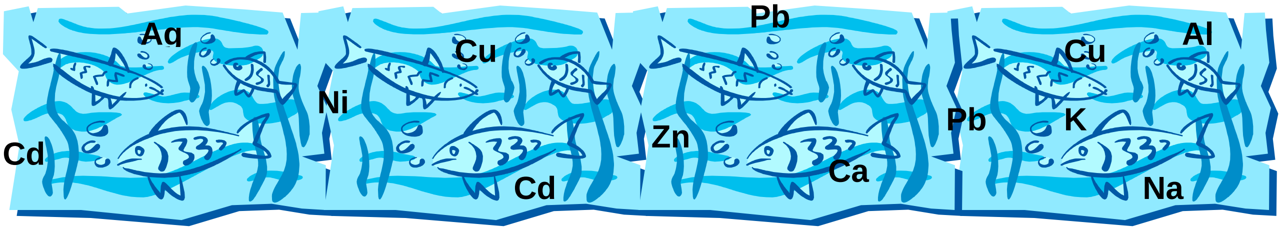
NSERC – Industry Strategic Project on Metal Bioavailability
Research Newsletter



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News

Farewell to Jasim*:* After three years at McMaster as a PDF, we are sad to say farewell to Dr Jasim Chowdury, who has recently taken up a position at the US-EPA lab in Duluth, Minnesota. Jasim was the senior PDF on the NSERC – Industry Strategic Project and worked primarily on the physiological and toxicological effects of metal acclimation in rainbow trout. At the US-EPA, he will be working with Dr. Sigmund Degitz on the effects of chemicals on thyroid axis disruption in amphibians at biochemical and molecular levels.

BLM short course*:* Several members of the lab recently attended the BLM course offered co-operatively by CDA/ICA and Hydroqual. This was a great introduction for some of the newer members in the lab, and a timely refresher course for others. Many thanks to the course instructors, Ray Arnold and Bob Santore, and the Canadian Centre for Inland Waters for hosting the event.

Conference presentations

The following paper was presented at the NIEHS-MFBSC/ARCH Science Symposium, University of Miami, Florida, March 18, 2004.

- **Wood, C.M**. The Biotic Ligand Model: putting comparative physiology into environmental regulations.

The following papers or posters were presented by the Metals Bioavailability Group at the Society of Environmental Toxicology and Chemistry (SETAC) Europe 14th Annual Meeting in Prague, Czech Republic, April 18-22, 2004.

- **Franklin, N.M., Glover, C.N., Baldiserotto, B., and Wood, CM.** Interactions between dietary and waterborne cadmium exposure in rainbow trout, and the modulating effect of dietary calcium
- **Gillis, P., Chow-Fraser, P., Sharma, S., Ranville, J.F., Ross, P.E. and Wood, C.M.** The importance of feeding strategy of benthic organisms for copper toxicity in sediments
- **Glover, C.N., Playle, R.C. and Wood, C.M.** Heterogeneity in the protection of natural organic against silver toxicity to *Daphnia magna*
- **Kjoss, V.A., Nadella, S., Pyle, G., Kamunde, C.N., Niyogi, S., Grosell, M. and Wood, C.M.** Dietary copper uptake by rainbow trout – interactions with sodium

- **Niyogi, S. and Wood, C.M.** Can the acute biotic ligand model approach for Cd, developed in a model sensitive salmonid, be extended to a resistant percid?
- **Pane, E.F., Bucking C., and Wood, C.M.** Renal function in rainbow trout (*Oncorhynchus mykiss*) acutely and chronically exposed to waterborne nickel
- **Wood, C.M.** The biotic ligand model: you are what you eat

The following paper will be presented at the MITE-RN 2004 Annual Research Symposium in Aylmer, Quebec, May 11-13, 2004.

- **Wood, C.M., Niyogi, S., Kamunde, C., Taylor, L., Baldosserotto, B., Grosell, M. and McDonald, D.G.** Extending the Biotic Ligand Model (BLM) approach for assessing chronic impacts of metals in freshwater fish: prospects and challenges

The following metals-related paper will be presented at the Canadian Society of Zoology Annual Meeting in Wolfville, Nova Scotia, May 11-15, 2004.

- **Nadella, S. and Wood, C.M.** Mechanism of intestinal copper transport in rainbow trout

The following paper will be presented at the International Association of Great Lakes Research, Waterloo, May 25-28, 2004.

- **Glover, C.N., Playle R.C. and Wood, C.M.** Ameliorative actions of NOM on silver toxicity to *Daphnia magna* are governed by NOM source

The following papers will be presented by the Metals Bioavailability Group at the VI International Congress on the Biology of Fish in Manaus, Brazil, August 1-5, 2004.

