiplying the tissue-specific Cd concentrations and weight of individual organs.

At the end of 4 weeks, 20 fish from each treatment were gut purged. Ten were dissected similarly for measurement of tissue-specific Cd accumulation whereas the other 10 were weighed and digested in 3 volumes of 1N HNO_3 for 48 h at 60°C . The digest was later diluted for total Cd measurement by graphic furnace atomic absorption spectrophotometry. Certified reference material TM-15 (National Water Research Institute, Environment Canada) was used for the quality control of the metal analysis and agreement was within 10%. The information on total Cd concentration in the tissue was used to estimate the ratio of total Cd and counts of ^{109}Cd in the fish for conversion of ^{109}Cd counts into Cd concentration in the organs and gut subcellular fractions. Trophic transfer (%) of Cd from the worms to the fish each week was calculated from the cumulative amount of Cd accumulated in the fish divided by the cumulative amount of Cd ingested from the worms over the experimental period. A similar efficiency for each week's cumulative calculation would mean Cd is transferred at a comparable efficiency even when more worms are eaten, whereas an increasing or decreasing efficiency would mean Cd is transferred more or less efficiently over time.

2.5. Subcellular Cd compartmentalization in oligochaete worms

Samples of worms used for feeding the fish were fractionated as to understand the correlation between Cd subcellular compartmentalization in the worms and the percentage transferred to the fish. Since the Cd accumulation in worms from different sets of exposure was similar, they were combined to form five replicates for the subcellular fractionation. The method for subcellular fractionation was modified from Wallace et al. (2003). Two to five volumes of Tris buffer (pH: 7.1, 25 mM) with 0.2 mM PMSF (phenylmethyl-sulphonyl fluoride)

and 2 mM 2-mercaptoethanol were added to the worms and homogenized under ice-cold conditions. The homogenate was centrifuged at $1450 \times g$ for 15 min at 4°C . The pellet was then dissolved in 2 mL sodium hydroxide (1N) and heated at 80°C for 15 min. Next, the mixture was spun at $5000 \times g$ for 10 min to separate the cellular debris (pellet) and metal-rich granules (supernatant). On the other hand, the supernatant from the first spin of the homogenate was spun at $100,000 \times g$ for 1 h at 4°C to separate the organelles (pellet) from the soluble proteins (heat-denaturable proteins + metallothionein-like proteins). Thereafter, heat-denaturable proteins (which appeared in the next pellet) were separated from the heat-stable metallothionein-like proteins (in the supernatant) by heating at 80°C for 10 min and subsequent centrifugation at $50,000 \times g$ for 10 min. In total, five subcellular fractions were collected: cellular debris, metal-rich granules, organelles, heat-denaturable proteins and metallothionein-like proteins. The organelles, heat-denaturable proteins and metallothionein-like proteins are considered trophically available fractions (Wallace and Luoma, 2003). The recovery of Cd from the worm fractions was $74.5 \pm 3.5\%$ (sum of amount of Cd in five fractions/amount of Cd in tissue before homogenization). Loss of Cd may occur during multiple transfer of tiny amounts of material between vials.

2.6. Ca uptake by the fish

Since Cd may interrupt the uptake of waterborne Ca by fish, Ca regulation was examined in the control and Cd-exposed fish by measuring the whole body Ca influx from the water and Ca concentration in the plasma of fish. The methods were established from Franklin et al. (2005) and Baldisserotto et al. (2005). Briefly, each week, 20 fish from each treatment in the 200 L tank were taken for gut purging. After 5 days in the 20 L tank, 10 of these fish were transferred to a plastic bag in a darkened container which was filled with 3 L aerated dechlorinated tapwater spiked with $5 \mu\text{Ci L}^{-1}$ ^{45}Ca . The fish were exposed to the water for 4 h, and water samples were taken from each bag before the fish were transferred and after the flux ended. After the flux, each fish was removed and rinsed in deionized water for 1 min to remove the surface-adsorbed ^{45}Ca . Then they were digested in 1N HNO_3 at 60°C for 48 h. Fish digest (2.5 mL) was added into 15 mL UltimaGold scintillation fluor (Packard Bioscience, Meriden, CT, USA) and stored in dark overnight before measurement of ^{45}Ca . In addition, about 1 mL blood was individually collected from 10 fish of each treatment after gut purging. The blood was spun at $18,000 \times g$ for 5 min at 4°C and plasma was collected for measuring total Ca concentration by flame atomic absorption spectrophotometry after appropriate dilution in 0.5% LaCl_3 and 1% HNO_3 .

2.7. Subcellular Cd concentration in the fish gut tissue

Subcellular Cd distribution in the rainbow trout can provide information on metal toxicity and detoxification in the fish. The above fractionation method, as described for the worms, was applied to the trout gut tissue, which was the largest site of accu-

mulation in the fish. For each of three replicates per treatment, three to four fish guts were combined from each treatment. Each fraction was later counted for ^{109}Cd and converted to $\mu\text{g Cd g}^{-1}$ wet weight fish by using the measured ratio of ^{109}Cd to total Cd. Concentrations of Cd ($\mu\text{g g}^{-1}$ wet weight fish gut tissue) in the metal sensitive fractions (organelles + heat-denaturable proteins) and biologically detoxified fractions (metal-rich granules + metallothionein-like proteins) were determined (Wallace et al., 2003). The recovery of Cd from the fish gut fractions was $79.5 \pm 3.6\%$.

2.8. Radioactivity measurement

Radioactivities of ^{109}Cd and ^{45}Ca were measured by a gamma counter (Wallac 1480 Wizard 3'') and liquid scintillation counter (LKB Wallac 1217 Rackbeta LSC) respectively. The counts were background and decay-corrected. Counting time was adjusted to $<5\%$ error for each sample. The gamma counter detected ^{109}Cd alone, since ^{45}Ca is only a beta-emitting radioisotope, but both ^{45}Ca and ^{109}Cd were detected by the scintillation counter. We determined the relative counting efficiency of ^{109}Cd on the scintillation counter versus the gamma counter under different degrees of quench. It allowed us to subtract the scintillation counts due to ^{109}Cd from the total, thereby yielding the counts solely due to ^{45}Ca . Separate quench curves were generated by measuring the counts of ^{45}Ca or ^{109}Cd after adding known amounts of external ^{45}Ca or external ^{109}Cd to the same amount of scintillation fluor and different dilutions of the fish digest. The procedure was validated by performing an additional quench series where known amounts of both external ^{45}Ca and external ^{109}Cd in combination were added to different dilutions of the fish tissue digest with the same amount of scintillation fluor. The counts were separated successfully.

2.9. Statistical analysis

Data are routinely reported as means ± 1 S.D. Data were checked for homogeneity of variance and normality before analysis. In addition, percentage data were arcsine transformed for analysis. One-way ANOVA was used to compare among treatments in each exposure time and differences between groups were identified by the Tukey *post hoc* test. When the data violated the assumptions, the non-parametric Kruskal–Wallis test, followed by the Dunnett T3 *post hoc* test were used for the analysis. Linear regression analysis was used to check the correlation between Cd in subcellular fractions of the worms and percentage Cd transferred to the fish. Significance for all tests was taken at $P < 0.05$.

