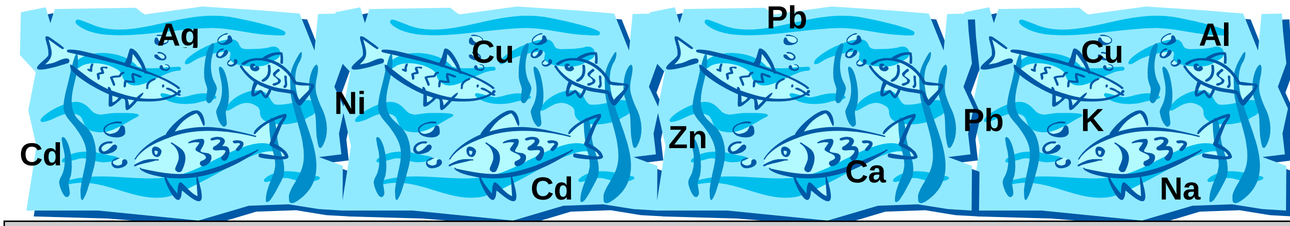
# NSERC – Industry Project on Metal Bioavailability
## Research Newsletter



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## ***News***

***Comings and Goings (2009-2010)***

**Scott Smith** has just wrapped up a 1-year sabbatical visit to Chris Wood's lab where he studied the in vitro Cu –binding characteristics of isolated gill cells and cultured gill epithelia.

Ph. D. students **Marianna Jorge** and **Lygia Noguiera**, and **PDF Dr. Camila Martins**, all from **Dr. Adalto Bianchini**'s lab at the University of Rio Grande in southern Brazil joined Chris Wood's lab this past spring, to work on marine and freshwater metals projects. Their visit is part of the 5-year IDRC International Research Chair Program awarded to Adalto Bianchini and Chris Wood. Marianna and Lygia will be in Canada until May (2011) while Camila returned to Brazil in August (2010) to take up a tenure-track faculty position which she won at the University of Rio Grande. Congratulations Camila! Marianna and Lygia are collaborating with **Dr. Patty Gillis** of Environment Canada in studies of metal toxicity to freshwater mussels, and therefore are dividing their time between McMaster University and the Canada Centre for Inland Waters in nearby Burlington, Ontario. The marine portions of the work of Lygia, Marianna, and Camila directly contribute to both CRD grant #1 and CRD grant #2.

As part of this same IDRC program, McMaster Ph.D. student **Erin Leonard** spent 4 months this past spring working on a marine metals project (nickel) in **Dr. Adalto Bianchini**'s lab at the University of Rio Grande. You can read about her research there in the Research Highlights section of the newsletter. This research directly contributes to CRD grant #2.

Recently, we welcomed another Brazilian visitor to the Wood lab, **Dr. Vania Lucia Loro**, who is a faculty member (Toxicology) in the Federal University of Santa Maria. Vania is a sabbatical visitor and will be at McMaster until August (2011). She is working on a marine metals project, specifically looking at bioaccumulation of zinc and biomarker responses as a function of salinity in fish and crabs. Her work directly contributes to marine CRD grant #2.

**Margaret Tellis**, earlier an undergraduate Honours thesis student, formally started her M.Sc. research in Chris Wood's lab at McMaster this past May (2010). Margaret is examining toxic mechanisms of Pb and Zn to marine invertebrate larvae as a function of salinity and DOC concentrations. Her work directly contributes to marine CRD grant #1.

**Adalto Bianchini, Margaret Tellis, Marianna Jorge, Lygia Noguiera, Sunita Nadella,** and **Chris Wood** spent an enjoyable month (June, 2010) working together at Bamfield Marine Station on the west coast of Vancouver Island, B.C. The research focused on the effects of DOC from various marine sources, some of it collected locally in the Bamfield area, on Pb and Zn toxicity to marine invertebrate larvae, and associated biomarkers responses. This preliminary project directly contributes to both CRD grant #1 and CRD grant #2..