- **Baldisserotto, B., Chowdhury, M.J., and Wood, C.M**. Dietary calcium affects dietary and waterborne cadmium contamination in rainbow trout
- **Galvez, F., Wong, D., and Wood, C.M**. Cadmium transport in isolated gill cell populations of freshwater rainbow trout
- **Grosell, M, Morgan, T.P., and Wood, C.M**. Copper uptake across freshwater fish gills – Cu(I) or Cu(II)?
- **Matsuo, A.Y.O., Wood, C.M., and Val., A.L.** No inhibition of Na^+ in tambaqui exposed to high copper concentrations in extremely soft water.
- **Pane, E.F., Bucking C., and Wood, C.M**. Renal function in rainbow trout acutely and chronically exposed to waterborne Ni
- **Sloman, K.A. and Wood, C.M.** Fish social behaviour and the effects of trace metal contaminants
- **Wood, C.M**. The Biotic Ligand Model: predicting metal toxicity to fish in the real world

Research Highlights

This issue will highlight research led by Rebecca Kent, a fourth year student in the lab of Chris Wood who worked with Dr Som Niyogi for her honours thesis research project this past winter. Rebecca is continuing as a summer student in the lab.

Effect of water chemistry on gill-Ca binding in rainbow trout: implications for Cd BLM approach

R. Kent, S. Niyogi and C.M. Wood

The Biotic Ligand Model (BLM) is a promising framework for predicting metal accumulation and toxicity for aquatic organisms in different water chemistries, based on the metal concentration and on the degrees of complexation and competition with environmental factors. Currently, there is no formal published BLM for Cd, but a Cd high-affinity gill-surface binding model was developed by Playle *et al.* (1993a,b) for fathead minnows in softwater. This model uses conditional metal-gill stability constants to predict how Ca^{2+}, DOC and pH will affect metal accumulation at the gill. However, the data used to develop the model were limited, and thus further comprehensive studies are needed to fully characterize the effects of all the key water quality parameters on Cd gill-binding properties (binding site density; $B_{max,}$ and binding site affinity; logK) and acute toxicity (96 h LC50). Therefore in the present study, our main objective was to characterize the effects of a broad range of Ca^{2+}, Mg^{2+}, Na^{+}, H^{+} (pH), alkalinity, and DOC concentrations on the acute binding of Cd to the gills of rainbow trout. Parallel toxicity work is planned for this summer.

Acute 3-h Cd gill-binding assays were carried out in synthetic softwater (US EPA, 1991) on rainbow trout acclimated to Hamilton tapwater (moderately hard water from Lake Ontario). Free Cd ion concentrations were estimated from the total Cd concentrations and the measured water chemistry using MINEQL+, in order to accurately determine the logK and B_{max}. These binding characteristics were calculated by Scatchard analysis.

Binding site characterization

Acute 3-h Cd gill-binding assays identified two types of binding sites for Cd: high affinity, low capacity sites that became saturated at around 20.1±1.1 μg.L^{-1} total Cd (N=4) (0.17 μM free Cd^{2+}), and lower affinity, higher capacity sites that bound Cd in a linear fashion beyond this point (Fig. 1). This result corroborates the findings of a number of other studies that demonstrated that the high-affinity, low-capacity sites become saturated at <0.24 μM of waterborne Cd during 3-h gill binding assays (Hollis *et al.* 2000a; Playle *et al.* 1993b). Scatchard analysis of bound/free versus bound Cd yielded $\log K_{Cd\text{-}Gill} = 7.52$ and $B_{max} = 0.68$ nmol.g^{-1} wet weight for the saturable high affinity sites. This affinity constant is similar to the value of 7.6 obtained by Hollis *et al.* (1999) for rainbow trout exposed in hardwater, but is much lower than the value of 8.6 originally obtained by Playle *et al.* (1993b) for fathead minnows exposed in softwater. The binding site density obtained in the present study (0.68 nmol.g^{-1}) is also lower than the binding site densities calculated for both the Hollis *et al.* (1999) and Playle *et al.* (1993b) studies (B_{max} = 1.61 and 2.27 nmol.g^{-1} wet weight, respectively). A possible explanation for the apparent discrepancy in the Cd binding site characteristics may be due to differences in experimental conditions between the studies, including (i) acclimation water hardness (soft vs. hardwater), (ii) ionic concentrations of the synthetic water, and (iii) analytical sensitivities of the methods used to measure Cd (cold Cd vs. Cd radioisotope). Alternatively, it has been suggested that differences in the history of the fish (batch, size, age, feeding regime, etc.) could be responsible for large discrepancies in calculated B_{max} (reviewed by Hollis *et al.* 1999).

