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Daphnia need to be gut-cleared too: the effect of exposure to and ingestion of metal-contaminated sediment on the gut-clearance patterns of *D. magna*

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Abstract

The presence of sediment particles in the gut indicated that *Daphnia magna* used in whole-sediment bioassays ingest sediment. If gut contents are not removed prior to whole-body tissue-burden analysis, then the bioavailability of any sediment-associated contaminants (e.g. metals) can be overestimated. Gut clearing patterns were determined for *D. magna* after exposure to both clean and metal-contaminated (Cu and Zn) field-collected sediments. *D. magna* exposed to reference sediment had fuller guts than those exposed to metal-contaminated sediment (95% versus 60% full). Neither reference- nor metal-exposed *D. magna* could clear their gut completely of sediment particles when held in clean water for 24 h. When *Daphnia* were transferred to clean water after exposure to metal-contaminated sediment, there was no significant decrease in gut-fullness ($P > 0.05$) even after 48 h of purging. By comparison, animals transferred to water containing 5×10^5 cells of algae (*Pseudokircheriella subcapita*) after exposure to contaminated sediment showed a significant drop in gut fullness from 56% immediately after exposure to 17% after 4 h of gut-clearance. Although gut fullness did not change significantly beyond 2 h of purging, data were much less variable after 8 h of gut-clearance than after 2 h or 4 h. The depuration of Cu was well described with a two-compartment first-order kinetic model ($r^2 = 0.78$, $P < 0.0001$) indicating that *D. magna* exposed to metal-contaminated sediment have one pool of Cu that is quickly depurated ($0.2\ h^{-1}$), and one that has been incorporated into the tissues ($\ll 0.00001\ h^{-1}$). Assuming tissue background of 48 μg/g, an exposed animal which has not been depurated or which has been purged with water alone would yield whole-body tissue Cu concentrations that are 5.6- and 4-fold higher, respectively, than that purged with algae + water (8 h). We recommend that *D. magna* used to estimate metal bioavailability from sediment be gut-cleared in the presence of algae for 8 h prior to determination of whole-body metal concentrations.

Keywords: *D. magna*; Gut-clearance; Metal bioavailability; Sediment exposures

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1. Introduction

Daphnia magna is routinely used as a model organism for investigating environmental exposure because of its sensitivity to environmental pollutants and ease to maintain in laboratory cultures. As filter-feeders, they sieve large quantities of water to collect suspended particles, and although their preferred food is phytoplankton, they will ingest any suspended particle that can be retained by their filtering appendages (i.e. > 0.45 μm; Brendelberger, 1985). If food levels fall below a threshold, they will also scrape the bottom or stir up sediments to feed on resuspended particles (Horton et al., 1979; Lampert, 1987). Therefore, although *D. magna* feeds mainly on plankton (algae and bacteria), they may ingest sediment and any associated contaminants (including metals) by inadvertently sieving resuspended material or by actively browsing at the sediment–water interface.

Given that *D. magna* can ingest both algae and sediment, it is an ideal test organism for studying the effects of metal-contaminated sediment on the planktonic food-web. In standard protocols (ASTM, 2003) with benthic deposit feeders, animals are exposed to contaminated sediment, and then held in clean water or clean sediment for a suitable period (8–24 h) to purge their guts, before they are analyzed for whole-body contaminant burden (Hare et al., 1989; Phipps et al., 1993; Krantzberg, 1994; Amyot et al., 1996; Brooke et al., 1996; Neumann et al., 1999; Gillis et al., 2004). The purging ensures that metal-contaminated particles in the animal's gut do not lead to overestimation of metal bioavailability. Although it is not standard practice to clear the gut of planktonic organisms before analyzing their tissues for whole-body metal accumulation, some investigators do transfer animals to clean algae to purge their gut after they have been fed metal-contaminated algae. For instance, Munger and Hare (1997) fed clean algae to *Ceriodaphnia dubia* for 30 min after exposing them to Cd-contaminated algae. Hooke and Fisher (2001) transferred *C. dubia* to clean algae for 4 h after exposure to Ag-labelled algae, and Barata et al. (2002) transferred *D. magna* to clean algae for 8 h to purge Cd-labelled algae. However, we have found no reports in the literature that document the amount of time required to purge the guts of *Daphnia* after they have fed on contaminated sediment.

The gut residence time of *Daphnia* can vary from 2 to 55 min, with gut passage time being inversely related to food concentration (Bond, 1973; Bourne, 1959; Rigler, 1961; Schindler, 1968; McMahon, 1970; Gliwicz, 1986; Cauchie et al., 2000). Gut passage time in these studies was determined while the animals were feeding on suspended algae, glass beads or clay particles. Since unassimilated algae are excreted from the hind-gut as more algae are ingested and packed into the fore-gut, Gophen and Gold (1981) have suggested that starved animals can retain feces in the mid-gut indefinitely. Particles that are resistant to digestion may also accumulate in the hind-gut when all other materials are digested (Lampert, 1987). Although 15–30 min were adequate to clear the gut of animals exposed to uncontaminated algae, Barata et al. (2002) found that 3–6 h were needed when animals fed on metal-contaminated algae. Therefore, exposure to and ingestion of metal-contaminated sediment particles could substantially lengthen the gut passage time in *D. magna*.

