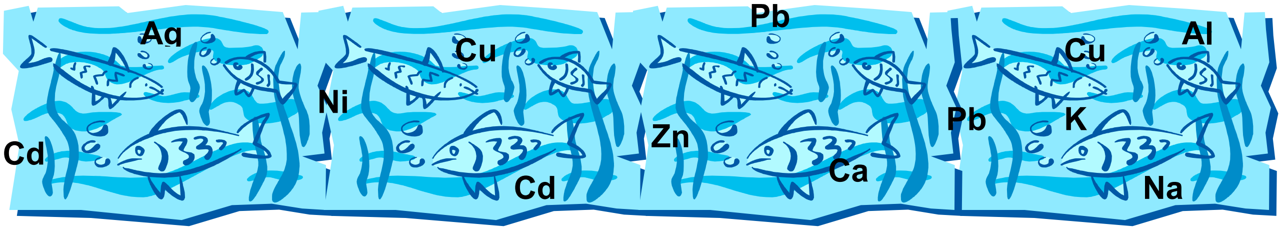
NSERC – Industry Project on Metal Bioavailability
Research Newsletter



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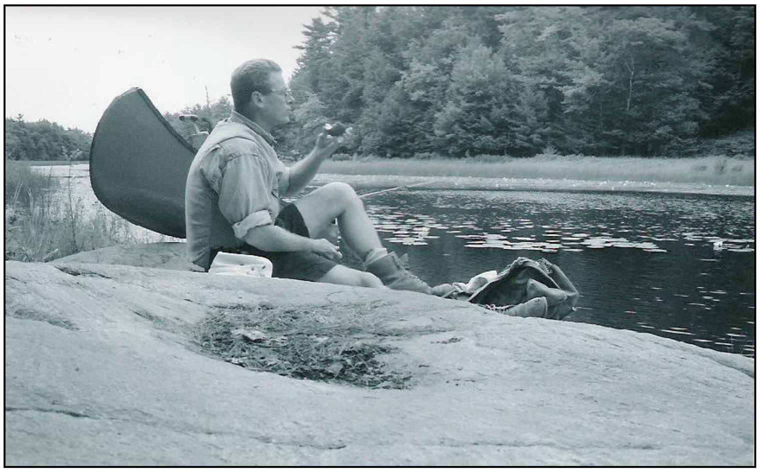
News

Congratulations
We are pleased to announce that Adeola Ojo has recently completed her M.Sc. at McMaster. Her thesis is entitled "Bioavailability and Metal Interactions in the Gastrointestinal Tract of Rainbow Trout." Adeola is now pursuing a Ph.D. at the University of Regina with Richard Manzon. Lara Alves (featured in this issue's Research Highlight) has also just defended her M.Sc. thesis and has begun a Ph.D. with George Dixon and Uwe Borgmann at the Canadian Center for Inland Waters in conjunction with the University of Waterloo. Lara's M.Sc. thesis is entitled "The Chronic Effects of Dietary Lead on Juvenile Rainbow Trout".

New members in the lab
With the beginning of a new school year and the ramping up of a new grant (NSERC-CRD) we have a number of new people in the lab this fall. Karen De Clerk who did her undergraduate at Queens University, in Kingston ON, has joined the lab as a M.Sc. student. Karen will be evaluating the aquatic invertebrate, *Chironomus riparius* as model organism for BLM-type exposures. Also this year we have two fourth year thesis students, Erin Leonard and Joanne Kam who are working on metals projects. We are now, or have hosted a number of international visiting scientists and students this year. Dr. Ibrahim Messaad, a visiting professor from King Khalid University in Saudi Arabia, will be working in the Wood lab for the coming year. Ibrahim will be looking at the interaction of dietary and waterborne metals exposure on Rainbow Trout. In particular, Ibrahim will be looking at the possible protective role of Ca in diet against the uptake of Cu from both the diet and the water. Pierre Laurent and Claudine Chevalier, from Strasbourg, France are once again visiting the lab to continue their work on morphological adaptation of the gills of killifish to different salinities. Finally, Viviane Prodocimo, from the Universidade Federal do Parana, Curibita in Brazil has been visiting the lab for the past five months as part of her Ph.D. research. Viviane investigated the response of rainbow trout and killifish, to acute, short-term salinity challenges. Her study monitored changes in Na and Ca regulation during a salinity transfer protocol that was designed to mimic a portion of a typical tidal cycle.