3. Results

3.1. Exposure of oligochaete worms to Cd

Cd concentrations (wet weight) in the worms after 1 week of waterborne exposure were $0.1 \pm 0.01 \mu\text{g g}^{-1}$ (Control); $0.6 \pm 0.1 \mu\text{g g}^{-1}$ (Cd5); $2.2 \pm 0.6 \mu\text{g g}^{-1}$ (Cd20); and $30.3 \pm 9.7 \mu\text{g g}^{-1}$ (Cd200) (Fig. 1a). Under the same laboratory

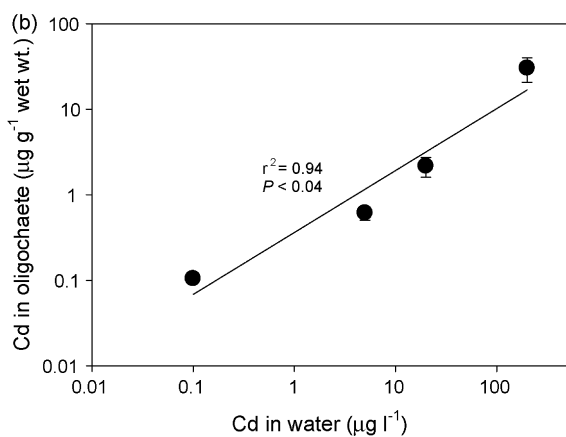
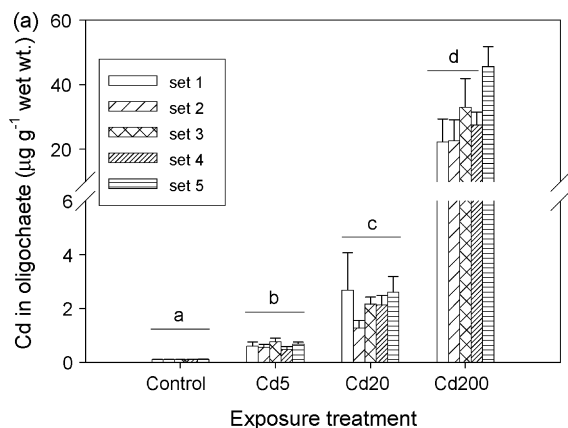


Fig. 1. (a) Cd concentration in oligochaete *L. variegatus* ($\mu\text{g g}^{-1}$ wet weight) after 1 week exposure to waterborne Cd. Mean \pm standard deviation ($n = 5$). The worms were exposed to control water (spiked with a trace of ^{109}Cd), $5 \mu\text{g L}^{-1}$ (Cd5), $20 \mu\text{g L}^{-1}$ (Cd20), and $200 \mu\text{g L}^{-1}$ Cd (Cd200) for 7 days. Significant differences in Cd concentration were tested by the non-parametric Kruskal–Wallis test, followed by the Dunnnett's T3 *post hoc* test. Different underlined letters indicate significant difference ($P < 0.01$) among treatments, but comparable concentrations in each treatment among sets of exposure ($P > 0.05$). (b) \log_{10} scale plot of Cd concentration in oligochaete *L. variegatus* ($\mu\text{g g}^{-1}$ wet weight) against Cd concentration in water ($\mu\text{g l}^{-1}$). Mean \pm standard deviation ($n = 5$). r^2 (0.94): coefficient of determination, and significance ($P < 0.04$) of the correlation was tested by linear regression analysis.

conditions, oligochaete worms from different sets of exposure accumulated similar amounts of Cd except that worms from set 5, exposed to $200 \mu\text{g L}^{-1}$ Cd had a higher Cd concentration compared to other sets. Since the variation among sets of exposure was in general small, we combined worms from all five sets of exposure for later feeding and subcellular analysis. Concentrations of Cd accumulated in the worms were in general proportional to the waterborne Cd concentrations (log scale, Fig. 1b).

3.2. Growth and mortality of fish

Dietary exposure to the Cd contaminated worms did not affect survival of the fish which averaged 95.7%. Individual specific growth rates of the fish were about 0.8–2.9% per day over the dietary exposure period, with a daily feeding rate of 3.5% body weight of fish (Fig. 2a). When comparing growth of the fish that were fed the same Cd diet, fish that were fed Cd5

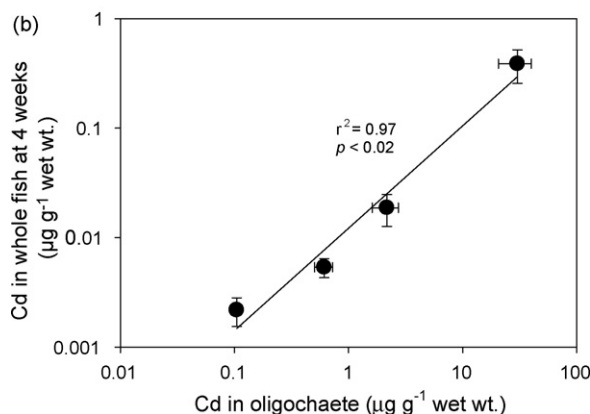
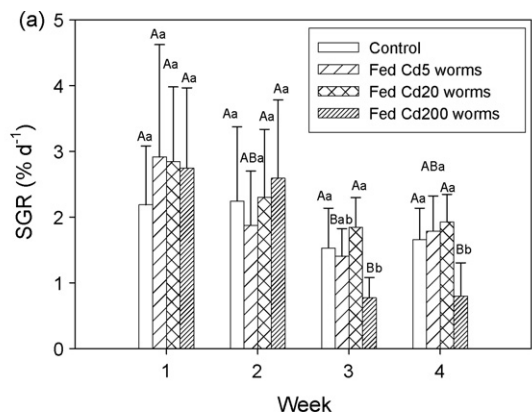


Fig. 2. (a) Individual specific growth rates (SGR, $\% \text{ day}^{-1}$) of *O. mykiss* that were fed Cd-contaminated oligochaete worms for 1 month. Mean \pm standard deviation ($n =$ approximately 58, 49, 39, 27 per treatment for weeks 1, 2, 3, 4, respectively). The fish were fed worms pre-exposed to control water (spiked with a trace of ^{109}Cd), $5 \mu\text{g L}^{-1}$ (Cd5), $20 \mu\text{g L}^{-1}$ (Cd20), and $200 \mu\text{g L}^{-1}$ Cd (Cd200) for 7 days. Significant differences ($P < 0.05$) were tested by one-way ANOVA, followed by the Tukey *post hoc* test. Means not sharing the same letter in the same case indicate significant differences among treatments in the same week (lower case) and among weeks in the same treatment (upper case). (b) \log_{10} scale plot of Cd concentration in the whole bodies of *O. mykiss* ($\mu\text{g g}^{-1}$ wet weight) at 4 weeks of dietary exposure against Cd concentration in the oligochaete *L. variegatus* worms ($\mu\text{g g}^{-1}$ wet weight). Mean \pm standard deviation (fish: $n = 10$; worms: $n = 5$). r^2 (0.97): coefficient of determination. Significance ($P < 0.02$) of the correlation was tested by linear regression analysis.

worms grew 2-fold slower in week 3 than week 1 ($P < 0.04$), but they recovered in week 4 ($P < 0.08$). Growth however, was more severely depressed in trout that were fed Cd200 worms in weeks 3 ($P < 0.001$) and 4 ($P < 0.001$), both relative to previous weeks and relative to the control group ($P < 0.04$), without any indication of recovery. In general, dietary exposure to Cd200 worms reduced the growth of fish by about 50% in week 3 and 4, compared to the control.

