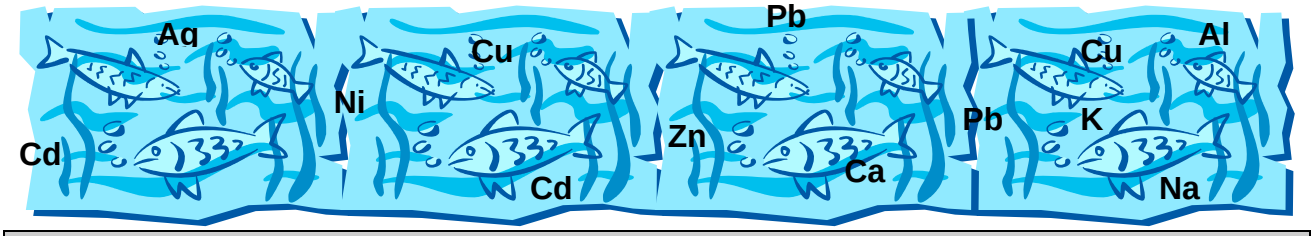
NSERC – Industry Strategic Project on Metal Bioavailability
Research Newsletter



Vol. 8: No. 3 ***McMaster University*** **September 2004**

News

Farewells: August saw the departure of a number of members of the lab who have taken on a variety of new experiences both in Canada and abroad. We wish Dr. Chris Glover all the best in his new position at the National Institute of Nutrition and Seafood Research in Norway. His project there will examine the toxicological effects of dietary methylmercury exposure via seafood on a mammalian model using gene expression profiling, with specific emphasis on the nutritional modification of toxicological actions provided by different seafood matrices. During his two-year tenure at McMaster Chris was primarily involved in research examining the role of natural organic matter in aquatic systems, primarily its ameliorative actions against silver toxicity, and its physiological impact upon ion regulation, in *Daphnia magna*.

Tammie Morgan, who completed her M.Sc. at McMaster on mechanisms of acute silver toxicity in trout, has recently taken up a position at the National Water Research Institute (Burlington, ON) with Dr. Mark Hewitt. Tammie is currently assisting in field collections of fish from Great Lakes Areas of Concern (AOCs). Her work will involve deploying semi-permeable-membrane-devices for passive sampling of substances within AOCs and setting up fish sex steroid receptor binding assays to evaluate those substances for hormonally active compounds.

Vicky Kjoss, both a technician in the lab and Chris Wood's secretary, has recently moved to Regina, Sasketchewan with husband Chris Somers (newly Dr. Somers!) where Chris has taken up a postdoctoral position. Vicky is enjoying life in the prairies and some well-deserved time off before contemplating her next adventure. We wish them all the very best. Monika Patel has taken over Vicky's duties for the next 4 months.

Visiting Ph.D. student: Victoria Tkatcheva, a Ph.D. student from the University of Joensuu, Finland, has joined the lab to complete her project on "Effects of mining wastewaters on fish, Northwest Karelia, Russia". Here at McMaster she is jointly supervised by Chris Wood and Dr. Grant McClelland, and will be investigating the response of trout gills to subacute Li toxicity.

Travel: Over the last few months Chris and several other members of the lab have been busy away from McMaster conducting research and attending conferences.

Over the summer, Dr Soumya Niyogi (a PDF in the lab) has been working with Dr. Greg Pyle at the Biology Dept. of Nipissing University. There he was investigating the effects of metal pollution on the olfactory reception (using electro-olfactogram apparatus) of the wild yellow perch population in Sudbury region of Northern Ontario. This is an ongoing project, and will be continued under newly started MITHE (Metals in the Holistic Environment) Research Network Funding.

In July, Chris and Joe Rogers were involved in a research trip to China where Chris gave a lecture on the Biotic Ligand Model (with slides in Chinese!) at Zheijang University in Hangzhou (close to Shanghai). Afterwards, Chris and Joe, together with a team of Canadian and Chinese scientists proceeded to the high plateau region of Northwest China for a 2 week field project on the physiology of the unusual "scaleless carp", an endangered (but recovering) species which is endemic to Lake Quinhai, China's largest inland water body.

