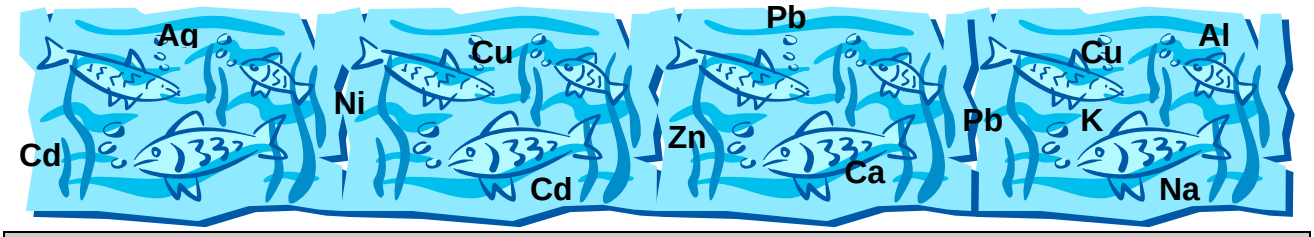
NSERC – Industry Project on Metal Bioavailability
Research Newsletter



Vol. 9: No. 1 ***McMaster University*** **May 2005**

News

Completion of NSERC Strategic Project

December saw the completion of our NSERC Strategic Grants Project entitled "Mechanistic Knowledge of the Chronic Effects of Metals on Aquatic Organisms – An Antidote to the Misuse of the Precautionary Principle". This 4-year project aimed at further developing the Biotic Ligand Model (BLM) for deriving Ambient Water Quality Criteria for metals, by providing research on the bioavailability, physiology, and acute and chronic toxic effects of 5 metals considered of greatest regulatory concern at present – Cu, Cd, Zn, Pb and Ni. This was both a highly productive and diverse collaboration involving active partnership between a university, a government laboratory (Natural Resources Canada – CANMET, through Dr Jim McGeer), four international industrial research organizations (International Copper Association, Copper Development Association, Nickel Producers Environmental Research Association, International Lead Zinc Research Organization), two Canadian mining/smelting corporations (Noranda-Falconbridge, Teck Cominco), and a Canadian consulting company (EVS, through Dr Peter Chapman). In total, we produced close to 50 primary research papers and 5 review articles originating from this work in international peer-reviewed journals, and participated in over 100 conference, workshop, and seminar presentations. We are extremely grateful to all those involved, which included a total of 8 postdoctoral fellows, 5 Ph.D. and 5 M.Sc. students, 13 undergraduate students, 7 technicians, and several visiting scientists. The collective efforts of our entire research team and industrial partners have made this work a very useful foundation for BLM-focused research in Canada and abroad.

New NSERC CRD Project (2005-2008)

In order to fully develop the BLM as a regulatory tool for effective water quality management, it was clear that additional "follow-up" research was required. This led two of the current members of our Strategic research team, Dr Chris Wood (Principal Investigator) and Dr Jim McGeer (co-investigator), in addition to Dr Grant McClelland (new co-investigator, McMaster University), to apply for an NSERC CRD Project Grant, for which we were recently successful (Jan 05–Dec 08). The project is entitled "The Science Needed for Site-Specific Regulation of Metals in the Aquatic Environment – Improving and Extending the Biotic Ligand Model for Ambient Water Quality Criteria". Specifically, the goal of this project is to do the critical research needed to: (i) extend the BLM to a wider range of target organisms; (ii) extend the BLM to the marine environments; (iii) better incorporate dissolved organic matter into the BLM; (iv) extend the BLM to predict chronic toxicity; (v) incorporate the effects of food-borne metals into the BLM; (iv) extend the BLM to multiple metal mixtures; and (vii) incorporate new proteomic and genomic endpoints. This CRD research is supported by the same partners involved in our previous Strategic grant (e.g. R. Dwyer-ICA; R.

Arnold-CDA; C. Schlekat-NiPERA; A. Green-ILZRO; W. Kuit-Teck Cominco; R. Prairie and D. Kemp-Noranda-Falconbridge; P. Chapman-Golder Associates) with the addition of Inco. Ltd (B. Conard). The metals under study will be the same as in our previous NSERC Strategic-Industry Project (Zn, Cd, Ni, Cu, and Pb), with particular emphasis on the latter three. We look forward to this new and exciting chapter of metals research.