Travel
This past August several members of our McMaster research group (Chris Wood, Natasha Franklin, Makiko Kajimura and Carol Bucking, along with a number of other colleges including, Pat Walsh (University of Miami), Gudrun De Boeck and Jasper Hattink (University of Antwerp) spent a month at Bamfield Marine Station on Vancouver Island, BC. The studies undertaken included an investigation into using *Mytilus* as a model marine organism for further BLM development. Early life stages of these marine bivalves were found to be highly sensitive to Cu, and to a lesser extent Zn, with more detailed BLM characterizations continuing each summer over the next 3 years. The group also looked at the impact of waterborne Cu on the physiology of elasmobranchs, a fish group which is usually sensitive to the some metals.



Rick Playle, January 1956 - July 2005

Although most you will know this by now, it is with great sadness that we must acknowledge the passing of Rick Playle, who died this past July after a brief illness. Over the past 20 years, Rick has played many roles to those of us in this metals group including, Ph.D. student, collaborator, supervisor, mentor, artist (see below) and of course, friend. A number of eloquent tributes have been written about Rick by his peers and friends, so in this newsletter we would just like to include a few quotes written by some of Rick's close colleagues at the time they heard that he had died.

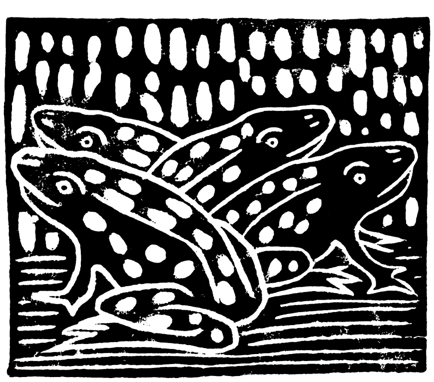
"Although still very young, Rick has made major contributions to environmental science & the development of metal toxicity models of which he was and is one of the most eminent forerunners & catalysers. I was always looking forward to his next paper, & we will now have to learn to live without his warm personality and scientific inspiration." Ronny Blust, University of Antwerp

"Your scientific legacy is written, buts it's your warmth & generosity that I'll remember."
Nic Bury, University of London.

"I will always remember Rick as incredibly generous, friendly, & helpful."
Martin Grosell, University of Miami

"To all of us, Rick embodied the image of health & balance between work & personal life. He was a great colleague & friend." Colin Brauner, University of British Columbia

"Rick was one of the finest individuals I have ever known, and easily my best Ph.D. student. He had poise, balance, perspective, and a truly healthy outlook on life, as well as on science. Rick truly cared about ideas, about principles and about individuals. He was generous and selfless. He always had time for students and helped so many of my grad students and PDFs both formally and informally. He was a brilliant scientist, and his metal modeling work has had a vast impact in environmental science, and in environmental regulation." Chris Wood, McMaster University



Praying mantis in wild asparagus. R.P.

Conference presentations

The following papers were presented by the Metals Bioavailability Group at the annual general meeting of the Laurentian Chapter of the Society of Environmental Toxicology and Chemistry (SETAC) in Burlington, Canada, June 3rd 2005.

- **Franklin N.M., Glover C.N., Wood C.M.** Identifying the source of cadmium accumulation from combined waterborne and dietborne cadmium exposures to juvenile rainbow trout.

- **Gillis P.L., Wood C.M., Chow-Fraser P.** Assessing the bioavailability of sediment-associated metals using *Daphnia magna*.

The following papers were presented by the Metals Bioavailability Group at the 31st Aquatic Toxicology Workshop (ATW) in Waterloo, Canada, October 2-5th 2005.

- **Franklin N.M., McClelland G., Wood C.M.** A Biotic Ligand Model Approach to Cu toxicity in the zebrafish *Danio rerio*.

- **Gillis P.L., Wood C.M.** The influence of water chemistry on the uptake and toxicity of cadmium in *Chironomus riparius*.

- **Green W.W., Mirza R.S., Wood C.M., Pyle G.G.** Olfactory sensitivity of wild yellow perch (*Perca flavescens*) from along a metal contamination gradient.

- **McGeer J., Nadella S., Wood C.M.** Influence of acclimation to Cu or Cd on the bioaccumulation of Cu and Cd.

- **Wood C.M.** Pathways of metal uptake and toxicity in fish: putting physiology into environmental regulations.

The following papers will be presented by the Metals Bioavailability Group at the 26th Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC) in Baltimore MD, USA, Nov. 13-17th 2005.