In August, Chris and several members of the lab (Eric Pane, Dr. Fernando Galvez, Dr. Richard Smith & Dr. Makiko Kajimura) attended the VI International Congress on the Biology of Fish in Manaus, Brazil. There, Dr. Kath Sloman (our former senior PDF, now appointed as a faculty member at the University of Plymouth, U.K.) was a plenary speaker, and gave a well-received presentation describing her work on behavioural endpoints in aquatic toxicology. Kath and Chris also organized and chaired a two day symposium entitled "*Behaviour, Physiology, and Toxicology Interactions in Fish*" with invited speakers from North America, Latin America, and Europe. More than 30 oral and 30 poster presentations were given, many of them focusing on the interactions of metals. Recent work on the Biotic Ligand Model, endpoints of chronic metal toxicity, and the trophic transfer of metals were all discussed in great detail before an international audience averaging more than 80 on each of the two days. Chris and Kath are very grateful to Kodak Brasil, The International Copper Association (ICA), The Nickel Producers Environmental Research Association (NiPERA), the International Lead Zinc Research Organization (ILZRO), and Elsevier, whose generous financial support made the symposium possible.

Following the conference, Chris and Kath spent three weeks working with Dr. Adalberto Luis Val at INPA, Manaus, on the ionoregulatory and respiratory physiology of fish endemic to the dilute, ion poor, NOM-rich waters of the Amazon basin. At the same time, Eric Pane spent several weeks at the University of Miami (RSMAS) working with Dr. Martin Grosell investigating possible Ni/Mg interactions in marine gulf toadfish.

In October, Chris heads from Hamilton, Canada to Hamilton, New Zealand to begin a three-month sabbatical at the National Institute of Water and Atmospheric Research. At NIWA, Chris will be working with Dr. Sue Clearwater on dietary Cd transfer through the food chain.

Fish facility renovation: After several weeks of laboratory renovations throughout August our fish facilities are now completed and the group is soon to be back into full swing.

Conference presentations

The following papers will be presented by the Metals Bioavailability Group at the Society of Environmental Toxicology and Chemistry, Fourth SETAC World Congress, Portland, Oregon, November 14-18, 2004.

- **Nadella, S. Kjoss V.A., Grosell, M. and Wood, C.M.** Dietary uptake of copper in the rainbow trout: an analysis of mechanisms

- **Gillis, P., Chow-Fraser, P., Ranville, J.R., Ross, P.E. and Wood, C.M.** Toxicity and bioavailability of sediment-associated Cu to *Daphnia magna*.

Research Highlights

This issue will highlight research conducted by Patricia Gillis, a PDF at McMaster in the laboratory of Pat Chow-Fraser and co-supervised by Chris Wood. The work was done in collaboration with the Colorado School of Mines.

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

Gillis P.L., Chow-Fraser P., Ranville J.F., Ross P.E., and C.M. Wood

As filter-feeders, *Daphnia magna* 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; Brendlerger 1985). If food levels fall below a threshold, they will also scrape the bottom or stir up sediments to feed on resuspended particles (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.

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. The purging ensures that metal-contaminated particles in the animal's gut do not lead to overestimation of metal bioavailability (Hare et al. 1989). Although it is not standard practice to clear the gut of planktonic organisms before analyzing their tissues for whole-body metal accumulation, some investigators (Hooke and Fisher 2001; Barata et al. 2002) transfer animals to clean algae to purge their guts after they have been fed metal-contaminated 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 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 (YCT). In the second experiment, reference sediment was used in place of metal-contaminated sediment to determine if *D. magna* were able to clear sediment from their gut when held in clean water. 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 was to determine the effect of different gut-clearance protocols on calculation of whole-body tissue metal concentrations in *D. magna.*

Juvenile *D. magna* were 'exposed' to either: regular food; or field-collected sediment. The metal-contaminated sediment was collected from Clear Creek, CO, USA, while reference sediment was collected from Long Point, Lake Erie. Following exposure, *D. magna* were transferred to 250 mL glass beakers for a designated gut clearing time. Water, and when necessary algae, in the gut clearing vessels were replaced every 4 h to prevent cophrophagy. After the appropriate gut clearing time the percentage of gut fullness was visually determined using depression slides and a stereo-compound microscope according to the designations in Figure 1.