The goals of this study were to determine the length of time required for *D. magna* to clear its gut after exposure to metal-contaminated sediment and to determine if the presence of food (algae) during gut-clearance would alter gut passage time. Three experiments were conducted to investigate gut-clearance patterns in *D. magna*. In the first experiment, the gut-clearance rate of *D. magna* exposed to metal-contaminated sediment was compared to that of *D. magna* exposed only to food. In the second experiment, reference sediment was used in place of metal-contaminated sediment to determine if *D. magna* were able to clear clean sediment from their gut when held in clean water for up to 48 h. A third experiment was conducted to determine if *D. magna* exposed to metal-contaminated sediment were able to clear their gut of sediment if they were fed algae during the gut-clearance period. The ultimate goal of these experiments was to determine the effect of different gut-clearance protocols on calculation of whole-body tissue metal concentrations in *D. magna*.

In this paper, we use the term 'gut-clearance' to describe the purging of gut contents in *D. magna*. Therefore, 'gut-clearance rate' is the rate at which the gut contents are purged and should not be confused with 'clearance rate' which has often been used in the *Daphnia* literature to describe the rate at which suspended algae is filtered from the water column.

2. Materials and methods

2.1. Sediments

The metal-contaminated sediment used in this study was collected from Clear Creek, CO, USA (39°44′54″ N, 105°23′55″ W). Clear Creek is a high-gradient stream, which receives metal-rich effluent from a number of mining sites, and has elevated metal levels in both the water and sediment. Sediment was collected from a site referred to as Beaver Dam on Clear Creek and shipped to McMaster University, Hamilton, Ont., Canada. Since Walski (2002) reported that there was significant bioavailable Zn and Cu associated with the Clear Creek sediment, we focused on the accumulation and depuration of these metals in *D. magna*. An earlier study (Gillis P.L., Wood C.M., Ranville J.F., Chow-Fraser P., unpublished data), found that exposure to this sediment was acutely toxic to *D. magna*; therefore, we attempted to remove any easily mobilized metals from the sediments by repetitive rinsing prior to use in these exposures. Sediment metal concentrations in the 'washed' sediment are reported in Table 1. The average organic carbon content of Clear Creek sediment was 2% (Walski, 2002). The reference sediment used in this study was collected from Long Point, Lake Erie (42°33′54″ N, 80°02′28″ W) and has been used as a 'clean' sediment by Environment Canada (referred to as Lake Erie 303). The organic carbon content of the reference sediment was 0.3%. Bulk sediments were digested using sequential acid digestion and analyzed by ICPMS.

2.2. Algae cultures

A pure culture of *Pseudokircheriella subcapita* purchased from University of Toronto Culture Collection (Toronto, Ont., Canada) in November 2002, was cultured using Bristol's medium according to USEPA protocol 16.60049002F (1993). Algae were cultured in 3-L volumetric flasks and held under 24 h light in climate-controlled chambers at 22 ± 2 °C.

2.3. D. magna cultures

A *D. magna* clone (lot #090600 DM) purchased from Aquatic Research Organisms (Hampton, NH, USA) in November 2002 was held in continuous culture according to USEPA protocol 16.60049002F (1993). *Daphnia* were fed a combined diet of yeast, cerophyll and trout chow (YCT) and algae (*P. subcapita*) daily. Culture media were changed three times per week. Neonates were used to initiate new cultures once a week. Dechlorinated Hamilton city tap water (Lake Ontario) was used as culture water, the overlying water in exposures, and the water for gut clearing. This water (herein referred to as reference water) was dechlorinated on site and routinely monitored for chlorine, metals and major ions. Ionic composition in mM was $[Na^+] = 0.6$, $[Cl^-] = 0.8$, $[Ca^{2+}] = 1.8$, $[K^+] = 0.4$, $[Mg^{2+}] = 0.5$. Hardness was approximately 140 mg/L (as $CaCO_3$), pH was 7.8–8 and DOC was approximately 3.0 mg C/L. Background Cu in the water was 2–4.0 μg/L and Zn was less than 60 μg/L.

We chose to use juvenile *D. magna* rather than neonates because their larger size was more suitable for tissue analysis, and rather than adults because this would avoid the changes in size and ionic status that accompany brood production and release.

2.4. Exposures

Prior to the gut-clearance experiments, juvenile *D. magna* (5-day-old) were 'exposed' to either: regular food (YCT); or field-collected sediment (reference or metal-contaminated). The specific exposure regime de-

Table 1
Metal concentrations (μg/g) of sediments used in exposures, Clear Creek (metal-contaminated, Experiment I), Lake Erie 303 (reference, Experiment II) and Clear Creek diluted with reference (Experiment III)

Sediment	Al	Ba	Ca	Cd	Co	Cu	Fe	K	Mg	Mn	Pb	Zn
Clear Creek[a]	9368.3	128.6	3878.9	25.2	41.1	1061.2	71010.2	654.8	1069.4	5166.6	190.4	4752.4
Lake Erie 303[b]	3637.6	21.3	73184.7	0.7	5.3	12.6	9642.7	482.4	NA	412.2	BD	32.6
Mixed[b]	10184.3	118.8	59056.0	11.4	25.3	469.0	53381.4	2844.6	13050.7	2327.0	140.7	2162.6

Sediment [a] was analyzed at a different time than sediment [b]. Pb detection limit was 1.2 μg/g. Ag (<0.24 μg/g) and As (<1.8 μg/g) were below detection in all sediments. NA: not available, BD: below detection.

pended upon the goal of the experiment (see below). All exposures were conducted in 5-L glass aquaria held at ambient (22 °C) room temperature (possible variation ±2 °C) for 24 or 48 h depending on the experiment. No supplemental food was given to *D. magna* exposed to sediments with the aim that hunger would motivate the animals to browse at the sediment–water interface. We also wanted to avoid any possible binding of dissolved metals with supplemental food, which could potentially affect metal uptake and toxicity.