**Dr. Paul Craig**, formerly a Ph.D. student in **Dr. Grant McClelland**'s lab studying the toxicological genomics of Cu to zebrafish, has been awarded a prestigious NSERC Postdoctoral fellowship to work with **Dr. Tom Moon** at the University of Ottawa. Congratulations Paul!

**Dr. Farhan Khan**, formerly a PDF in **Dr. Jim McGeer**'s lab studying the use of modeling techniques to track the recovery of acidified, metal-impacted lakes in Ontario, has received a prestigious fellowship to work with **Dr. Phil Rainbow** at the British Museum of Natural History. Congratulations Farhan!

**Dr. Tania Ng**, formerly the Senior Research Associate on the NSERC Strategic-Rio Tinto Alcan funded Tissue Residue Approach project of Chris Wood and Jim McGeer has accepted a sessional teaching position at Redeemer College in Hamilton. Congratulations Tania!

**Dr. Ishaq Ahmed**, formerly a PDF working with **Drs. Greg Pyle** and Chris Wood on an NSERC-MITHE network project studying genomic responses of fathead minnows to waterborne and dietary Cu exposure has accepted a postdoctoral fellowship at the University of British Columbia. Congratulations Ishaq!

At Scott Smith's lab **Rachael Diamond** is starting her MSc project on marine metal speciation funded by CRD #1. Rachael did her undergraduate thesis in Scott's lab but left for a year to study at the University of Guelph. Welcome back Rachael! **Sarah DePalma**, after completing her MSc, is now at Environment Canada as a Physical Scientist. **Dr. Cristina Gheorghiu** recently finished her two year PDF with Mike Wilkie and Scott Smith at Laurier. Good luck Cristina!

At Jim McGeer's lab, **Jess Milne** wrapped up her MSc research on the time-course of subcellular Cd uptake and distribution in trout tissues. She has recently started her program in Dentistry at U of T. Two new students, **John Ellis** and **Katherine Chan** joined the lab this September. John will continue work on the subcellular metal accumulation and ROS production, with Ni. Katherine will be working on the chronic effects of Ni on *Hyalella azteca*.

***New funding***

**Adalto Bianchini (P.I.)** and **Chris Wood** have just completed the first year of a 5-year IDRC International Research Chair Program funded by IDRC and the Canada Research Chair Program. This research and social development project supports a multi-stakeholder project on Coastal Zone Management in Brazil, with a strong focus on metals.

**Chris Wood (P.I.)** and **Jim McGeer** are just wrapping up a 2-year NSERC Strategic Grant, co-funded by Rio Tinto Alcan with in kind support from Environment Canada. The project focused on further development of the Tissue Residue Approach for Risk Assessment of Metals.

**Chris Wood (P.I.)** and **Scott Smith** received a 2-year NSERC CRD grant, co-funded by ILZRO and IZA, to study the Interactive Effects of Salinity and DOC on Pb and Zn Toxicity to Marine Invertebrate Larvae. This started in May (2010) and is referred to as CRD #1 above.

**Scott Smith (P.I.)**, **Chris Wood**, **Grant McClelland**, and **Jim McGeer** received a 4-year NSERC CRD project, co-funded by ICA./CDA, NiPERA, IZA, ILZRO, Teck Resources, and Vale, with in kind support form Xstrata Zinc. The research project focuses on Research towards Biotic Ligand Models for Metals in Saltwater Environments from both Chemical and Biological Perspectives. The NSERC funding will start in Jan. 2011, but significant progress has already been made thanks to the early arrival of industrial co-funding. This is a much larger project than CRD #1, with a much broader scope, and considers Cu, and Ni as well as Zn and Pb. It is referred to as CRD # 2 above.

**Scott Smith (PI)** is just wrapping up a project funded by CDA. The experimental work should be completed early in 2011 and involves multi-method (voltammetry, competitive ligand, ion selective electrode) comparisons of copper speciation for marine samples. Saltwater metal speciation method comparisons continue in CRD #1 and CRD #2.