Cu and Zn tissue concentrations in *D. magna* from the third experiment were determined throughout the gut clearing period. A two-

compartment first-order kinetic model (2CFOK) was used to characterize metal kinetics during gut clearance of algae-cleared *D. magna*.

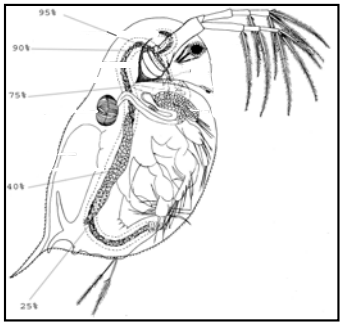


Figure 1. Designations of percent gut fullness used for visual (microscopic) determination. *Daphnia* diagram was modified from Pennak (1989).

Gut clearance following exposure to metal-contaminated sediments (Experiment 1)

Metal exposed *D. magna.* Were unable to clear their guts after a 12 h period. Indeed, there was no significant difference in gut fullness across the different gut clearance periods (i.e. 0 to 12 h). After 12 h in reference water, metal-exposed *D. magna* still had a mean gut fullness of 62%. In contrast, *D. magna* that had only been fed YCT, gut fullness was significantly lower after 6 and 12 h of purging compared to uncleared (t=0) individuals. After 12 h in reference water the unexposed *D. magna* had a mean gut fullness of 19%. 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 (Lambert 1987).

Gut clearance following exposure to reference sediments (Experiment 2)

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

This experiment demonstrated that even uncontaminated sediment cannot be cleared from the gut in unfed *D. magna* after two 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 (Experiment 1) but not clean sediment (Experiment 2), it is likely that it is the nature of the particles that prevents *D. magna* from clearing sediment from its gut whereas the lack of food may slow down the passage of digestible food.

Gut clearance in the presence of algae (Experiment 3)

Overall, visual inspection found that *D. magna* that had been fed algae (Fig. 2a) during the gut clearance period were able to purge their gut contents better than those that were held in reference water only (Fig. 3a). Mean gut fullness was 58% immediately after removal from exposure sediment (t=0). The first significant decline in gut fullness for algae-cleared *D. magna* was observed after 2 h (28% full) with a continuing trend and decreasing variability up to 8 h (17% full) 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. Even after *D. magna* were gut cleared in the presence of algae it appeared that approximately 20% of the gut still contained either sediment or partially digested algae. To verify the origin of the residual gut content, tissue solubilizer was applied to several specimens. Visual inspection found that >90% of the darkened contents in the hind-gut were digested, indicating that most of the gut content in question was in fact algae. A very small amount of undigested, irregularly shaped particles that looked like sediment particles remained after the tissues were dissolved, which

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

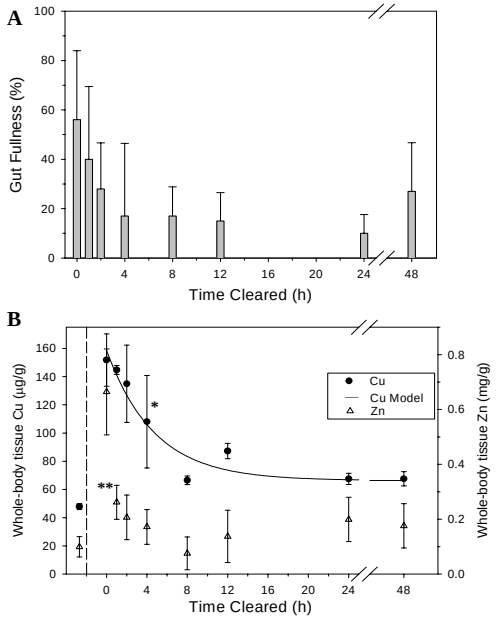


Figure 2. Visually determined percent gut fullness (A) and whole-body tissue Cu and Zn concentrations (B) in *D. magna* following transfer to reference water containing $5x10^5$ cells of *P. subcapitta*. *D. magna* had previously been exposed to metal-contaminated field sediment for 48 h. Symbols located to the left of the dotted line represent the Cu and Zn levels in unexposed *D. magna*. Curve is the result of nonlinear regression of tissue Cu data. For (A) n= 35 and for (B) n=5. Error bars are SD.