Following exposure, *D. magna* were transferred to 250 mL glass beakers for the designated gut clearing time as detailed below. Water, and when necessary algae, in the gut clearing vessels were replaced every 4 h to prevent cophrophagy.

2.5. Gut fullness determination

After the appropriate gut clearing time (0 to 48 h), individual *D. magna* were rinsed in deionized water prior to visual estimation of the percentage of gut fullness using depression slides and a compound stereomicroscope according to the designations in Fig. 1. The actual estimate in each case was an interpolation between the reference points marked in Fig. 1.

2.6. Gut-clearance experiments

2.6.1. Gut-clearance following exposure to metal-contaminated sediments (Experiment I)

D. magna were either fed YCT (7.5 mL/L) or exposed to metal-contaminated field sediment (water to sediment ratio 4:1, v:v) for 24 h before transfer to reference water for: 0, 1, 2, 4, 6, 8, or 12 h to purge their gut contents. Although the initial aim was to examine at least 10 *D. magna* at each time interval, because of mortality experienced during the exposure and gut clearing period, only 5 individuals were examined at each designated time. After the appropriate gut-clearance period, gut fullness was determined as described above.

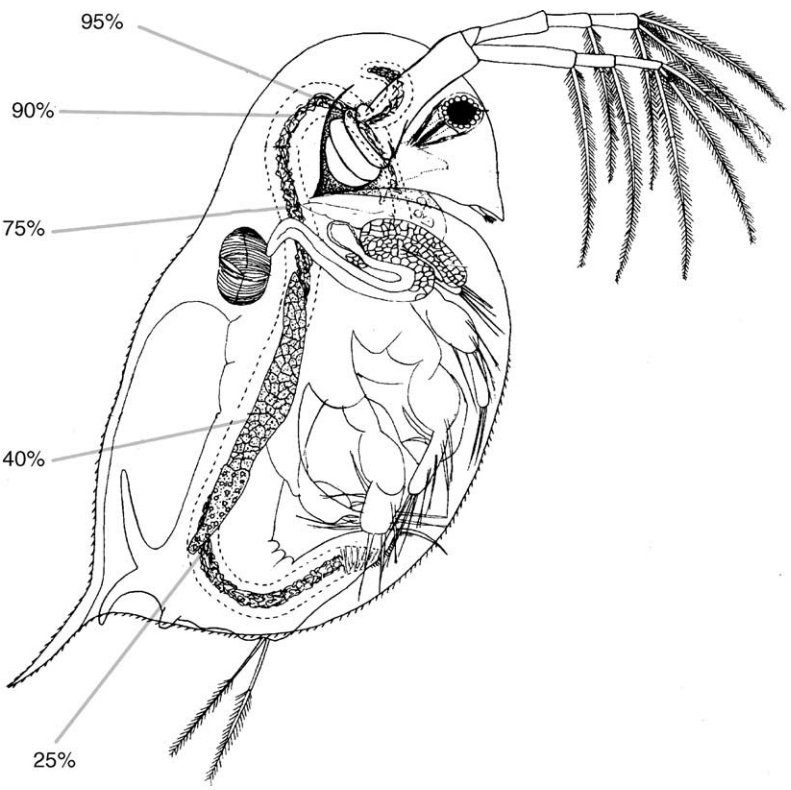

Fig. 1. Designations of percent gut fullness used for visual (microscopic) determination. *Daphnia* diagram was modified from Pennak (1989).

In all three experiments, the $t = 0$ samples were processed immediately after removal from the exposure aquarium (i.e. no gut-clearance).

2.6.2. Gut-clearance following exposure to reference sediments (Experiment II)

D. magna were exposed to reference field sediment for 24 h prior to transfer to reference water for: 0, 12, 24, or 48 h. For each of the designated time periods, 10 *D. magna* were placed in each of 3 replicate beakers. After the appropriate gut-clearance period, gut fullness was determined as described above.

2.6.3. Gut-clearance in the presence of algae (Experiment III)

Based on an earlier study (Gillis P.L., Wood C.M., Ranville J.F., Chow-Fraser P., unpublished data), we suspect that the mortality observed in the first experiment was a result of exposure to toxic levels of Cu and Zn in the overlying water. Therefore, for this experiment, the Clear Creek sediment was diluted 50:50 with reference sediment. Dissolved (filtered through an Acrodisc 0.45 μm syringe-tip in-line filter) and total (unfiltered) Zn and Cu concentrations in the overlying water were measured at initiation of the exposure. Following 48 h of exposure, the animals were transferred to beakers containing either reference water only, or reference water with 5×10^5 cells of *P. subcapita* added. *D. magna* were then held for: 0, 1, 2, 4, 8, 12, 24, or 48 h. For each of the designated time periods, seven *D. magna* were placed in each of five replicate beakers. After the appropriate gut-clearance period, gut fullness was determined and all *D. magna* from one replicate beaker were combined into a single sample for metal analysis (due to minimum requirement, approximately 0.3 mg). Tissues were dried at 60 °C for 24 h, weighed, and then digested with 50 μL of concentrated, metals grade nitric acid (2 mL DiaMed™ polypropylene centrifuge tubes) for 24 h at 60 °C. Samples were brought up to a final volume of 1.5 mL with 1% nitric acid. Tissue and water concentrations of Cu were measured using Graphite Furnace Atomic Absorption Spectroscopy (220, Varian) and Zn concentrations were determined using Flame Atomic Absorption Spectroscopy (220 FS, Varian). Method blanks (5) and Fisher Scientific calibration standards (every 20 samples) were included in every run. The detection limit for Zn was 60 μg/L and the detection limit for Cu was 2 μg/L.