- **Chowdhury M., Pane E., Wood C.M.** Chronic exposure of rainbow trout to dietary cadmium: physiological responses and acclimation.

- **Clearwater S., Ellwood M.J., Wood C.M.** Does bioincorporation of dietary cadmium alter its toxicity to fish?

- **Franklin N.M., Galvez F., Wood C.M.** Chronic waterborne and dietborne cadmium exposures to juvenile rainbow trout: physiological and molecular endpoints.

- **Franklin N.M., McClelland G., Wood C.M.** Extending the Biotic Ligand Model: characterizing Cu gill binding and acute toxicity to the zebrafish *Danio rerio*.

- **Galvez F., Donini A., Diao L., Playle R., Smith S., Wood C.M.** The effects of natural organic matter on fish gill epithelia.

- **Gillis P.L., Chow-Fraser P., Ranville J.R., Wood C.M.** Are ingested sediments a significant source of bioavailable Cu to *Daphnia magna*?

- **Gillis P.L., Wood C.M.** Comparing the effect of varying water chemistry on the acute toxicity of cadmium to *Chironomus riparius*.

- **Kajimura M., Iwata K., Sakamoto T., Iwata I., Nishiguchi E., Smith R.W., Wood C.M.** High ambient ammonia promotes growth in an ureogenic goby, *Mugilogobius abei*.

- **McGeer J., Nadella S., Wood C.M.** The influence of acclimation to Cu or Cd on the toxicity and bioaccumulation of Cu and Cd in rainbow trout and *Daphnia magna*.

- **Pane E., McDonald M., Curry H., Wood C.M., Grosell M.** Hydromineral balance in the marine gulf toadfish exposed to nickel via two routes.

- **Wood C.M., Franklin N.F., Alves L., Ojo A., Niyogi S., Kamunde C., Pyle G.G., Chowdhury M., Kjoss V., Nadella S.** How hard is that diet? Implications for metal accumulation and toxicity.

Research Highlights

This issue will highlight research conducted by Lara Alves 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.

Dietary Pb accumulation in juvenile freshwater rainbow trout (*Oncorhynchus mykiss)*

Alves L.C., Glover C.N., and C.M. Wood

Lead (Pb) is a non-nutrient metal found in the earth's crust, which can enter the aquatic environment through natural processes of geological weathering, volcanic emissions and well as through anthropogenic practices such as the mining, refining and smelting of Pb. In fish, the primary site of Pb toxicity is at the gills (Varanasi and Gmur 1978), where inhibitory actions of Pb on Ca^{2+}, Na^{+}, and Cl^{-} uptake have recently been documented (Rogers et al. 2003, 2005; Rogers and Wood 2004). However, Crespo et al. (1986) showed that toxicity may also occur via the gastrointestinal tract since rainbow trout that were administered with Pb in the diet (10 μg Pb/g dry weight/fish/day) had morphological alterations of the intestinal brush border which resulted in an impairment of intestinal absorption. Typically, Pb levels in uncontaminated benthic invertebrates are less than 1 μg/g dw, however body burdens up to

792 μg/g dw have been reported in contaminated sites (Farag et al. 1994, 1999). While these studies using naturally contaminated diets have displayed evidence of dietary Pb uptake and toxic effects to fish, the laboratory study of Hodson et al. (1978) reported that rainbow trout fed diets contaminated with up to 118 μg Pb/g did not accumulate Pb in internal tissues. The objectives of this study were i) to determine if juvenile rainbow trout fed a Pb-contaminated diet accumulated Pb, and ii) to evaluate the pattern of tissue-specific accumulation over time, while looking for any evidence of regulation or depuration. We also investigated possible hematological abnormalities in the exposed fish as well as any alterations in plasma Ca and Mg regulation, and Na and Ca influx rates.

Methods

Fish were assigned to one of four treatment groups; either a control (0.06 ± 0.004), low (7.2 ± 0.9), intermediate (76.5 ± 6.7), or a high Pb diet (519.8 ± 50.0) μg Pb/g dw. These concentrations were specifically selected to cut across the range used by Hodson et al. (1978; 4-118 μg Pb/g dw) and the concentrations found in benthic invertebrates (Farag et al. 1994, 1999). Pb-enriched diets were made by adding lead nitrate ($Pb(NO_3)_2$) to commercial salmon fry food. Each group was fed once daily to satiation, and pellets not eaten were removed to reduce any leaching of Pb. Waterborne Pb concentration, mean fish weight, condition factor, food consumption per fish, food conversion efficiency, and specific growth rates were determined throughout the experiment. Pb concentrations in whole blood, plasma, liver, kidney, intestine, and gills as well as plasma Ca and Mg levels were determined weekly from day 0 to day 21. On day 0, 8 and 22, six fish each were randomly selected from each exposure to measure the unidirectional Na and Ca influx rates from the water.