Whole-body tissue concentrations of Zn and Cu in *D. magna* immediately after exposure were significantly ($p<0.01$) higher than levels in unexposed animals. The elevated tissue levels may indicate that these sediments had high amounts of bioavailable metal associated with them or simply that the animals had ingested metal-contaminated sediment. Tissue metal concentrations decreased rapidly in *D. magna* that were fed algae during gut clearance (Fig. 2b). In the algae-cleared animal the first significant decline in Zn and Cu tissue concentrations occurred after 1 and 4 h, respectively. There was no significant difference in tissue Zn or Cu concentration across all times in the water-cleared *D. magna* (Fig. 3b).

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 2CFOK model. According to the model, Cu in the gut component will be cleared at a rate of 20% h^{-1} whereas Cu in the tissues is depurated much slower (<<0.0001% h^{-1}). Although we assumed that the quickly depurated pool is sediment in the gut, 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 in the gut contents, and 42% in the tissue. Since Cu is an essential metal, the tissue compartment is comprised of both newly accumulated and background Cu. Considering that only 64 µg/g of the total Cu body burden of an uncleared animal (152 µg/g) is tissue Cu, and 48 µg/g of that is background Cu, the actual amount of newly acquired Cu was relatively small (16 µg/g). Without taking into account the amount of Cu in the gut, the body burden of Cu could be overestimated by 6 fold. Visual inspection confirmed that *D. magna* were unable to purge their gut of sediment particles (Fig. 3) if held in water alone, even after 8 h. Therefore holding *D. magna* in water alone for gut clearance could result in an overestimation of Cu accumulation by 4 fold compared those that cleared their guts in the presence of algae for the same duration.

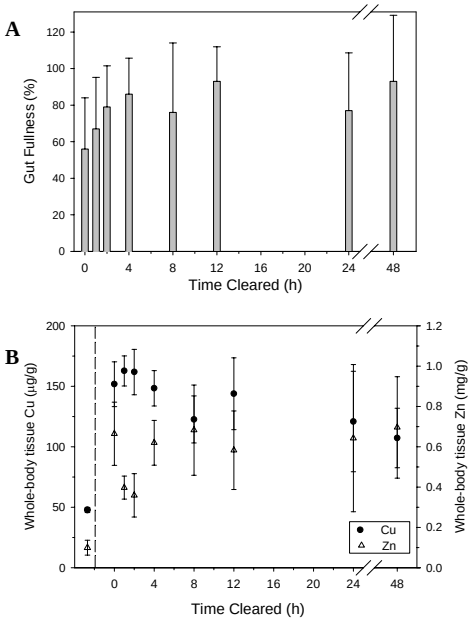


Figure 3. Visually determined percent gut fullness (A) and whole body-tissue Cu and Zn concentrations (B) in *D. magna* following transfer to reference water. For (A) n= 35 and for (B) n=5 (composite tissue samples).

The depuration of Zn could not be described with the 2CFOK model. Whole body tissue concentration of Zn sharply declined within the first hour of gut clearance. The rapid drop in tissue Zn after one hour of gut clearance in algae indicates that there was only one pool of newly acquired Zn that could be quickly depurated in the metal-exposed *D. magna*. Unlike Cu, 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 decline in tissue Cu concentration corresponded well with the visually observed gut fullness in algae-fed animals for the first 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). 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.

The results from 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 full guts after 48 h of gut clearance. 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 hours, respectively.

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. 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) has cautioned against using a gut clearance time associated with high variability, we suggest a gut clearance time (8 h) where the gut fullness has stabilized over an earlier time.

This study was funded by the Center for the study of Metal in the Environment (USEPA). An extended version of this paper has been submitted for publication.

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***Vox Salmonis*:** The lab of Chris Wood hosts a weekly seminar series entitled "*Vox salmonis*." Presentations cover a range of topics in physiology, toxicology, and behaviour of aquatic organisms. We cordially invite anyone who is interested in attending and/or presenting a talk to join "*Vox*" on Tuesdays from 12:00-13:30 on the campus of McMaster University. Please contact Dr. Patricia Gillis (email: gillisp@mcmaster.ca) for more information.

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