Even after *D. magna* were gut-cleared in the presence of algae, it appeared from observations under stereo-microscope that approximately 20% of the gut still contained either sediment or partially digested algae. To verify the origin of the residual gut content, NCS-II Tissue Solubilizer (Amersham Corp., IL, USA) was applied to several specimens; the residual material was assumed to be sediment if it was still visible after tissue solubilization.

2.6.4. Statistical analysis

Means are given ±standard deviation. Statistical analyses were conducted using the software SPSS version 10.0. Comparisons between treatments (depuration times) were made using analysis of variance. Dunnett's Test was used to determine the first clearance time that gut fullness and metal concentrations were different from the control, and Tukey's multiple comparison test was used to determine differences between consecutive clearance times ($P < 0.05$).

A two-compartment first-order kinetic (2CFOK) model after Newman (1998) and Neumann et al. (1999) was used to characterize metal kinetics during gut-clearance of algae-cleared *D. magna* as:

$$C_{TB} = G_0 e^{(-k_g t)} + C_B e^{(-k_e t)} \quad (1)$$

where C_{TB} is the whole-body concentration at time t (μmol/g dry tissue), G_0 the contribution of gut contents to whole-body concentration at start of depuration (μmol/g dry tissue), C_B the whole-body concentration of metal in the body excluding the gut content at the start of depuration (μmol/g dry tissue), k_g the gut-clearance rate (h^{-1}), k_e the depuration rate from body (h^{-1}) and t is the time (h).

The first compartment ($G_0 e^{(-k_g t)}$) represents the total body concentration of metal that is easily mobilized. This compartment is used to predict the proportion of a metal contained in the gut at time 0 and the rate that the metal is cleared from the gut. The second compartment ($C_B e^{(-k_e t)}$) represents the total body concentration of a metal that is more tightly bound which is presumed to be the metal that has been incorporated into the tissues. This compartment is used to predict the proportion of a metal that is present in tissues at time 0 and the rate at which the metal is depurated from the tissues.

3. Results

3.1. Gut-clearance following exposure to metal-contaminated sediments (Experiment I)

D. magna that had been exposed to metal-contaminated sediment exhibited significant (50%) mortality during the exposure. Cu and Zn in the exposure sediment (washed Clear Creek sediment) were 1061 and 4752 μg/g, respectively. Gut fullness in exposed *D. magna* was highly variable (range 40–80%) (Fig. 2). There was no significant difference in gut fullness across the different gut-clearance periods (i.e. 0–12 h) in metal-exposed *D. magna*. After 12 h in reference water, metal-exposed *D. magna* had a mean gut fullness of 62 (±26)%. In *D. magna* that had only been fed YCT, gut fullness was significantly lower after 6 and 12 h of purging (but not 8 h) as compared to uncleared ($t = 0$) individuals. After 12 h in reference water, the unexposed *D. magna* had a mean gut fullness of 19 (±17)% although there was great variability (0–50%).

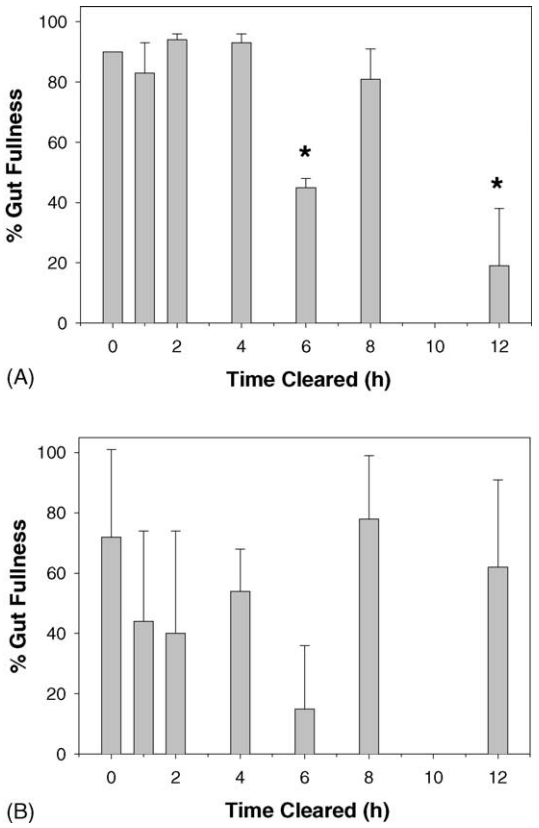

Fig. 2. Visually determined percent gut fullness of *D. magna* following transfer to clean water after (A) regular feeding on YCT (yeast, cerophyll, trout chow) and (B) exposure to metal-contaminated field sediment. Asterisk indicates significant decline compared to $t = 0$. Error bars represent standard deviations, $n = 5$, except for metal-exposed 6 h where $n = 2$.