**Scott Smith** and **Mike Wilkie** (co-PIs) recently completed a CDA funded project focused on Cu and DOC that had co-funding from Wilfrid Laurier University, MITHE, as well as the Ontario Government Ministry of Research and Innovation. This project funded the postdoctoral position of Dr. Cristina Gheorghiu.

**Jim McGeer and the research team of John Gunn (P.I.), Norm Yan, Dave Kreutzweiser** and **Shaun Watmough** received a 5-year NSERC CRD project, co-funded by Vale and Xstrata. The research project focuses on the role of organic matter in the recovery of damaged ecosystems. Jim's work on this project will characterize the influence of NOM quantity and quality on Ni and Cu toxicity.

**Jim McGeer**, **Scott Smith**, **Mike Wilkie** and 6 others co-applicants (PI Bill Quinton) were successful in developing a 6.3 million $ infrastructure grant under the New Initiatives Fund of the Canadian Foundation for Innovation (with matching funds from the Government of the Northwest Territories. The funding will develop the Canadian Aquatic Laboratories for Interdisciplinary Boreal Ecosystem Research (CALIBER) and provide a dramatic enhancement of the research capacities at Laurier. Metals bio-geochemistry in aquatic systems is central to the research that will be developed.

## Conference presentations

*The following papers are going to be presented by the Metals Bioavailability Group in the 31st Annual SETAC North America Meeting, Portland, Oregon. Nov 7- 11, 2010.*

- **Martins, C.D.M.G., de Menezes, E., Giacomin, M.M., Wood, C.M., and Bianchini, A. 2010.** Toxicity and tissue distribution and accumulation of copper in the blue crab *Callinectes sapidus* acclimated to different salinities: *In vivo and in vitro* studies.
  Poster: Exhibit Hall, Wednesday.

- **Cunningham, J., McGeer. J. 2010.** The effects of chronic cadmium exposure on the repeat swimming performance in three species of salmonids.
  Presentation, Portland Ballroom 252 Tuesday 8:50 AM

- **Sandhu, N., McGeer, J., Vijayan, M. 2010.** Cadmium impacts the cortisol stress axis in rainbow trout.
  Presentation, Portland Ballroom 252 Tuesday 11:05 AM

- **Costa, E-J., McGeer, J. 2010.** The Effect of metal-oxide nanoparticles on *Daphnia pulex* and *Hydra Attenuata*

Presentation, Portland Ballroom 253 Wednesday 5:25 PM

- **McGeer, J., Pais, N., Straus, T. 2010.** Bioaccumulation of Cd in *Lumbriculus vaiegatus, Lymnaea stagnalis* and *Hyalella azteca* and development of a novel tissue residue approach. Poster: Exhibit Hall Wednesday

- **Leonard, E.M., Barcarolli, I., Silva, K.R., Wasielesky, W., Wood, C.M., Bianchini, A. 2010.** Evaluating the effects of salinity on chronic Ni toxicity and bioaccumulation in two euryhaline crustaceans: *L. vannamei* and *E. armata*.
Portland Ballroom 252 Monday 1:55 PM

- **Smith, D.S. 2010.** Comparison between electrochemical methods of copper speciation determinations in salt water and implications for toxicity.
Poster. Exhibit Hall Tuesday.

- **Smith, D.S., Diao, L. Wood, C. M. 2010.** Copper interactions with isolated gill cells and with gill cell inserts: testing of Biotic Ligand Model parameters.
Presentation, Portland Ballroom 252 Wednesday 9:15 AM

*Other presentation (2010):*

- **D'Silva, J., Leonard, E.M., Wood, C.M. 2010.** Chronic nickel toxicity and bioaccumulation in rainbow trout (*Oncorhynchus mykiss*). Comparative Physiology and Biochemistry Workshop, Feb 5-7, 2010. Rice Lake, Ontario, Canada. Poster Presentation.

- **Dhaliwal, T., Ng, T. and Wood, C.M. 2010.** Chronic Cu toxicity and bioaccumulation in *Chironomous riparius.* $19^{th}$ Annual Comparative Physiology and Biochemistry Workshop, Rice Lake, Ontario, February 5-7, 2010. Poster Presentation.