3.3. Tissue-specific Cd accumulation in fish

There was on average 30% Cd leached from the worms to the water when they were thawed and fed to the fish. After the 70% water replacement, the ^{109}Cd cpm in the water were greatly reduced, and became undetectable after continuous water flow for 4 h.

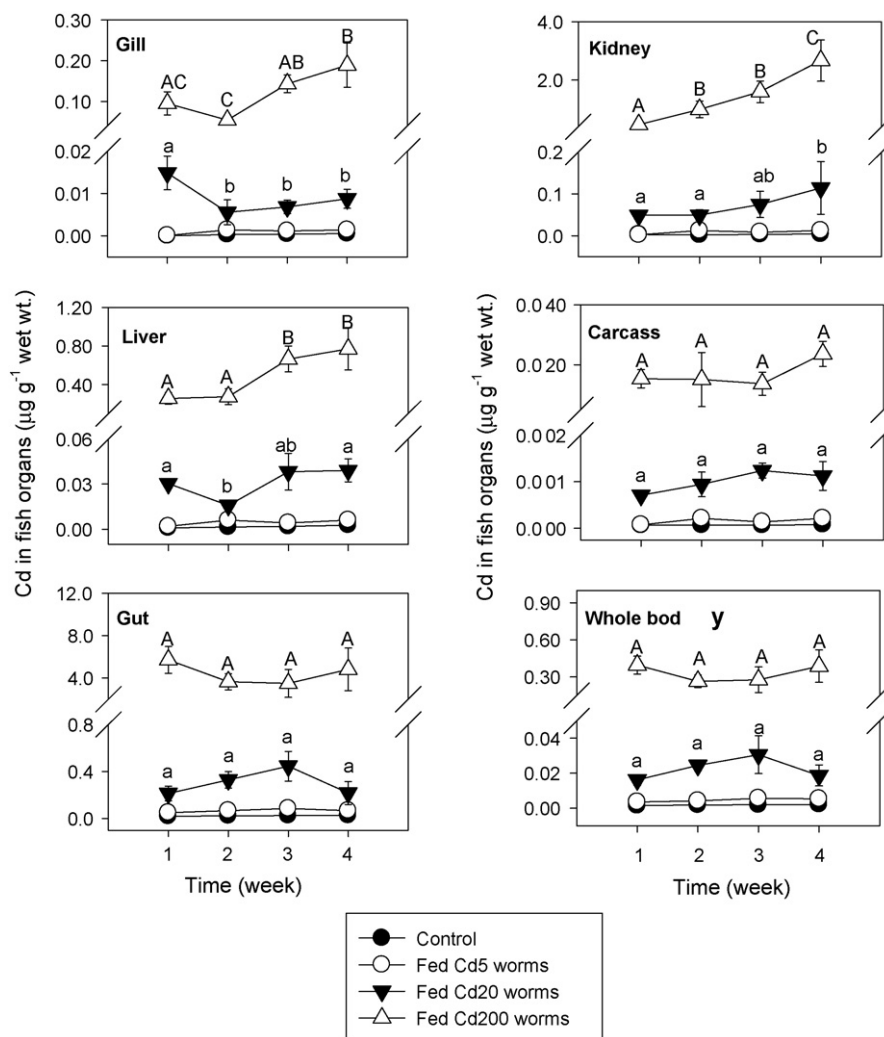


Fig. 3. Tissue-specific concentration of Cd ($\mu\text{g g}^{-1}$ wet weight) in *O. mykiss* after feeding on Cd-contaminated oligochaete worms for 1 month. Mean \pm standard deviation ($n=10$). The fish were fed worms pre-exposed to control water (spiked with a trace of ^{109}Cd), $5 \mu\text{g L}^{-1}$ (Cd5), $20 \mu\text{g L}^{-1}$ (Cd20), and $200 \mu\text{g L}^{-1}$ Cd (Cd200) for 7 days. Significant differences ($P < 0.05$) were tested by the non-parametric Kruskal–Wallis test, followed by the Dunnett's T3 *post hoc* test. Significant differences ($P < 0.01$) were found in Cd tissue-specific concentrations amongst all treatments. The Cd tissue-specific concentration in the fish fed control and Cd5 worms was constant over time, whereas the differences between weeks for the fish fed Cd20 and Cd200 worms are indicated by letters. Means not sharing the same letter in the same case indicate significant difference between weeks.

The whole fish accumulated 0.002, 0.005, 0.02, $0.4 \mu\text{g Cd g}^{-1}$ wet weight respectively, after feeding on control, Cd5, Cd20 and Cd200 worms for 4 weeks (Fig. 2b). Again, the whole body Cd burden in fish corresponded well with the Cd concentration in the worms, i.e., the higher Cd concentration in the worms the fish were fed, the higher amount of Cd the fish accumulated. Uptake and transport of Cd from the diet to internal organs was observed with significant accumulation in all measured tissues for the fish fed with Cd200 worms and slight accumulation in fish fed with Cd20 worms (Fig. 3). The gut had the highest Cd concentration, followed sequentially by the kidney, liver, gill and carcass. Cd levels remained similar over time in gut, carcass and whole body even though the fish were fed Cd-contaminated worms continuously for a month ($P > 0.05$). However, Cd load increased steadily over time in kidney (by 2–3-fold) ($P < 0.05$) and liver (by 4-fold) ($P < 0.05$) of the fish fed Cd20 and Cd200 worms, and slightly in gill (by

2-fold) ($P < 0.001$) in the fish that were fed Cd200 worms. The order of percentage of total Cd distributed among the organs was gut (81.3–92.7%) \gg kidney (0.9–6.4%) \sim liver (1.0–4.4%) \sim carcass (1.9–5.7%) \sim gill (0.1–3.3%) (Table 1). The percentage of Cd in the fish organs in general, was independent of the Cd concentration in the diet and the length of dietary exposure, but it increased 3-fold in the liver ($P < 0.001$) and 5-fold in the kidney ($P < 0.001$) in week 4 relative to week 1 in the Cd200 treatment.

The efficiency of Cd transferred from the oligochaete worms to the fish varied significantly with respect to weeks of dietary exposure, and also between the fish fed the control and Cd contaminated worms. The Cd transfer ranged from 0.9 to 6.4% (Table 2) and it was higher in the first week compared to later weeks ($P < 0.05$). In general, Cd transfer percentage from the Cd contaminated worms was about half of that from the control worms after week 1, but the transfer percentage was comparable among the Cd treatments.