Results

Waterborne Pb values in the low and intermediate exposures were not significantly above background, but waterborne Pb (10 μg/L) in the high dietary Pb exposure was significantly

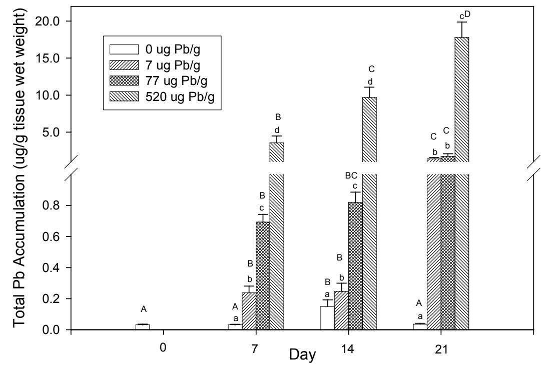


Figure 1. Lead accumulation in the intestine of rainbow trout exposed to different levels of Pb in the diet over 21 days. Data represented as mean ± 1 SEM, N=12, except the 77 μg Pb/g treatment, N=6. Lower case letters indicate significant differences (P<0.05) between treatment means within a day and upper case letters indicate significant differences between days within treatment means.

elevated, presumably due to the leaching from the diet and/or feces. No mortality was associated with the dietary Pb treatments throughout the course of the experiment. Specific growth rates, total food eaten, food conversion efficiency, and condition factor also did not differ among treatments.

Pb accumulation was measured in the gills, intestine (Fig. 1), liver (Fig. 2), kidney and carcass during the course of the experiment at all doses. The intestine (17.8 μg Pb/g tissue wet weight (ww)), carcass (2.7 μg Pb/g ww), kidney (2.4 μg Pb/g ww) and the liver (1.9 μg Pb/g ww) all exhibited their highest Pb burdens on day 21. In contrast, the gills, at least at the highest dose, had the greatest Pb accumulation on day 7 (8.0 μg Pb/g ww) and a much lower Pb burden (2.2 μg Pb/g ww) by day 21. Pb burden in the intestine increased with time in all dietary Pb treatments. The carcass accumulated about 80%, of the Pb burden, the intestine about 10%, while the gills, kidney and liver made up the remaining 10% of the Pb burden. Pb in RBC increased from a background of 0.05 μg Pb/g to about 1.5 μg Pb/g in the highest dose by day 21 (Fig. 3), which is about 105 times more Pb than was seen in the plasma. For the major ions, plasma Mg and Ca levels fell significantly in the intermediate and high Pb treatments at mid-time points in the exposure period, although both had stabilized by the end of the experiment. Neither hematocrit nor plasma protein were affected by dietary Pb exposure.

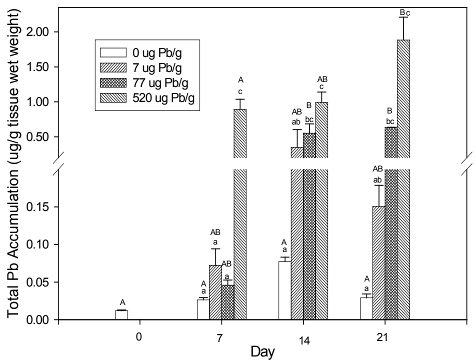


Figure 2. Lead accumulation in the liver of juvenile rainbow trout exposed to different levels of Pb in the diet for 21 days. Other details as in Fig. 1.

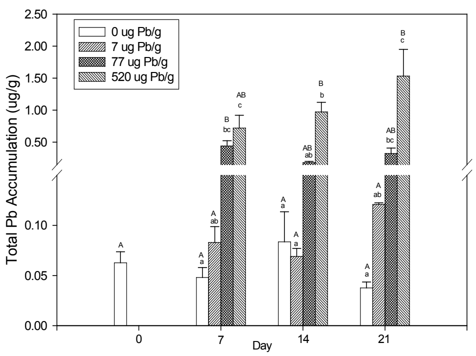


Figure 3. Lead accumulation in the red blood cells of juvenile rainbow trout exposed to different levels of Pb in the diet for 21 days. Other details as in Fig. 1.