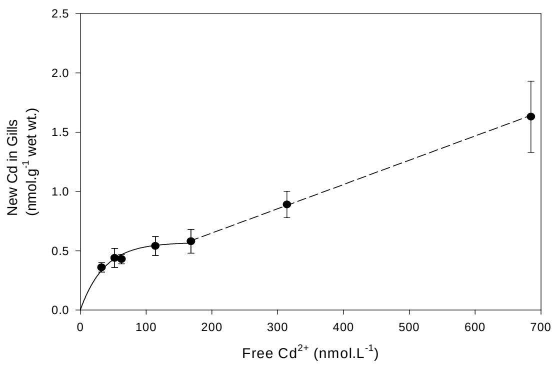

Figure 1. The effects of various water Cd concentrations on the amount of newly accumulated gill Cd in rainbow trout exposed for 3 h in synthetic soft water (Ca^{2+} = 0.220mM±0.007, N=16). Data presented as means ± SEM (N=7 fish). The solid lines represent show the high affinity, low capacity binding sites, while the dotted lines represent the low affinity, high capacity sites.

Effects of water hardness – Ca^{2+} and Mg^{2+}

Cd accumulation on the gill at 18.4 µg.L^{-1} total Cd decreased with increasing water Ca^{2+} levels (Fig. 2). Gill Cd was significantly higher at 0.040mM Ca^{2+} compared to the control ($p<0.05$, 3.81 vs. 2.06 nmol.g^{-1}) and significantly lower at 1.33mM Ca^{2+} compared to the control ($p<0.05$, 0.84 vs. 2.06 nmol.g^{-1}). The calculated free Cd ion did not vary from ~95% of total Cd species across the Ca^{2+} range tested, suggesting that Ca^{2+} kept Cd off the gills through competition for binding sites rather than through changing the speciation. This supports a number of other studies that have shown a protective effect of Ca^{2+} against both Cd gill binding and toxicity (Spry and Wiener 1991; Hollis *et al.* 2000b; Pagenkopf 1983; Playle *et al.* 1993 a,b). Using $\log K_{Cd\text{-}Gill} = 7.52$ (as calculated above), $\log K_{Ca\text{-}Gill}$ (the affinity of Ca^{2+} for the same binding sites as Cd^{2+}) was estimated to be approximately 4.48, which is in reasonable agreement with $\log K_{Ca\text{-}Gill} = 5.0$ estimated by Playle *et al.* (1993b) over a narrower range of Ca^{2+} levels. This indicates that the Cd binding sites (which make up a fraction of the Ca^{2+} transporters) at the gill have a much greater affinity for Cd than for Ca^{2+}.

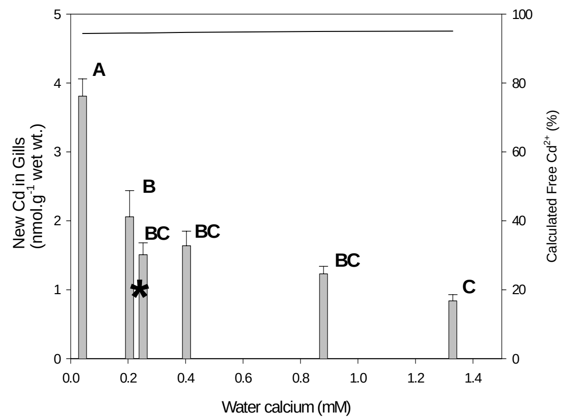

Figure 2. The effects of varying Ca^{2+}on the accumulation of Cd by gills of rainbow trout exposed for 3 h to 18.4µg.L^{-1} Cd in synthetic soft water. Data presented as means ± SEM (N=7). * represents the control value and the solid line indicates the amount of free Cd ion estimated by MINEQL+ as a percentage of total Cd species present. Bars not showing the same letter are significantly different ($p<0.05$).

The other major water hardness cation Mg^{2+} had no significant effect on either Cd accumulation or Cd speciation over the range tested (not shown). Thus, Ca^{2+} is the far more important water hardness factor involved in reducing Cd uptake and toxicity. This is significant because current acute water quality criteria (AWQC) consider the effects of water hardness on acute Cd toxicity, which can be due to Mg^{2+} and / or Ca^{2+} concentrations. Our study shows that it is important to take into account the relative contributions of Ca^{2+} and Mg^{2+} to water hardness when generating water quality criteria, since Cd gill accumulation is only affected by Ca^{2+}, not Mg^{2+}.

Effects of DOC

The levels of DOC (Aldrich Humic Acid) used (~5-38 mg C.L^{-1}) were able to complex 18.4 µg.L^{-1} Cd to varying degrees (from ~8 to 41% Cd-humate). The resulting change in free Cd ion (from ~94 to 56%) corresponded to a significant decrease in Cd gill accumulation at 19.3 and 37.7 mg C.L^{-1} DOC compared to controls ($p<0.05$, 1.06 to 0.55 and 0.25 nmol.g^{-1} wet weight, respectively; Fig. 3). This findings support the BLM assumption that DOC reduces

Cd gill accumulation and toxicity by forming complexes (Cd-humate in this study) with free Cd^{2+}, thus reducing the amount of Cd that is available for binding to the gill. Previous studies have also shown that DOM has a protective effect against Cd gill accumulation and toxicity (Van Ginneken *et al.* 2001, Winner 1984), but to a lesser extent than calcium (Playle *et al.* 1993a,b).