Table 1
Percentage of whole body Cd in the organs of *O. mykiss* that were fed Cd- contaminated oligochaete (*L. variegatus*) for one month

Organ	Treatment	Week 1	Week 2	Week 3	Week 4
Gill	Control	<0.00 ^{Aa}	0.46 ± 0.15 ^{Ba}	0.72 ± 0.31 ^{Ba}	0.82 ± 0.40 ^{Ba}
	Fed Cd5 worms	0.09 ± 0.17 ^{Aa}	1.42 ± 0.55 ^{Bb}	0.62 ± 0.13 ^{Ca}	0.80 ± 0.13 ^{BCa}
	Fed Cd20 worms	3.27 ± 1.38 ^{Ab}	0.74 ± 0.32 ^{Bab}	0.75 ± 0.32 ^{Ba}	1.53 ± 0.52 ^{Ba}
	Fed Cd200 worms	0.83 ± 0.30 ^{ABb}	0.64 ± 0.19 ^{Bab}	2.02 ± 1.29 ^{ABa}	1.70 ± 0.74 ^{Aa}
Liver	Control	1.19 ± 0.86 ^{Aa}	1.23 ± 0.59 ^{Aa}	1.79 ± 0.40 ^{Aa}	1.92 ± 0.57 ^{Aa}
	Fed Cd5 worms	0.96 ± 0.51 ^{Aa}	2.80 ± 1.40 ^{Ba}	1.17 ± 0.31 ^{Aa}	1.58 ± 0.50 ^{ABa}
	Fed Cd20 worms	3.79 ± 0.79 ^{Ab}	1.32 ± 0.25 ^{Ca}	2.00 ± 0.54 ^{BCab}	3.29 ± 0.99 ^{ABa}
	Fed Cd200 worms	1.09 ± 0.36 ^{Aa}	1.44 ± 0.46 ^{Aa}	4.39 ± 1.78 ^{Bb}	3.26 ± 1.43 ^{Ba}
Gut	Control	85.75 ± 7.23 ^{Ab}	92.64 ± 2.84 ^{Aa}	91.31 ± 2.97 ^{Aa}	90.43 ± 2.91 ^{Aa}
	Fed Cd5 worms	94.51 ± 3.41 ^{Ac}	86.93 ± 3.97 ^{Ba}	94.47 ± 1.59 ^{Aa}	91.22 ± 1.36 ^{ABa}
	Fed Cd20 worms	81.90 ± 4.91 ^{Aa}	92.66 ± 1.60 ^{Ba}	89.18 ± 3.48 ^{Bab}	81.29 ± 5.21 ^{Ab}
	Fed Cd200 worms	92.06 ± 2.50 ^{Abc}	90.04 ± 3.09 ^{Aa}	82.28 ± 5.41 ^{Bb}	82.50 ± 3.70 ^{Bb}
Kidney	Control	2.46 ± 2.29 ^{Ab}	1.29 ± 0.66 ^{Aa}	1.96 ± 0.77 ^{Aa}	2.43 ± 0.89 ^{Aa}
	Fed Cd5 worms	0.87 ± 0.53 ^{Aa}	3.07 ± 1.26 ^{Cab}	1.40 ± 0.75 ^{ABa}	2.48 ± 0.17 ^{BCa}
	Fed Cd20 worms	3.57 ± 1.41 ^{ABb}	1.78 ± 0.49 ^{ABb}	2.78 ± 1.58 ^{Aa}	7.28 ± 2.93 ^{Bb}
	Fed Cd200 worms	1.34 ± 0.32 ^{ABb}	3.62 ± 0.89 ^{Bb}	6.15 ± 2.02 ^{Cb}	6.40 ± 1.19 ^{Cb}
Carcass	Control	4.26 ± 2.68 ^{Aa}	3.48 ± 1.37 ^{Aa}	3.18 ± 1.12 ^{Aa}	3.32 ± 1.16 ^{Aa}
	Fed Cd5 worms	1.93 ± 0.98 ^{Aa}	4.06 ± 0.91 ^{Ba}	2.18 ± 0.81 ^{Aa}	3.62 ± 1.46 ^{ABa}
	Fed Cd20 worms	3.92 ± 0.92 ^{Aa}	3.38 ± 1.06 ^{Aa}	3.97 ± 1.61 ^{Aa}	5.31 ± 1.54 ^{Aa}
	Fed Cd200 worms	3.56 ± 1.27 ^{Aa}	3.68 ± 1.67 ^{Aa}	4.52 ± 1.02 ^{Aa}	5.67 ± 1.25 ^{Aa}

Mean ± standard deviation ($n = 10$). Significant differences ($P < 0.05$) were tested by one-way ANOVA followed by Tukey *post hoc* tests. Means not sharing the same letter in the same case indicate significant differences among treatments in the same week (lower case) or among weeks in the same treatment (upper case).

3.4. Subcellular Cd compartmentalization in oligochaete worms

In general, worms exposed to Cd exhibited subcellular distributions comparable to those of the control group. A high percentage of Cd was associated with the trophically available fractions (metallothionein-like proteins + heat-denaturable proteins + organelles) of the worms (Control: $80.2 \pm 1.5\%$; Cd5: $76.5 \pm 3.1\%$; Cd20: $76.6 \pm 2.4\%$; Cd200: $72.4 \pm 2.8\%$) (Table 3) and among that, most Cd was distributed in the metallothionein-like proteins ($\geq 50\%$). The worms exposed to $200 \mu\text{g L}^{-1}$ Cd had significantly lower Cd in the trophically available fractions compared to the Control ($P < 0.01$), possibly due to a slower, but not statistically significant Cd distribution in the metallothionein-like proteins and organelles of the Cd-exposed worms.

Since the percentage of Cd transfer from the worms to the fish in the first week had different pattern from the other weeks, correlations between Cd storage in the worms and transfer percentage were plotted by weeks (Fig. 4). No significant relationship between the percentage of Cd transfer to the fish and Cd percentage in the trophically available frac-

tions of worms was demonstrated in the first week ($P > 0.05$). In the second and third weeks, the higher percentage of Cd in the trophically available fraction apparently was associated with a higher Cd transfer percentage to the fish (second week: $r^2 = 0.53$, $P > 0.05$; third week: $r^2 = 0.60$, $P > 0.05$); however, the correlations were not significant and similarly, no correlation was found for the fourth week of dietary exposure ($r^2 = 0.52$, $P > 0.05$).