MITHE-RN Project (2005-2009)

January also saw the start of our involvement the new NSERC Metals in the Holistic Environment Research Network (MITHE-RN), which builds on the accomplishments of the MITE-RN (1999-2004). This project, partnered by Chris Wood (McMaster University) and Dr. Greg Pyle at Nipissing University, field-tests the applicability of the BLM for fish. Those involved in the project also include, M.Sc. student Warren Green (who is supported by a NSERC postgraduate scholarship), and postdoctoral fellow Dr Reehan Mirza, both from Nipissing University.

Congratulations

We are pleased to announce that two of our graduate students from the Metals Bioavailabity Group, Eric Pane and Sunita Nadella, recently completed their degrees at McMaster. Eric successfully defended his Ph.D. thesis in January entitled "*Mechanistic analysis of the effects of Ni on Daphnia magna and rainbow trout*". Eric produced an impressive number of publications from his thesis and was awarded the SETAC/ICA Chris Lee Award for Metals Research at the recent SETAC World Congress in Portland, Oregon. He has now returned to sunny California where he is continuing with research. We wish him all the best!

Sunita Nadella (who's featured in this issues Research Highlight) also defended her M.Sc. thesis in January, entitled "*Dietary uptake of copper in rainbow trout (O. mykiss): a study of mechanisms*". We are fortunate to have Sunita remain in the lab in both a technical role and as Chris Wood's secretary.

Congratulations also to Chris Wood who has recently been appointed as "Distinguished University Professor" at McMaster University. This is a great honor for Chris and for the Biology Department as there are only 8 such positions at the University at any given time and the title carries for life. Well done Chris!

New members in the lab

Paul Craig, from Ottawa, recently joined the lab as a PhD candidate. Paul received his B.Sc. in zoology at the University of Guelph in 2001, and recently completed his M.Sc. work at Guelph under the supervision of Dr. Nicholas Bernier. The title of his M.Sc. thesis was "*The Effects of Seawater Transfer on the Corticotropin Releasing Factor Family of Peptides*". At McMaster, Paul will be working under the guidance of Dr. Grant McClelland looking at the chronic effects of Cu and Cd exposure on zebrafish, with an emphasis on molecular endpoints of chronic toxicity.

Tatania Kozlova also joins the Metals Bioavailablity Group as a M.Sc student working on the trophic transfer of metals. Tatania will be based primarily at CANMET MMSL, in Ottawa, under the supervision of Dr. Jim McGeer, but she will also spend some time in the lab at McMaster. She has a B.Sc. from Kuban State University in Russia with her thesis on "*Ichthyoplankton in Oil Polluted Tsemess Bay (Black See)*". Welcome aboard!

Travel

From October 04 through until Christmas 04, Chris Wood spent a productive and enjoyable mini-sabbatical working with Dr. Sue Clearwater at the National Institute of Water and Atmospheric Research, in Hamilton, New Zealand. Chris' travel was funded by the Royal Society of New Zealand. At NIWA, Chris and Sue worked on a project studying the dietary transfer of Cd through the aquatic food chain, supported by a Marsden Fund Fast Start Research Grant to Sue, again from the Royal Society of New Zealand. A lot of preliminary data were obtained, and the project is ongoing under Sue's able leadership.

Conference presentations

The following paper was presented by Greg Pyle at the MITHE-RN Annual Research Symposium, Ottawa, Canada, March 1-2, 2005.

- **Pyle, G. and Wood, C.M.** Generation and field validation of chronic Biotic Ligand Models for fish

The following Plenary Session was presented by Chris Wood at the 8th International Conference on the Biogeochemistry of Trace Elements (ICOBTE) in Adelaide, Australia, April 3-7 2005.

- **Wood, C.M.** Uptake and toxicity of trace elements to aquatic organisms

The following papers were presented by the Metals Bioavailability Group at the annual meeting of the Canadian Society Zoologists (CSZ) in Kingston, Canada, May 10-14 2005.

- **Alves, L. and Wood, C.M.** The chronic effects of dietary lead on juvenile rainbow trout (*Oncorhynchus mykiss*)

- **Franklin, N.M., Glover, C.N. and Wood, C.M.** Radiotracer analysis to assess cadmium accumulation associated with individual and combined waterborne and dietborne cadmium exposures to juvenile rainbow trout

- **Gillis, P.L., Chow-Fraser, P. and Wood, C.M.** Bioavailability of sediment-associated metals to *Daphnia magna*

- **Nadella, S., Bucking, C. and Wood, C.M.** Digestive handling of copper in the trout gut and physical constants governing transport kinetics

- **Ojo, A. and Wood, C.M.** Bioavailability and interactions of metals via the intestinal tract of rainbow trout (*Oncorhynchus mykiss*)

- **Patel, M., Rogers, J.T., Pane, E.F., and Wood, C.M.** Renal responses to acute lead exposure in the freshwater rainbow trout (*Oncorhynchus mykiss*)

The following invited seminar was presented by Chris Wood at the Canadian Centre for Inland Waters, Burlington, Ontario, May 19, 2005.