- **Belowitz, R., Leonard, E.M., Agema, P., O'Donnell, M.J. 2010.** Competition between physiologically relevant ions and toxic metals in *C. riparius*. Comparative Physiology and Biochemistry Workshop, Feb 5-7. Rice Lake, Ontario, Canada. Platform presentation.

- **Tellis, M., Alsop, D., Wood, C.M. 2010.** The effect of Cu on cortisol release in rainbow trout. $19^{th}$ Annual Comparative Physiology and Biochemistry Workshop, Rice Lake, Ontario, February 5-7, 2010. Platform presentation.

- **Wood, C.M. 2010.** Regulation and toxicity of metals in aquatic ecosystems. Annual Meeting of the Soc. Exp. Biol., June 30- July 3, 2010, Prague. Invited Platform presentation.

- **Wood, C.M., Grosell, M., McDonald, M.D., Playle, R.C., Walsh, P.J. 2010.** Effects of waterborne silver in a marine teleost, the gulf toadfish (*Opsanus beta*): Effects of feeding and chronic exposure on bioaccumulation and physiological responses. 49th Annual Meeting of the Can. Soc. Zool, May 17-21 2010. U.B.C, Vancouver. Platform presentation.

- **Wilkie, M. P., Gheorghiu, C., Kara, Y., and Smith, D. S. 2010.** The influence of NOM quality on metal-gill binding in fish exposed to metals and metal mixtures. SEB Annual Main Meeting, June 2010. Prague, Czech Republic. Platform presentation.

- **Gheorghiu, C., Smith, D. S., Kara, Y., and Wilkie, M. P. 2010.** Source of NOM differently affects metal-gill binding in rainbow trout (*Oncorhynchus mykiss*) exposed to Pb-Cd mixture. 49th Annual Meeting, Canadian Society of Zoologists, Vancouver, BC, Canada. Platform presentation.

- **Cunningham, J., McGeer, J.C. 2010.** Effects of chronic exposure to cadmium on the swimming performance in brown trout and the rainbow trout. 49th Canadian Society of Zoologists Annual Meeting, May 17-21 2010, Vancouver BC. Oral presentation.

- **Straus, A., McGeer, J.C. 2010.** Linkages between exposure, effect and accumulation of cadmium in *Lumbriculus variegatus* and *Hyalella azteca*. 49th Canadian Society of Zoologists Annual Meeting, May 17-21 2010, Vancouver BC. Oral presentation.

- **Costa, E-J., McGeer, J.C. 2010.** The effect of metal-oxide nanoparticles on *Daphnia pulex* and *Hydra attenuata*. 49th Canadian Society of Zoologists Annual Meeting, May 17-21 2010, Vancouver BC. Oral presentation.

- **Mancini, A., Milne, J.L., McGeer, J.C. 2010.** Physiological effects of chronic Cd exposure in rainbow trout. 49th Canadian Society of Zoologists Annual Meeting, May 17-21 2010, Vancouver BC. Oral presentation.

- **Pais, N., McGeer, J.C. 2010.** Acute and chronic effects of waterborne cadmium on *Lymnaea stagnalis*. 49th Canadian Society of Zoologists Annual Meeting, May 17-21 2010, Vancouver BC. Oral presentation.

- **Pais, N., Costa, E-J., Straus, A., McGeer, J.C. 2010.** Acute and chronic effects of bioaccumulated Cd on *Lumbriculus variegatus, Lymnaea stagnalis, Hyalella azteca* and *Daphnia pulex*. 37th Annual Aquatic Toxicity Workshop Oct 5-8, 2010. Toronto. Oral presentation.

- **Hassan Al-Reasi, Scott Smith, Chris Wood. 2010.** Ameliorative effect of natural organic matter (NOM) on metal toxicity to aquatic organisms: Evaluating the influence of NOM quality. The Aquatic Toxicology Workshop (ATW), October 3-6, 2010. Toronto, Ontario, Canada.