3.5. Ca uptake by the fish

The Ca concentration in the dechlorinated hardwater was $831.5 \pm 40.5 \mu\text{M}$ and the Ca influx from the water to the whole body of fish was $30.9 \pm 8.3 \mu\text{mol}^{-1} \text{kg}^{-1} \text{h}^{-1}$ (Fig. 5). Ca influx in the fish fed the same Cd diet exhibited some variation from week to week. For example, Ca influx decreased in the control fish in week 4 compared to week 3 ($P < 0.001$), and increased in fish fed Cd200 worms in week 2 compared to week 1 ($P < 0.04$). However, in general, Ca influx was not affected by the dietary Cd exposure, except for a significant reduction of 10% in the highest Cd treatment in the third week, compared to the control ($P < 0.02$) and fish fed Cd5 worms ($P < 0.04$). A

Table 2
Trophic transfer (%) of Cd from the oligochaete *L. variegatus* to *O. mykiss*

Treatment	Week 1	Week 2	Week 3	Week 4
Control	5.35 ± 2.72 ^{ABab}	3.59 ± 0.59 ^{Aa}	2.49 ± 0.64 ^{ABa}	2.05 ± 0.60 ^{Ba}
Fed Cd5 worms	2.36 ± 1.05 ^{Aa}	1.32 ± 0.38 ^{ABb}	1.20 ± 0.21 ^{ABb}	0.90 ± 0.17 ^{Bb}
Fed Cd20 worms	2.52 ± 0.48 ^{Aa}	2.34 ± 0.29 ^{Ac}	1.90 ± 0.67 ^{ABb}	0.89 ± 0.29 ^{Bb}
Fed Cd200 worms	6.37 ± 1.16 ^{Ab}	1.82 ± 0.35 ^{Bbc}	1.38 ± 0.42 ^{Bb}	1.37 ± 0.46 ^{Bb}

Mean ± standard deviation ($n = 10$). Significant differences ($P < 0.05$) were tested by one-way ANOVA followed by Tukey *post hoc* tests. Means not sharing the same letter in the same case indicate significant differences among treatments in the same week (lower case) or among weeks in the same treatment (upper case).

Table 3
Subcellular Cd distribution (%) in the oligochaete *L. variegatus* and the gut tissue of *O. mykiss* after 1 and 4-week dietary exposure to Cd

Worms	MTLP	HDP	ORG	CD	MRG
Control	58.2 ± 5.4 ^{ab}	10.3 ± 0.6 ^{ab}	11.8 ± 3.5 ^a	17.8 ± 1.6 ^a	2.0 ± 0.5 ^a
Cd5	52.7 ± 5.6 ^{ab}	14.6 ± 3.2 ^a	9.1 ± 0.9 ^a	19.2 ± 4.8 ^a	4.3 ± 2.2 ^a
Cd20	58.5 ± 2.2 ^a	8.9 ± 1.0 ^b	9.2 ± 0.9 ^a	19.4 ± 1.6 ^a	4.1 ± 0.9 ^a
Cd200	49.7 ± 2.0 ^b	13.6 ± 2.7 ^a	9.2 ± 2.7 ^a	23.7 ± 3.1 ^a	3.8 ± 1.3 ^a
Fish (week 1)					
Control	26.0 ± 10.8 ^{Aa}	8.9 ± 3.7 ^{Aab}	18.0 ± 6.3 ^{Aa}	23.5 ± 12.6 ^{Aa}	22.8 ± 20.2 ^{Aa}
Fed Cd5 worms	45.7 ± 2.8 ^{Aa}	10.1 ± 1.0 ^{Aa}	7.9 ± 0.4 ^{Aa}	3.3 ± 0.7 ^{Aa}	32.9 ± 4.1 ^{Aa}
Fed Cd20 worms	26.4 ± 13.9 ^{Aa}	15.1 ± 12.4 ^{Aab}	9.9 ± 2.2 ^{Aa}	7.1 ± 4.7 ^{Aa}	41.5 ± 4.0 ^{Aa}
Fed Cd200 worms	18.4 ± 0.9 ^{Aa}	21.5 ± 2.0 ^{Ab}	9.9 ± 5.4 ^{Aa}	16.7 ± 6.7 ^{Aa}	33.5 ± 4.7 ^{Aa}
Fish (week 4)					
Control	23.2 ± 5.5 ^{Aab}	4.4 ± 0.8 ^{Aa}	12.1 ± 1.7 ^{Aa}	1.8 ± 0.2 ^{Aa}	58.4 ± 7.8 ^{Aa}
Fed Cd5 worms	18.1 ± 2.4 ^{Ba}	2.6 ± 0.1 ^{Ba}	9.9 ± 1.3 ^{Aa}	3.4 ± 0.7 ^{Aa}	65.9 ± 3.2 ^{Ba}
Fed Cd20 worms	29.9 ± 3.9 ^{Aab}	5.7 ± 1.5 ^{Aa}	14.7 ± 2.0 ^{Aa}	2.7 ± 0.7 ^{Aa}	47.1 ± 6.6 ^{Aab}
Fed Cd200 worms	35.8 ± 4.3 ^{Bb}	3.8 ± 1.6 ^{Ba}	13.3 ± 1.3 ^{Aa}	11.0 ± 4.5 ^{Aa}	36.1 ± 0.02 ^{Ab}

Mean ± standard deviation (worms: $n = 5$; fish gut tissue: $n = 3$). Significant differences ($P < 0.05$) were tested by one-way ANOVA followed by Tukey *post hoc* tests. Means not sharing the same letter in the same case indicate a significant difference among treatments in the same week (lower case) or between weeks in the same treatment (upper case). MTLP: metallothionein-like proteins; HDP: heat-denaturable proteins; ORG: organelles; CD: cellular debris; MRG: metal-rich granules.

difference in Ca influx may affect the subsequent Ca concentration in the fish plasma, but in this study, Ca concentration in the plasma was not significantly different among treatments over the entire exposure period, with an overall mean of $2705 \pm 312 \mu\text{M}$ ($P > 0.05$).

3.6. Subcellular Cd concentration in the fish gut tissue

The higher the Cd concentration in the diet, the higher amount of Cd accumulated in the subcellular fractions of the gut tissue of the rainbow trout (Fig. 6). Over the dietary exposure period, the amount of Cd associated with the biologically detoxified fraction (for example, metallothionein-like proteins) in the fish fed with the higher Cd contaminated worms increased (1.5–3-fold) (fish fed Cd20 worms: $P < 0.02$; fish fed Cd200 worms: $P < 0.002$), while that associated with the metal-sensitive fraction (for example, heat-denaturable proteins) decreased slightly (≤ 2 -fold) (fish fed Cd200 worms: $P < 0.01$). In terms of distribution, the percentage of Cd associated with the biologically detoxified ligands (metallothionein-like proteins + metal-rich granules, 48–84%) was higher than in the metal-sensitive fractions (heat-denaturable proteins + organelles, 12–31%) of the fish gut tissue (Table 3). Over time, fish in the highest Cd treatment increased Cd distribution in the metallothionein-like proteins (1 week versus 4 week, $P < 0.004$), and decreased that in the heat-denaturable proteins (1 week versus 4 week, $P < 0.002$). Generally, fish exposed to different Cd diets had comparable Cd subcellular distribution in the gut tissue at the beginning and end of the feeding period ($P > 0.05$), except that fish fed Cd200 worms had 12% less Cd in metal-rich granules than control ($P < 0.03$) and fish fed Cd5 worms in week 4 ($P < 0.02$). Compared to worms, the fish gut tissue stored less Cd in the trophically available fractions (fish: 49.8–63.7%; worms: 72.4–80.2%) and more in the metal-rich granules (fish: 22.8–65.9%; worms: 1.96–4.32%).