3.2. Gut-clearance following exposure to reference sediments (Experiment II)

In *D. magna* that had been held in reference sediment, gut fullness remained relatively constant from 0 to 24 h after transfer to reference water (Fig. 3). Mean gut fullness was near 100% (97–99) for 0–24 h. Although the mean fullness had dropped to 74% by 48 h, the variability was very high (range 0–100%) as compared to shorter gut clearing periods.

3.3. Gut-clearance in the presence of algae (Experiment III)

Total (unfiltered) and dissolved (filtered 0.45 μm) Zn concentrations were 71 and 61 μg/L, respectively. Total Cu concentration was 16.7 μg/L and the dissolved Cu was 15.7 μg/L. Cu and Zn in the exposure sediment (diluted Clear Creek sediment) were 469 and 2163 μg/g, respectively.

Overall, visual inspection found that *D. magna* that had been fed algae (Fig. 4A) during the gut-

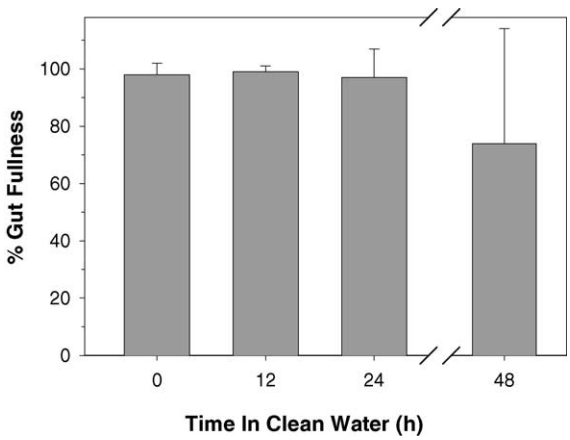

Fig. 3. Visually determined (mean ± S.D., $n = 30$) percent gut fullness of *D. magna* following transfer to clean water after exposure to reference field sediment.

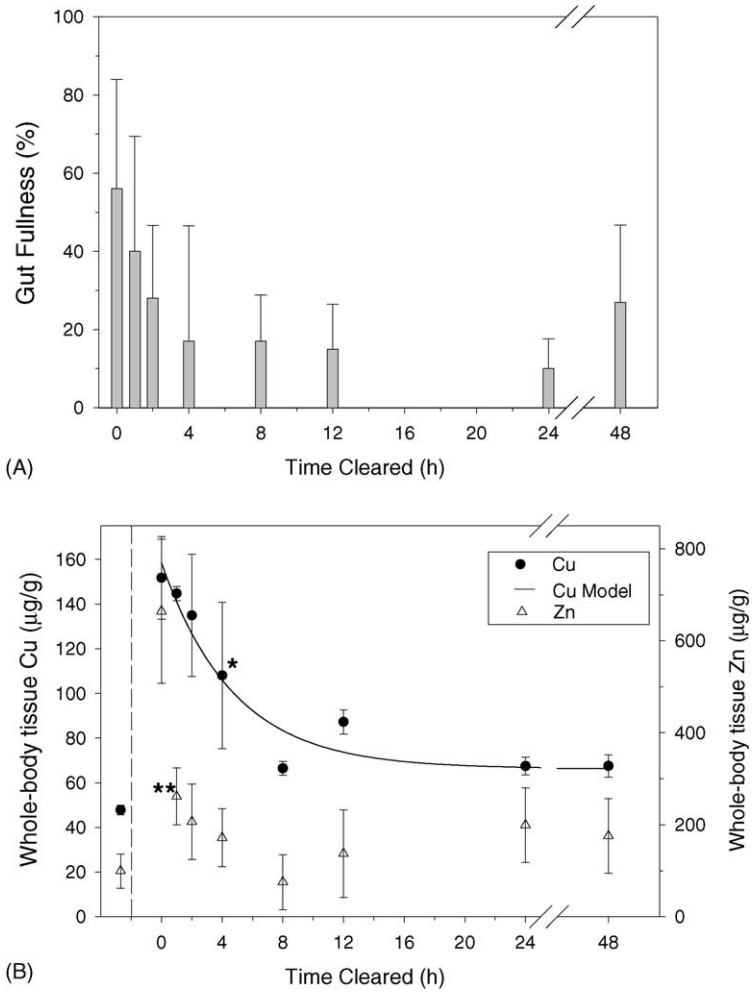

Fig. 4. Visually determined percent gut fullness (A) and whole-body tissue Cu and Zn concentrations (B) in *D. magna* following transfer to reference water containing 5×10^5 cells of *P. subcapita*. *D. magna* had previously been exposed to metal-contaminated field sediment for 48 h. Symbols located to the left of the dotted line represent the Cu and Zn levels in unexposed *D. magna*. Asterisk indicates first significant decline (single Cu, double Zn). Curve is the result of nonlinear regression of Eq. (1) for tissue Cu data. Error bars represent standard deviations. For (A) $n = 35$ and for (B) $n = 5$ (composite tissue samples).

clearance period were able to purge their gut contents better than those that were held in reference water only (Fig. 5A). Mean gut fullness was 56 (±28)% immediately after removal from the exposure sediment ($t = 0$). The first significant decline in gut fullness for algae-cleared *D. magna* was observed after 2 h (28 ± 18)%. Although we could not detect any further reduction in gut fullness after 2 h, variability in the data continued to decline up to 8 h (17 ± 12%) of gut-clearance in algae. *D. magna* that had been held in water only during the gut-clearance period had gut fullness ranging from 60–80%, even after 48 h in reference water.