Calcium influx rates remained stable throughout the experiment. However, on day 8, Na influx rates were significantly elevated in all treatments, and this occurred to the greatest extent in the high Pb diet treatment, although these effects had disappeared by day 22.

Discussion

Dietary exposure to Pb had few apparent adverse effects on rainbow trout during the 21 day experiment. The lack of mortality and changes in specific growth rate were consistent with Mount et al. (1994) who reported no effects on survival and growth of rainbow trout fed a dietary concentration as high as 170 μg Pb/g. Pb body burdens in the low dietary treatment were close to background levels in all tissues, suggesting that a dietary concentration greater than 7 μg Pb/g was necessary for Pb to accumulate in the internal tissues. In the present study the gills accumulated Pb (up to 2.1 μg Pb/g), mainly in the high dietary Pb treatment, although this may be a result of the additional route of waterborne exposure in this treatment. The high Pb accumulation in the intestine suggests that this tissue may be a potential site for chronic Pb toxicity via the diet in accord with the physiology results of Crespo et al. (1986). This is also consistent with Farag et al. (1994) who found that adult rainbow trout fed metal-contaminated benthic invertebrates exhibited substantial metal accumulation in the gut tissues. The high Pb burden in the intestine in this study may be due at least in part to binding of Pb from the diet by mucus (Powell et al. 1999), whose secretion may be stimulated by dietary metals (Glover and Hogstrand 2002). This mucus layer can act to sequester high levels of metals, and thus prevent the exposure of the underlying epithelial tissue to potentially toxic metal levels. Such a scenario was observed in fish exposed to intestinally-perfused zinc (Glover and Hogstrand 2002). They reported that the trapped metal burden may subsequently be sloughed off as a result of movement of food through the intestine. Therefore, the high levels of Pb associated with the intestine in this study, at least in part, may represent Pb that is adsorbed, but not absorbed.

This study demonstrated that dietary Pb can cross the intestinal epithelium and significantly accumulate in internal soft tissues such as the liver, kidney, and RBCs. There was significant Pb accumulation on day 21 in the high dietary Pb treatment in the kidney. Since Ca and Pb are believed to be antagonists of one another, these ions may be competing for transport sites at renal tubule cells. This could lead to Pb becoming trapped in the tubule cells and result in considerable Pb tissue burden in this tissue. The low levels of Pb in the liver may be explained by the fact that Pb is not able to induce hepatic detoxifying proteins, namely metallothioneins (Reichert et al. 1979; Campana et al. 2003).

In mammals, under steady state conditions, about 96% of Pb in the whole blood is in the red blood cells (WHO 1995). In this study 99% of Pb was in the RBCs on day 21 in fish fed the high Pb diet.

Ca, a tightly regulated ion in freshwater fish, is continuously absorbed from the water via the gills (Flik and Verbost 1993). A significant decrease in plasma Ca levels in the intermediate and high Pb diets occurred on day 7. However, Ca levels recovered thereafter. Studies have shown decreased plasma Ca levels in rainbow trout exposed to waterborne Pb, indicating the presence of a Pb/Ca interaction at the gills (Rogers et al. 2003, 2005; Rogers and Wood 2004). The significant decrease in plasma Ca levels in this study may be the result of a Ca/Pb interaction at the intestine, which in addition to the gill may act as an important Ca uptake route (Flik and Verbost 1993).

In summary, the present study provides evidence that dietary Pb accumulates in the internal tissues of rainbow trout and that the intestine has a potential role in sublethal Pb toxicity via the diet. Nevertheless, physiological disturbances were minimal, and feeding, growth, and food conversion efficiency were relatively unaffected over the 21 days of the experiment. This study has provided a basis to explore Pb effects on membrane transport in the intestine, and given the high accumulation in RBCs, its effect on the heme synthesis pathway in future studies.

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Editor's Desk: This newsletter is distributed by the Metals Bioavailability Group, Department of Biology, McMaster University. Beginning with this issue Dr. Natasha Franklin has passed the editor's job to Dr. Patricia Gillis. If you know of others who would enjoy this newsletter, or if you no longer wish to receive it yourself, please contact**: Patricia Gillis,** Department of Biology, McMaster University, 1280 Main St. West, Hamilton, Ontario L8S 4K1, Canada. Tel.: 905-525-9140 ext.23237; fax: 905-522-6066; e-mail: gillisp@mcmaster.ca