4. Discussion

4.1. Cd accumulation in the oligochaete worms

Most studies on Cd accumulation by *L. variegatus* have been primarily on sediment exposure (Chapman et al., 1999; Hansen et al., 2004). Waterborne exposure may also be an important metal exposure pathway for oligochaete worms. In this study, the oligochaete worm *L. variegatus* had a similar Cd bioconcentration factor ($134.9 \pm 34.6 \text{ L kg}^{-1}$) from the water as another oligochaete species *Tubifex tubifex* commonly used in toxicity testing (Redeker et al., 2004, 2007). *L. variegatus* accumulated different levels of Cd according to the exposure concentration; these tissue concentrations fit into the range which has been recorded in aquatic organisms ($1\text{--}29 \mu\text{g Cd g}^{-1}$) from clean or contaminated freshwater environments (Farag et al., 1999).

4.2. Cd bioavailability and accumulation in the rainbow trout

Worms leached 30% Cd into the water and the short-term waterborne Cd exposure would have been expected to exert the greatest effect on Cd accumulation in the Control fish as they were kept in the same tank with Cd-dosed fish. Despite this complication, Cd concentrations in the whole bodies and gills in the Control fish (whole body: $0.002 \mu\text{g g}^{-1}$ wet weight; gill: $0.0005 \mu\text{g g}^{-1}$ wet weight) were still comparable or lower than the background Cd levels in rainbow trout of the other studies (whole body: $0.002 \mu\text{g g}^{-1}$ wet weight; gill: $0.005 \mu\text{g g}^{-1}$ wet weight) (Baldisserotto et al., 2005; Franklin et al., 2005). These other studies used commercial pellet food that did not leach Cd. Thus, the effect of waterborne Cd exposure on the total Cd accumulation in the fish was likely negligible in the present study.

The trout which were fed Cd-contaminated worms, had a great increase of tissue-specific Cd concentration after the first

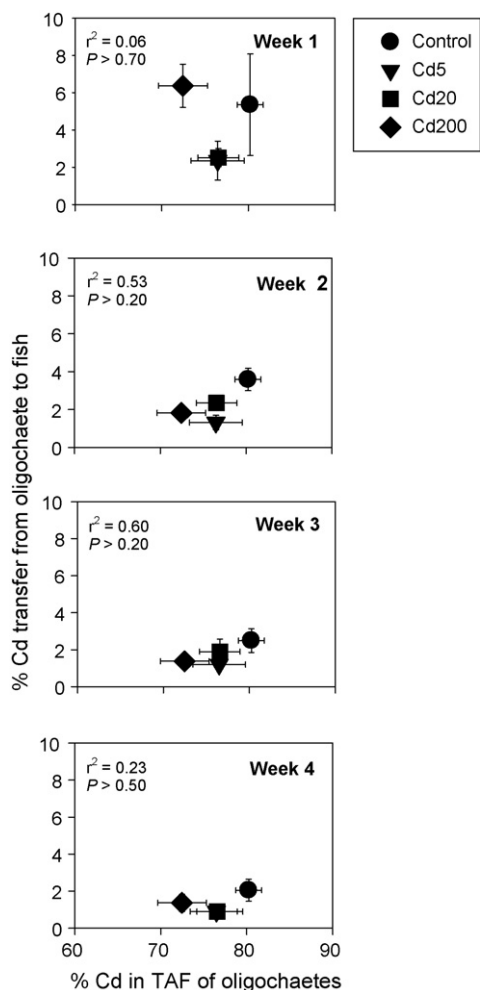


Fig. 4. Correlation between percentage of Cd transfer from the oligochaete *L. variegatus* to *O. mykiss* and the Cd distribution in trophically available fractions (TAF) of the worms. Mean \pm standard deviation (TAF: $n=5$; transfer efficiency: $n=10$). Different symbols indicate Cd percentage in TAF of worms that were exposed to different Cd doses plotted against trophic transfer efficiency of Cd for that particular treatment r^2 : coefficient of determination. Significance of the correlation was tested by the linear regression analysis; none were significant ($P > 0.05$).

week of dietary exposure. This indicates a higher Cd uptake than loss by the fish in the first week. In terms of tissue-specific Cd distribution, rainbow trout fed Cd-contaminated worms retained most Cd in the gut tissues ($\geq 80\%$). A similar study using commercial pellet food demonstrated only 20–35% Cd accumulation in the gut tissue (Franklin et al., 2005). Gastric-infused Cd (Chowdhury et al., 2004) or Cd from the commercial diet (Franklin et al., 2005) is significantly absorbed in the stomach of rainbow trout but there is little information on the absorption of Cd in the gut from the natural diet. Our study demonstrated that despite a high percentage of Cd in the gut, a low percentage of Cd was internalized to other tissue. This agrees with Chowdhury et al. (2004) that only 2.4% gastric-infused Cd was internalized across the gut wall of rainbow trout whereas the rest was bound in the gut tissue, remained in the lumen or was lost from the fish. The preferential accumulation of Cd in the liver and kidney over time in the current study has been reported elsewhere

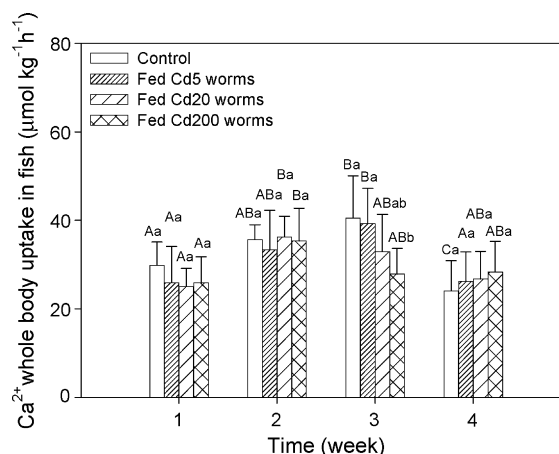


Fig. 5. Whole body uptake rate of Ca ($\mu\text{mol kg}^{-1} \text{h}^{-1}$) in *O. mykiss* that were fed Cd-contaminated oligochaete worms for various periods of time. Mean \pm standard deviation ($n=10$). The fish were fed worms pre-exposed to control water (spiked with a trace of ^{109}Cd), $5 \mu\text{g L}^{-1}$ (Cd5), $20 \mu\text{g L}^{-1}$ (Cd20), and $200 \mu\text{g L}^{-1}$ Cd (Cd200) for 7 days. Significant differences ($P < 0.05$) were tested by one-way ANOVA, followed by the Tukey *post hoc* test. Means not sharing the same letter in the same case indicate significant differences among treatments in the same week (lower case) and among weeks in the same treatment (upper case).

(Szebedinszky et al., 2001; Chowdhury et al., 2004; Franklin et al., 2005) and may relate to the metal sequestration abilities of these tissues.