When algae-cleared *D. magna* were treated with tissue solubilizer visual inspection revealed that almost all (>90%) of the darkened contents in the hind-gut were digested, indicating that most of the gut content in question (20%) was in fact algae. A very small amount of undigested, irregularly shaped particles that looked like sediment particles remained after the tissues were dis-

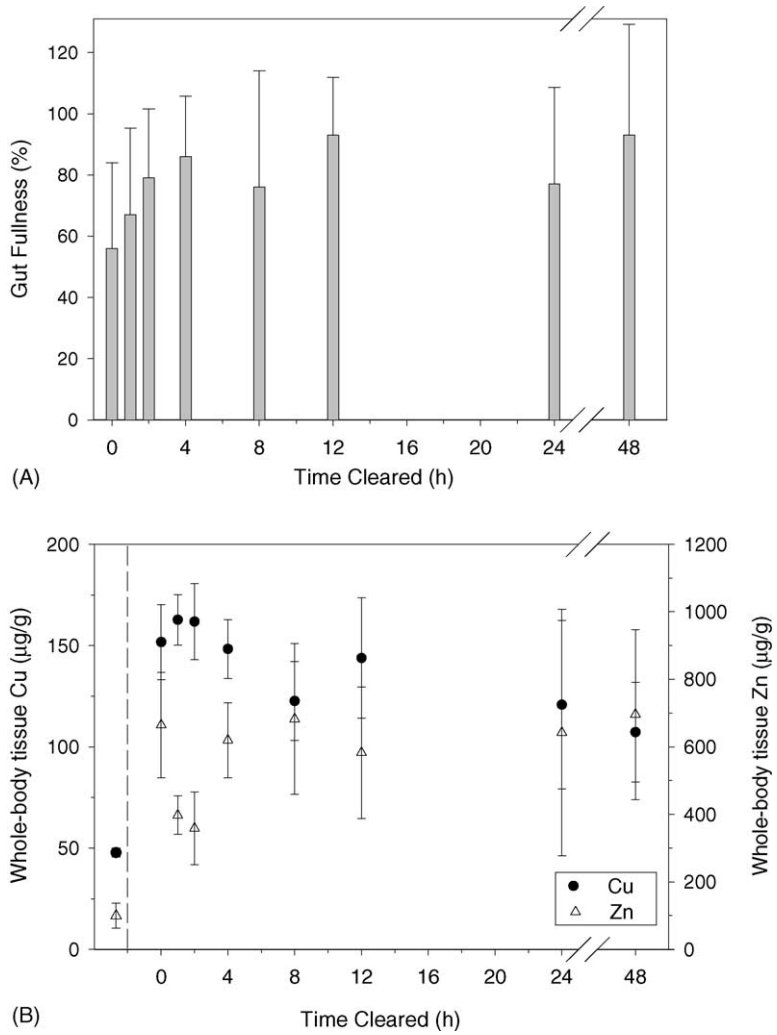

Fig. 5. Visually determined percent gut fullness (A) and whole-body tissue Cu (circles) and Zn (triangles) concentrations (B) in *D. magna* following transfer to reference water after 48 h exposure to metal-contaminated field sediment. Symbols located to the left of the dotted line represent the Cu and Zn levels in unexposed *D. magna*. Error bars represent standard deviations. For (A) $n = 35$ and for (B) $n = 5$ (composite tissue samples).

solved, which would represent approximately 2% of the total gut contents of *D. magna*.

Tissue concentrations of Zn and Cu in *D. magna* immediately after exposure were significantly ($P < 0.01$) higher than levels in unexposed animals. Whole-body Cu concentration was 47.8 (±2.1) μg/g prior to exposure and 151.7 (±18.5) μg/g following exposure. Before the sediment exposure, whole-body Zn concentration was 99.5 (±37.2) μg/g and it was 664.3 (±156.6) μg/g after the exposure. Because these post-exposure measurements were made on undepurated individuals, we cannot differentiate whether this increase in whole-body metal concentration indicates that there is significant bioavailable metal associated with this sediment or whether the increase is simply due to the presence of metal-contaminated sediment in the gut of the animal. Whole-body tissue metal concentrations decreased rapidly in *D. magna* that were fed algae during gut-clearance (Fig. 4B). In the algae-cleared animals the first significant de-

cline in Zn and Cu tissue concentrations occurred after 1 and 4 h, respectively. There was no significant difference in whole-body tissue Zn or Cu concentration across all times in the water-cleared *D. magna* (Fig. 5B).

The depuration of Cu in *D. magna* gut-cleared in the presence of algae was well described ($r^2 = 0.78$, $P < 0.0001$) with a two-compartment first-order kinetic model (Eq. (1)) (Fig. 4B). According to the model ($C_{TB} = 92.18\,e^{(-0.211t)} + 66.32\,e^{(-2.37E-11t)}$), the amount of Cu in the gut prior to gut clearing (G_0) was estimated to be 92.18 μg/g and C_B or the amount of Cu present in the tissue at $t = 0$ was predicted to be 66.32 μg/g. Copper in the gut component will be cleared at a rate of 20% h^{-1};whereas, Cu in the tissues is depurated more slowly ($\ll 0.0001\%\,h^{-1}$). The depuration of Zn could not be described with the 2CFOK model. Whole-body tissue concentration of Zn sharply declined from 664.3 (±156.6) μg/g to 261 (±62) μg/g after 1 h of gut-clearance and did not appear to change after the first hour.