- **Wood, C.M.** The physiology of metal uptake and the Biotic Ligand Model for predicting metal toxicity to aquatic organisms.

Research Highlights

This issue will highlight research conducted by Sunita Nadella who recently completed her M.Sc. at McMaster under the supervision of Chris Wood. An extended version of this paper has been recently submitted for publication.

Digestive processing of Cu *in vivo* and physical characterization of Cu uptake *in vitro* in freshwater rainbow trout.

Sunita Nadella, Carol Bucking, Martin Grosell and Chris M. Wood

Copper (Cu) is an essential element found in all living organisms in the oxidized Cu (II) and reduced Cu (I) states. The status of Cu as an essential nutrient as well as a potent toxicant has been established both in mammalian and piscine species. Fish are unique among the vertebrates, a consequence of having two routes of metal uptake, the gills and the gut. It is generally believed that fresh water teleosts have a daily dietary requirement of Cu in the region of 15-60 µmol kg^{-1} dry diet (1.4 µg g^{-1}dry diet) (Lanno *et al.*, 1985). However, Kamunde *et al.* (2002a) showed that by considering the input from water when determining Cu requirement in fish, as little as 0.8 µg g^{-1} Cu in the diet is adequate to support normal growth in rainbow trout juveniles at normal water Cu level of 3µg L^{-1}.

The mechanisms of waterborne Cu uptake and toxicity to fish are beginning to be well understood (Wood, 2001). While several studies have indicated the diet to be the major source of Cu for fish under optimal growth conditions (Handy, 1996), only Kamunde *et al.* (2002b) have directly measured the rate of Cu uptake from food. They reported that rainbow trout fed a control diet spiked with ^{64}Cu took up Cu at a rate of 0.9 ng g^{-1} h^{-1}, ten times higher than the rate of waterborne Cu uptake determined in control hard water during the same study. However the uptake of dietborne Cu in fish is not well characterized. This study examined the digestive processing of Cu *in vivo* in intact trout fed a normal diet to determine major sites of Cu absorption. *In vitro* preparations of freshly prepared intestinal segments were later utilized to gain insight into the physical processes governing transport kinetics. The aim of the study was to provide insight into the basic mechanism by which Cu is transported across the intestinal epithelium in fresh water trout.

To characterize the digestive processing of Cu in trout, a disappearance test was designed to follow the processing (uptake and secretion) of Cu along the digestive tract from its oral input (from food) to the anus. Trout were fed a specially prepared diet that contained X-ray dense glass beads. The beads act as a non-absorbable marker that can be used to quantify absorption. The percentage of absorption of a given nutrient in the meal can be calculated by comparing the nutrient:marker concentration in the chyme sample and in the test meal .

Intestinal sacs were used to investigate the mechanism of Cu uptake. Sacs were prepared from the anterior, mid and posterior segments of the intestine. The sacs were filled with 1 ml of appropriate mucosal saline labeled with 0.04 mCi ml^{-1} ^{64}Cu as $Cu(NO_3)_2$ or 0.04 µCi ml^{-1} ^{22}Na as NaCl . Sacs were sampled at the end of the flux period which was routinely 2 h in duration and counted for ^{64}Cu or ^{22}Na activity by gamma counting.

Site of Cu absorption

Cu-to-bead ratios in total chyme in the posterior intestine when compared to parallel values in the food indicate an apparent absorption of about 50% of the ingested Cu in the food after 72 h. By way of comparison, Berntssen *et al.* (1999) measured an apparent Cu

retention of 25% in Atlantic salmon fry reared for 3 months on a standard diet.

In the stomach contents, approximately 16% of the total Cu was present in the supernatant initially, the remainder in the solid phase. Cu-to-bead ratios in the supernatant from the stomach (Fig.1) progressively decreased, supporting the interpretation of significant Cu absorption through the gastric mucosa. Considering that Cu is not leached from the food prior to ingestion, the considerable decline in Cu-to-bead ratio from food to stomach implicates the stomach as an important site of Cu absorption in the rainbow trout.