The Cd concentrations in the liver, kidney, carcass and gill of the fish were 3–5-fold, 3–4-fold, 20–100-fold and 4–8-fold respectively, less than those of the trout exposed to Cd-supplemented pellet food at a similar ration and exposure period (Baldissarotto et al., 2005; Franklin et al., 2005). Largely, this difference reflected the much lower Cd dose in the present study (6–8-fold lower Cd daily dose). Nevertheless, the Cd concentrations in the gut and whole body of the present fish were comparable to those of the previous study. This may imply a higher Cd bioavailability across the gut wall of the fish from the natural diet. Harrison and Curtis (1992) suggested that the efficiency of metal absorption from commercial diets may differ from that of metals absorbed through the food chain. For example, both the sunfish *Lepomis gibbosus* (Merlini et al., 1976) and plaice *Pleuronectes platessa* (Pentreath, 1973) accumulated Zn from commercial food more efficiently than Zn incorporated into live foods. In contrast, the absorption efficiency coefficient for Cd was about five times greater for fish which were fed amphipods than for fish which were fed commercial trout diet (Harrison and Curtis, 1992). The mechanism for the difference in metal bioavailability between natural and commercial diets is still unclear and worthy of further study.

One possible explanation is that the high percentage of Cd bound to the proteins of the worms ($\sim 70\%$, strong sulfhydryl bond) may result in higher bioavailability than for metals loosely adsorbed on the commercial pellet food. The higher bioavailability of metals binding to proteins than metals binding to inorganic matter is also observed in other invertebrates (Wang and Fisher, 1996; Griscom et al., 2000). Another factor may be the ionic composition of the diet. Diets containing a higher Ca concentra-

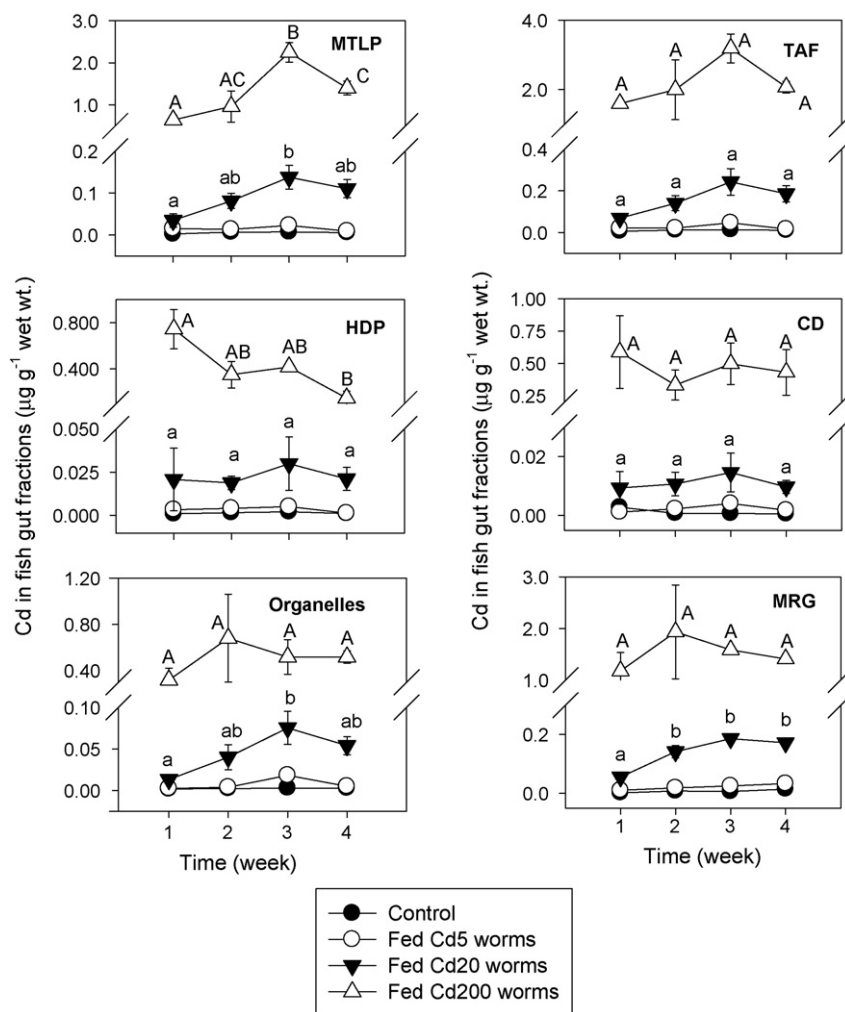


Fig. 6. Cd concentrations ($\mu\text{g g}^{-1}$ wet weight tissue) in subcellular fractions of the gut tissue of *O. mykiss* that were fed Cd-contaminated oligochaete worms for 1 month. Mean \pm standard deviation ($n=3-4$). The fish were fed worms pre-exposed to control water (spiked with a trace of ^{109}Cd), $5 \mu\text{g L}^{-1}$ (Cd5), $20 \mu\text{g L}^{-1}$ (Cd20), and $200 \mu\text{g L}^{-1}$ Cd (Cd200) for 7 days. MTLP: metallothionein-like proteins; HDP: heat-denaturable proteins; ORG: organelles; CD: cellular debris; MRG: metal-rich granules. The trophically available fractions (TAF) include MTLP, HDP and ORG. Significant differences ($P < 0.05$) were tested by the non-parametric Kruskal–Wallis test, followed by the Dunnett’s T3 *post hoc* test. Significant differences ($P < 0.01$) were found in Cd fraction-specific concentrations among all treatments. The Cd subcellular concentration in the fish fed control and Cd5 worms was constant over time, whereas the differences among weeks for the fish fed Cd20 and Cd200 worms are indicated by letters. Means not sharing the same letter in the same case indicate a significant difference between weeks.

tion may reduce Cd uptake since Ca and Cd appear to share the uptake transporters in the gastrointestinal tract of the freshwater fish (Wood et al., 2006). Trout commercial pellet food has a much higher Ca concentration ($\sim 20 \text{ mg g}^{-1}$ dry weight) (Franklin et al., 2005) than typical invertebrates in freshwater environments ($\sim 1 \text{ mg g}^{-1}$ wet weight) (Klinck et al., 2007). Investigations of diet composition and metal speciation in the diet may provide insights into the difference of metal bioavailability between the two diets.

4.3. Trophic transfer efficiency of Cd

Assimilation efficiency of Cd, which is a component of trophic transfer efficiency, is generally reported to be low ($< 10\%$) (Douben, 1989; Campenhout et al., 2007). This agrees with the low trophic transfer efficiency in this study. Nevertheless, the trophic transfer efficiency in this study was in general, 1–3-fold higher than Cd taken up from Cd-supplemented com-

mercial food or oral administration ($< 2\%$) (Haesloop and Schirmer, 1985; Harrison and Klaverkamp, 1989; Handy, 1992).

Cd trophic transfer efficiency was independent of the Cd concentrations in the Cd-dosed worms; however, it was reduced over the dietary exposure period. This may relate to the effect of dietary acclimation to Cd on the Cd assimilation efficiency and excretion by the fish. For example, Cd level was reduced by 71% in the posterior intestine of the rainbow trout that had acclimated to dietary Cd for 30 days, suggesting an enhanced sloughing of Cd bound to tissue materials in the gut (Chowdhury et al., 2005).