4. Discussion

In the first experiment (Fig. 2), the lack of a significant decline in gut fullness after 12 h of clearing in the *D. magna* exposed to metal-contaminated sediment could be due to any combination of the following factors (i) a slowing of gut passage resulting from metal exposure (Barata et al., 2002); (ii) the inability of starved animals to clear previously ingested food from the gut (Gophen and Gold, 1981) or (iii) a collection of hard to digest material in the gut (Lampert, 1987). Lampert (1987) found that when mineral particles fill the gut they influence the filtering rate 'in a way as if the food concentration was high'. Therefore, a gut full of sediment particles would decrease the rate of ingestion of new particles and thus further slow down the passage of sediment particles through the gut. *D. magna* fed only YCT had a fuller gut than those that had been exposed to metal-contaminated sediment (90% versus 60%). The apparent reduction in feeding could be due to the toxicity of the exposure or simply due to an insufficient amount of particles available for the sediment-exposed animals to ingest in order to produce a full gut (no supplemental food was given). The high variability in the gut fullness data illustrated the need for larger sample sizes in subsequent experiments.

The second experiment (Fig. 3) demonstrated that even uncontaminated sediment could not be cleared from the gut in unfed *D. magna* after 2 days in reference water. These results suggest that the slow passage time seen in the sediment-exposed animals in the first experiment was likely due to the nature of the particle (i.e. mostly inorganic) rather than any toxic properties of the sediment. Since *D. magna* can clear a typical food stuff (YCT) from their gut after 12 h in clean water (Fig. 2A) but not clean sediment (Fig. 2B), it is likely that the nature of the particles involved prevents *D. magna* from clearing sediment from its gut; whereas, the lack of new food may slow down the passage of digestible food. Caution should be used when comparing gut-clearance times between these two experiments because different sediments had been used in the two exposures (Experiment I: Clear Creek, Experiment II: Lake Erie).

The final experiment demonstrated that only when *D. magna* were provided with new food, could they clear their gut of previously ingested sediment. *D. magna* that were fed algae-cleared their gut within 4 h, whereas the water-only exposed animals still had a full gut after 48 h of gut-clearance (Figs. 4A and 5A). The amount of time required to clear metal-contaminated sediment from the gut reported in this study was within the range reported by other studies where *D. magna* had been exposed to metal-contaminated algae. Barata et al., (2002) and Hooke and Fisher (2001) found that *D. magna* that had been previously exposed to metal-contaminated algae were able to clear their guts in the presence of clean algae within 8 and 4 h, respectively, and they attributed these slower clearance times to the fact that metal exposure can slow down the animal's ability to clear their gut. Bianchini A., Rouleau C., Wood C.M. (unpublished data), however, found that the gut burden of silver, ingested as colloidal silver sulfide, was cleared in 5 h when *D. magna* were held in water alone.

Survival of exposed *D. magna* in the final experiment with diluted Clear Creek sediment was over >90%. Dissolved metals (Zn, 61 μg/L; Cu, 15.7 μg/L) were below levels reported to be toxic to *D. magna* (Muyssen and Janssen, 2001; Heijerick et al., 2003; De Schamphelaere and Janssen, 2002), although both Cu (469 μg/g) and Zn (2163 μg/g) in the exposure

sediment still exceed the 'severe effect levels' (Cu, 110 μg/g; Zn, 820 μg/g) of the Ontario sediment quality guidelines (Persaud et al., 1992).

The depuration of Cu in algae-cleared *D. magna* was well described by the two-compartment first-order kinetic model, indicating that *D. magna* has one pool of Cu that is easily depurated, and one pool that has accumulated in the tissues and therefore is depurated much more slowly. The quickly depurated pool is assumed to be the sediment in the gut, although it could also partially reflect metal adsorbed to the carapace. According to the model, in the absence of gut-clearance an individual would have 58% of its total body Cu concentration present in the gut contents, and 42% in the tissue. Since Cu is an essential metal, the tissue compartment (C_B) is comprised of both newly accumulated and background Cu. Considering that only 66 μg/g of the total Cu body burden of an uncleared *Daphnia* (158 μg/g, model predicted $t = 0$) is tissue Cu, and 48 μg/g of that is background Cu (measured pre-exposed *D. magna*), the actual amount of newly acquired Cu was relatively small (18 μg/g). Without taking into account the amount of Cu in the gut, the body burden of Cu could be overestimated by 5.6-fold. Visual inspection confirmed that *D. magna* were unable to purge their gut of sediment particles (Fig. 5) if held in water alone, even after 48 h. Therefore, holding *D. magna* in water alone for gut-clearance could result in an overestimation of Cu accumulation by 4-fold compared with those that cleared their guts in the presence of algae for the same duration.

One of the difficulties associated with purging of gut contents is the potential to lose contaminants that had accumulated in the tissues during the gut clearing period (Hare et al., 1989; Amyot et al., 1996; Neumann et al., 1999). However, based on the very slow depuration rate for Cu from the tissues ($k_e = 2.37 \times 10^{-11}$), we suggest that the amount of true body burden lost during the gut clearing period, at least for Cu, would be negligible ($\ll$1%).

The digestive tract of *D. magna* exposed to metal-contaminated sediment was only 50–60% full as compared to animals that had been exposed to clean sediment (90%) or YCT food (97%). This apparent reduction in feeding in exposed animals is consistent with Taylor et al. (1998) who suggested that metal exposure can result in reduced ingestion rate in *D. magna*. Alternatively, the difference in gut fullness may be related to differences in sediment composition (Lake Erie versus Clear Creek).