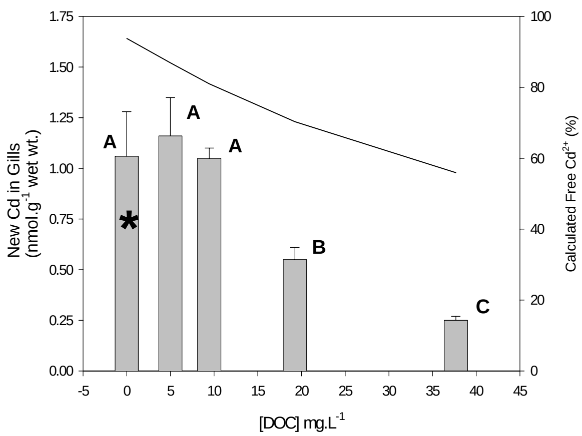

Figure 3. The effects of varying DOC on the accumulation of Cd by gills of rainbow trout exposed for 3 h to 18.4µg.L^{-1} Cd in synthetic soft water. Data presented as means ± SEM (N=7). * represents the control value and the solid line indicates the amount of free Cd ion estimated by MINEQL+ as a percentage of total cadmium species present. Bars not showing the same letter are significantly different ($p<0.05$).

The calculated $\log K_{Cd\text{-}Gill} = 7.52$ was used to indirectly estimate $\log K_{Cd\text{-}DOC}$ (the affinity of Cd for DOC) to be 7.10, which is in reasonable agreement with the $\log K_{Cd\text{-}DOC} = 7.4$ from Playle *et al.* (1993b) and the $\log K_{Cd\text{-}DOC} = 7.52$ (for high affinity sites on Aldrich HA) from Van Ginneken *et al.* (2001). In addition, both our study and the study by Playle *et al.* (1993b) yielded $\log K_{Cd\text{-}Gill}$ values that were greater than the estimated $\log K_{Cd\text{-}DOC}$ values. This means that Cd binds more strongly to the gill than it does to DOC, perhaps explaining in part the high DOC concentrations needed to keep Cd off the gill.

Effects of pH and Alkalinity

We found no significant effect of pH or alkalinity on Cd gill accumulation over the ranges tested (pH 4.6-9.4, alkalinity 13.7-85.5 mg.L^{-1} as $CaCO_3$, respectively). However, the calculated free Cd^{2+} ion decreased above pH 8.0 and at 61.6 and 85.5 mg.L^{-1} alkalinity. According to the BLM theory, we would expect such a decrease in the amount of bioavailable Cd to correspond to a decrease in Cd gill accumulation. One possible explanation for this apparent discrepancy is that the pH of the gill micro-environment can be different from the pH of the water itself due to expired ammonia and carbon dioxide (Playle and Wood 1989), which would alter the metal speciation near the gill. For instance, in poorly buffered water Playle and Wood (1989) predict that inspired bulk water pH's of 8.1 and 9.2 would correspond to pH's of 6.8 and 7.8 in the gill micro-environment, respectively. In both cases, this would increase the amount of free Cd^{2+} calculated by MINEQL+ to ~94% of total Cd species, which is equal to the other pH levels. In other words, the amount of free Cd^{2+} in the gill micro-environment would actually remain constant across the entire pH range tested, due to differences between the gill micro-environment and the bulk water itself. This shows that bulk water chemistry alone may not be sufficient for predicting Cd gill accumulation from Cd speciation at pH>8.0, which has significant implications for the present ongoing effort in our laboratory and elsewhere of developing an acute Cd-BLM for fish, since the BLMs are developed primarily based on studies in poorly buffered water. The cause of the decrease in free Cd^{2+} ion at increased alkalinity needs to be explored further.

Future Research

The characterization of the effects of water chemistry on Cd gill-binding properties in rainbow trout is an important first step towards our effort to develop an acute Cd-BLM for fish. Our next step will be to determine whether the observed changes in the binding profile as a result of water chemistry can be correlated with changes in acute Cd toxicity (96h LC50).

(We thank Chris Glover at McMaster University for his help with the DOC portion of this study, Dr. Richard Playle at Wilfred Laurier University for analyzing samples, and the NSERC Strategic Grants Program, the ILZRO, the ICA, the CDA, the NiPERA, Teck Cominco Ltd., Noranda-Falconbridge Ltd., and MITE-RN for their financial support).

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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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