Trophic transfer efficiency of Cd in the rainbow trout was only weakly correlated to the internal Cd storage in the worms. Aquatic organisms have a variety of metal detoxification strategies and metals in different intracellular pools are not equally bioavailable to the predators (Rainbow, 2007). Wallace and Luoma (2003) first suggested that organelles, heat-denaturable proteins and metallothionein-like proteins are trophically avail-

able fractions and Cd in these fractions of the bivalve were 100% assimilated by the grass shrimp. Linear relationships between percentage of metals in the trophically available fractions of the prey and metals assimilated by the predators were found in many invertebrate prey, predators and fish (Wallace et al., 1998; Seebaugh and Wallace, 2004; Seebaugh et al., 2006; Zhang and Wang, 2006b). However, trophically available fractions cannot be totally accountable for the metal bioavailability. Seebaugh et al. (2005) reported that 68% Cd was trophically available in the grass shrimp *Palaemonetes pugio*, but only 3–19% Cd was assimilated by the predator fish *Fundulus heteroclitus*. Similarly, Redeker et al. (2007) reported that 72% trophically available Cd in *T. tubifex* was only associated with 9.8% assimilation efficiency by the carp *Cyprinus carpio*. Even when isolated and pure trophically fractions were fed to the grunt *Terapon jarbua*, the assimilation was lower than 100% (Zhang and Wang, 2006b). In addition, several studies have shown that not only trophically available fractions, but also the insoluble fractions such as metal-rich granules can be bioavailable to the predators (Cheung and Wang, 2005; Zhang and Wang, 2006b). The oligochaete worm *L. variegatus* in our study had 72–80% Cd in the trophically available fractions, which was comparable to *T. tubifex* (71%) under a similar waterborne exposure (Redeker et al., 2007); however, only a few percent were retained by the fish.

Explanations for low bioavailability of Cd in our study are complicated by the continuous dose of Cd and long observation period (1 month), in contrast to the single dose and short observation period (equal to or less than 1 week) of other assimilation studies. The low Cd bioavailability in our study may be caused by incomplete assimilation of Cd from the worms or regulation of Cd by reducing assimilation and increasing excretion in the rainbow trout over the dietary exposure time. Cd uptake and loss kinetics in the fish need to be examined to verify explanations. In addition, the minimal range of variation of Cd subcellular distribution in the worms, regardless of the Cd concentrations, may also lead to the weak correlation between the Cd subcellular distribution and Cd trophic transfer efficiency by the rainbow trout.

4.4. Toxicity of dietary Cd on the rainbow trout

In the present study, there was no overall effect of dietary Cd on whole body Ca uptake from the water, or Ca concentrations in the plasma of the rainbow trout. Hypocalcemia and disruption of Ca^{2+} transport following acute and chronic waterborne Cd exposure have been widely reported for several fish species (Pratap et al., 1989; Baldisserotto et al., 2004). This appears to be related to inhibition of the basolateral active Ca^{2+} extrusion mechanism, Ca^{2+} -ATPase, and associated closure of apical Ca^{2+} channels in gill ionocytes (Verbost et al., 1987, 1989). However, the literature is unclear about the effects of dietary Cd on Ca uptake from the water. Franklin et al. (2005) reported no significant inhibitory effect of dietary Cd ($500 \mu\text{g g}^{-1}$) on the whole body waterborne Ca^{2+} uptake rates in the rainbow trout, but Baldisserotto et al. (2005) found a 50% reduction in the fish exposed to a similar Cd diet. The effect of the dietary Cd was only transient, suggesting that the trout recovered or acclimated

during the extended exposures. It may be possible that dietary Cd acts at a different site from the waterborne Cd, and dietary Cd only transiently affected waterborne Ca uptake.

Waterborne Cd is well-known to reduce growth of the fish (Benoit et al., 1976; Peterson et al., 1983). In the present study, dietary Cd at the highest dose level ($30 \mu\text{g g}^{-1}$ in the worms) also reduced the growth of fish in the later weeks of exposure. This is possibly explained by the reduction of food/caloric conversion efficiency (Hansen et al., 2004) as energy is used for detoxifying Cd. Another possibility is an inhibition of Ca uptake from the diet resulting in limitation of skeletal growth. In contrast, Cd-supplemented pellet food does not produce any toxic effects on growth when the concentration is at or lower than $500 \mu\text{g g}^{-1}$ (Pratap et al., 1989; Szebedinszky et al., 2001; Baldisserotto et al., 2005; Franklin et al., 2005). This correlates with reports that growth of fish fed metal-contaminated invertebrates in the natural range of concentrations may be inhibited depending on the prey (Farag et al., 1994, 1999; Mount et al., 1994), and reinforces the conclusion that dietary toxicity of metals may be enhanced when metals are biologically incorporated.

Rainbow trout were efficient in detoxifying Cd. The increase of Cd concentrations transported to the kidney and liver and the fraction stored in the metallothionein-like proteins may facilitate the excretion of Cd and render it into a non-toxic form in the fish tissue. Metallothionein is widely recognized as important for detoxifying Cd in the rainbow trout. Waterborne exposure to $3 \mu\text{g L}^{-1}$ Cd for 30 days induced metallothionein in the kidney, liver and gill (Hollis et al., 2001; Chowdhury et al., 2005). In addition, dietary Cd induced the greatest increase of metallothionein in the kidney, followed by caeca, posterior intestine, liver and stomach, mid-intestine, and gill of the trout (Chowdhury et al., 2005). The marine black seabream *Acanthopagrus schlegeli* accumulated about 20–40% Cd in the insoluble fractions (including metal-rich granules, cellular debris and organelles), 10–40% Cd in the heat-denaturable proteins and 40–60% Cd in the heat-stable proteins or metallothionein-like proteins in the carcass and viscera. After waterborne and dietary Cd exposure, the percentage of Cd associated with the insoluble fractions of the carcass decreased; meanwhile, more Cd moved to the heat-denaturable proteins of the carcass and less was retained in those of the viscera (Zhang and Wang, 2006a). The subcellular Cd distribution in the gut tissue of the rainbow trout was slightly different, with a relatively higher percentage in the insoluble fractions (~55% in week 4) than the metallothionein-like proteins (~30% in week 4) at the beginning of the exposure. As the Cd dietary uptake increased over the exposure period, metallothionein played a more significant role and Cd levels were reduced in the heat-denaturable proteins, presumably to avoid the toxic effects of Cd.

4.5. Conclusions

To conclude, our study provides significant implications for understanding the dietary toxicity of Cd in the rainbow trout. Worms are one of the natural food sources of trout and Cd concentrations accumulated by *L. variegatus* in the present laboratory exposure were comparable to the Cd concentrations of

aquatic organisms recorded from contaminated environments. Trophic transfer efficiency of Cd from the oligochaete worms to the rainbow trout was low. Nevertheless, an environmentally relevant concentration of Cd in the worms inhibited growth of the fish, despite no observed effects on the Ca regulation. Most of the absorbed Cd was held in the gut tissue. The relatively higher transfer efficiency of Cd from natural food than from the commercial food used in other studies provides evidence that biologically incorporated Cd is more bioavailable and toxic to the fish. This finding may have great implications on the choice of diet for future dietary toxicity experiments.

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