Daphnids are known to 'drink' their surrounding water to facilitate digestion (Fox, 1952) and in some situations drinking can be a significant route of metal exposure. Stobbart et al., (1977), reported that *D. magna* replace a weight of fluid equivalent to 3.1% of their body weight every 10 min through drinking. Therefore, a *D. magna* with a mean wet weight of 0.67 mg, exposed to 17 μg/L Cu and 71 μg/L Zn in the overlying water of the final experiment (48 h), would have accumulated approximately 88 pg of Cu and 360 pg of Zn from drinking alone. Based on these figures, a *D. magna* that was gut-cleared in the presence of algae (8 h) could have acquired 7% of the newly accumulated Cu (18 μg/g dry weight) from drinking. Because Zn concentrations in *D. magna* had returned to background levels following gut clearing, we can conclude that neither the ingestion of metal-contaminated sediment, nor drinking resulted in an increase in the amount of Zn incorporated into the tissues of *D. magna*.

It should be noted that whole-body tissue metal concentrations could lead to an overestimation of the actual metal load of the animal if the animal was losing weight between measurements since calculations are based on a per weight basis (μg metal/g tissue). Because sediment is denser than algae, when *D. magna* replace the sediment in their gut with algae they could be losing weight. Even with this potential confounding factor, the drop in Cu concentration as the guts were purged was very pronounced, and we therefore conclude that any effect of weight loss on Cu concentration must have been negligible. Zn on the other hand remained relatively unchanged, after the initial drop in the first hour, but it is impossible to know whether *D. magna* weight loss was a factor in these results because of other complicating factors (approaching detection limit).

D. magna that were fed algae during gut-clearance could have potentially accumulated metal from the algae. But since *D. magna* replaced the metal-laden sediment in their gut (Cu, 469 μg/g; Zn, 2163 μg/g) with algae that had much lower metal concentrations (Cu, 17 μg/g; Zn < 60 μg/g), ingested algae would have only contributed minimally to the measured metal burdens.

The rapid drop in whole-body tissue Zn after 1 h of gut-clearance in algae indicated that there was only one pool of newly acquired Zn that could be quickly depurated in the metal-exposed *D. magna*. Unlike Cu,

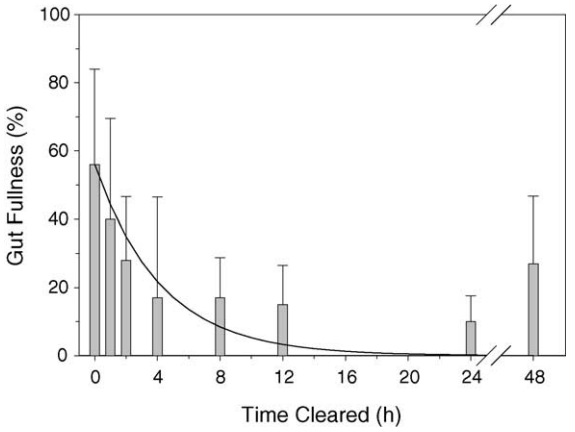

Fig. 6. Visually determined gut fullness (bars) overlaid with the model (Eq. (1)) predicted gut fullness based on Cu tissue concentrations (solid line).

Zn was probably weakly associated with the sediment particles. While whole-body Cu concentration did not decline until the gut was almost empty (4 h), the majority of Zn was depurated even before the gut contents had been purged (1 h). The great release of Zn over Cu during gut-clearance is consistent with the observed differences in their binding to mineral surfaces seen in both laboratory (Stumm, 1992) and field situations (Smith et al., 1998).

The decline in whole-body tissue Cu concentration corresponded relatively well with the visually observed gut fullness in algae-fed animals. Fig. 6 illustrates how the observed gut fullness compares with the rate predicted by the model (Eq. (1)) which was based on the concentration of Cu in the gut component (G_0). There is good agreement between the model and the observed gut fullness from 0 to 4 h. Although the model predicted that gut fullness would continue to decline until it approached zero fullness (empty) by 24 h, we did not observe any further reduction once the gut had reached 20% fullness (4 h). Even after 48 h of purging with algae, we observed a residual of unknown origin in the hind-gut. Closer examination with the aid of a tissue solubilizer, confirmed that most (>90%) of the residual gut content was partially digested algae and not sediment. The residual gut contents in algae-cleared *D. magna* (4–48 h) led to an overestimation of the visually determined gut fullness unlike the model, which predicted that gut fullness would approach zero.

5. Conclusions

The ASTM (2003) protocol E1688 for bioaccumulation studies with invertebrates calls for gut content to be purged to avoid overestimation of bioavailability when metals are the contaminant of concern. Based on our data, we recommend that any *Daphnia* that are to be used for whole-body tissue metal analysis be allowed to purge their gut in the presence of algae prior to analysis. This study found that *D. magna* held in clean algae for 8 h were able to clear their gut following exposure to metal-contaminated sediments. Even though there was no significant difference in gut fullness after 2 h, the variability of the data decreased with increased clearance time (up to 8 h). Since Hare et al. (1989) have cautioned against using a gut-clearance time associated with high variability, we therefore recommend a gut-clearance time of 8 h. Also, since the amount of time required to clear the gut may depend on the nature of the previously ingested particle and the particular metal(s) associated with the particles, we suggest that a preliminary study be carried out to determine the appropriate gut-clearance time for each exposure situation.

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