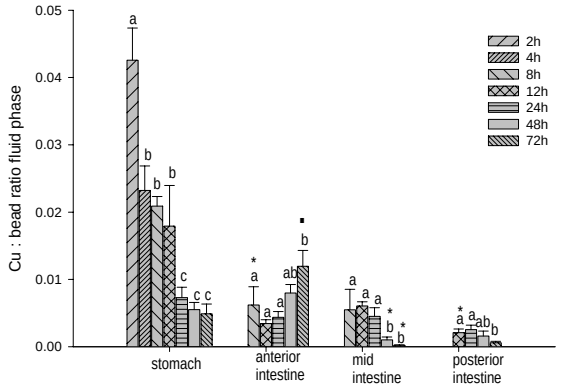


Figure 1. Cu:bead ratio in digestive tract contents of each section in the fluid phase. Values are means ± SEM (n = 6). Asterisks denote significant decrease from previous compartment at same time point and squares represent significant increase from previous compartment at same time point.

Examination of the spatial distribution of Cu *in vitro* reveals a 5-10 fold higher Cu uptake rate in the anterior intestine compared to the mid and posterior regions which is consistent with evidence from Clearwater *et al.* (2000) suggesting that ~ 80% of an absorbed ^{64}Cu dose originally infused into the stomach of adult rainbow trout is found in the anterior intestinal tissue, 20% in the mid- and posterior intestinal tissue and <1% in the stomach tissue after 72 h. Against this consistent background localizing the bulk of *unidirectional* Cu uptake to the anterior intestine, it is therefore very interesting that the *in vivo* "disappearance test" using the Cu-to-bead ratio analysis clearly demonstrated that the anterior intestine is not a site of *net* Cu uptake, but rather a site of *net* Cu addition to the chyme (Fig.1). The obvious conclusion is that although unidirectional uptake of Cu is high in this region, there is an even higher efflux component which significantly elevates the Cu-to-bead ratio in the chyme. Biliary secretions, which are rich in Cu, probably play the dominant role in this region.

In contrast to the anterior intestine, the present *in vivo* data clearly indicated a decline in the Cu-to-bead ratios in the mid and posterior intestinal regions relative to the anterior intestine, with progressive absorption from the fluid phase in these compartments (Fig.1). Substantial unidirectional Cu uptake was also measured in these regions *in vitro* suggesting an important role for the mid and posterior intestine in Cu uptake. Indeed, since the Cu-to-bead ratios in total chyme of the anterior intestine are the same as those in the original food it could be argued that all *net* Cu absorption occurs in the mid and posterior intestine *in vivo*.

Characterization of Cu uptake in the trout intestine. Over the range of Cu concentrations tested, Cu uptake appeared to be biphasic in both mid (Fig.2) and posterior intestine.

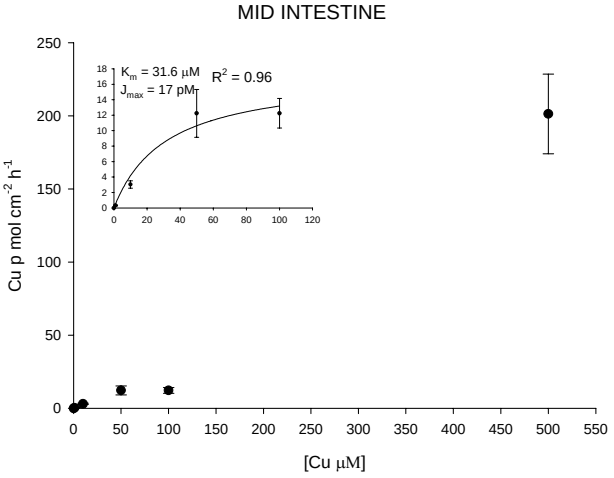


Figure 2. Cu uptake kinetics in the mid intestine of rainbow trout exposed to five different Cu concentrations (1-500 μM). Means ± SEM (n=3 per treatment).

The differential dose-response relationship observed (Fig.2) indicates a saturable component at low Cu concentrations (1-100 μM), suggesting a carrier-mediated process. This component was superceded by a possible

linear diffusive pathway at Cu concentrations of 500 µM. Similar Cu uptake characteristics have been determined for intestinal Cu transport in mammals and one other fish species **(**Bronner and Yost, 1985). Additional evidence for the presence of a carrier-mediated component to Cu uptake can be derived from the significant increase in Cu uptake with increase in temperature. Q_{10} values approaching or exceeding 2.0 for Cu uptake into the serosal and muscle compartments (Fig.3, i.e. basolaterally effluxed Cu) in both the mid and posterior intestine suggest that the basolateral transport of Cu is biologically mediated. Q_{10} values lower than 1.5 measured from the epithelial compartment indicate a non-mediated apical route of Cu uptake. Possibly, the latter may become the rate-limiting process for transport across the entire mucosal epithelium at higher Cu concentrations where the diffusive mechanism appears to predominate (Fig.2).

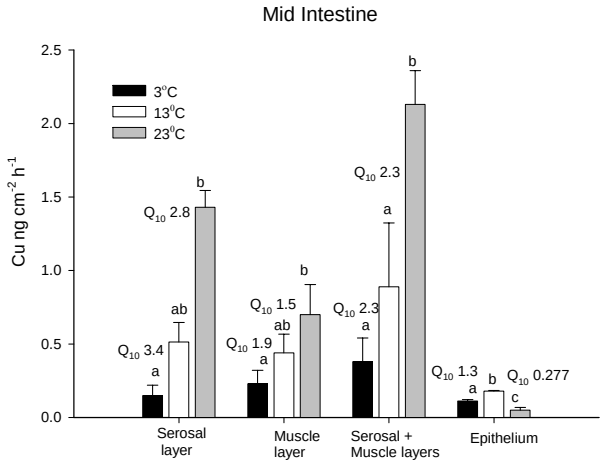


Figure 3. *In vitro* Cu uptake rates in the mid intestine of rainbow trout at 3ºC, 13ºC and 23ºC. Values are means ± SEM

The affinity of the saturable Cu uptake component determined in the present study for the mid and posterior intestine respectively (K_m = 31.6 µM in mid intestine, Fig. 2; 78 µM, or 18.9 µM with correction for linear component in posterior intestine) was in the range of Cu concentrations normally found in the fluid phase of the chyme *in vivo* (8-63 µM). These values are similar to that (K_m = 21 µM) obtained in the rat intestine (Wapnir & Steil, 1987) and comparable to the affinity of mouse embryonic cells for Ctr1-independent Cu transport, reported to be ~ 10 µM (Lee *et al.,* 2002). Interestingly, branchial Cu uptake in fresh water rainbow trout similarly reveals saturation kinetics at low Cu concentrations for both the Na-sensitive and insensitive components and a linear relationship when Cu concentrations were raised (Grosell & Wood, 2002). However, the branchial affinity for Cu uptake in trout as reported by Grosell & Wood, (2002) was approximately three orders of magnitude lower with K_m =7.1 nM – 9.6 nM for the Na-sensitive and insensitive components respectively. Branchial transporters have to work at the much lower Cu levels (nM) which are normally present in water while the trout intestine is a much lower affinity absorptive pathway that functions at the much higher Cu levels (µM) which are normally present in the fluid phase of the chyme.

Ascorbic acid was used to reduce $Cu(II)^{2+}$ to $Cu(I)^{1+}$ in the mucosal solution to test which form of Cu was the preferred form for transport (Zerounian *et al.,* 2003). However there was negligible effect of ascorbic acid on Cu uptake rates by the trout intestine over a large range of concentrations tested. This result suggests, either that the valence of Cu present does not matter or more likely that sufficient quantities of endogenous reductase are already present on the intestinal epithelium. Therefore exposure to additional levels of a reducing agent would not be expected to augment Cu uptake. The latter implies that $Cu(I)^{1+}$ is the transported form. Indeed the presence of endogenous plasma membrane reductases capable of reducing Cu has been reported in mammalian brush border membranes (Knopfel and Solioz, 2002). In either case, the results indicate that Cu uptake rates are not influenced by change in the redox state of the Cu ion in bulk solution.

In conclusion, our results confirm the mid and posterior regions of the intestine as important sites of Cu absorption, suggest that the anterior intestinal region plays a complex role in bidirectional Cu transport and indicate a need for further investigation into the possible role of the stomach in Cu absorption in the fresh water rainbow trout. As well, we provide

evidence that Cu uptake occurs via a carrier-mediated, saturable process which can be fueled (at least indirectly) by $Cu(II)^{2+}$ at concentrations which are typical of those in fluid phase of the chyme *in vivo* in the trout intestine. The study opens an opportunity to investigate the nature and identity of the specific carriers and /or ion-channels involved in this process and gain further insight into factors governing intestinal uptake of Cu in rainbow trout.

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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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