- **McGeer, J., Milne, J., Mancini, A. 2010.** Bioaccumulation and physiological effect of chronic sublethal Cd exposure in rainbow trout. 37th Annual Aquatic Toxicity Workshop Oct 5-8, 2010. Toronto. Oral presentation.

*The following peer reviewed papers were published by the Metals Bioavailability Group in 2009 - 2010*

- **Martins, C.M., Barcarolli, I.F., Menezes, E.J., Giacomin, M.M., Wood, C.M. Bianchini, A. 2010.** Acute toxicity, accumulation and tissue distribution of copper in the blue crab *Callinectes sapidus* acclimated to different salinities: *In vivo and in vitro* studies. *Aquat. Toxicol.* In Press.

- **Adams, W.J., Blust, R., Borgmann, U., Brix, K.V., Deforest, D.K., Green, A.S., Meyer, J., McGeer, J.C., Paquin, P., Rainbow, P.S., Wood, C.M. 2010.** Utility of tissue residues for predicting effects of metals on aquatic organisms. *Integr. Environ. Assess. Man.* In Press

- **Nadella, S.R., Hung, C.Y., Wood, C.M. 2010.** Mechanistic characterization of gastric copper transport in rainbow trout. *J.Comp. Physiol. B.* In Press.

- **Ng, T.Y.-T., Chowdhury, M.J., Wood, C.M. 2010.** Can the Biotic Ligand Model predict Cu toxicity across a range of pHs in softwater-acclimated rainbow trout? *Env. Sci. Technol.* 44 pp: 6263-6268.

- **Marentette, J.R., Gooderham, K.L., McMaster; M.E., Ng, T., Parrot, J.L., Wilson, J.Y., Wood, C.M., Balshine, S. 2010.** Signatures of contamination in invasive round gobies (*Neogobius melanostomus*): A double strike for ecosystem health? *Ecotox. and Environ Safety*. 13 pp: 1755-1764.

- **Wood, C.M., Grosell, M., McDonald, D.M. Playle, R.C., Walsh, P.J. 2010.** Effects of waterborne silver in a marine teleost, the gulf toadfish (*Opsanus beta*): Effects of feeding and chronic exposure on bioaccumulation and physiological responses. *Aquat. Toxicol.* 99 pp: 138-148.

- **Craig, P.M., Wood, C.M. and McClelland, G.B. 2010.** Water chemistry alters gene expression and physiological endpoints of chronic waterborne Cu exposure in zebrafish *Danio reiro*. *Env.Sci. Technol.* 44 pp: 2156-2162.

- **Green, W.W., Mirza, R. S., Wood, C.M., Pyle. G. G. 2010.** Copper binding dynamics and olfactory impairment in fathead minnows *Pimephales promelas*. *Env. Sci. Technol.* 44 pp: 1431-1437

- **Arnold, W. R., Diamond, R. L., Smith, D. S. 2010.** The acute and multi-generation chronic toxicity of copper to the rotifer, *Brachionus plicatilis* ("L" strain). *Archives of Environ. Contam. and Tox.*. In press.

- **DePalma, S. G. S., Arnold, W. R., McGeer, J. C., Dixon, D. G., Smith, D. S. 2010.** Variability in dissolved organic matter fluorescence & reduced sulphur concentration in coastal marine & estuarine environments. *Applied Geochemistry* (ms. APGEO-D-09-00355 accepted pending revisions 04/05/10)

- **DePalma, S. G. S., Arnold, W. R., McGeer, J. C., Dixon, D. G., Smith, D. S. 2010.** Protective effects of dissolved organic matter and reduced sulfur on copper toxicity in coastal marine environments. *Ecotoxicology and Environmental Safety* (ms. EES-10-41 accepted pending revisions 04/06/10)

- **Gheorghiu, C., Smith, D. S., Al-Reasi, H., C., M. J., Wilkie, M. P. 2010.** Influence of natural organic matter (NOM) quality on Cu-gill binding in the rainbow trout (*Oncorhynchus mykiss*). *Aquatic Tox*. 97 pp: 343-352.

- **Arnold, W. R., Diamond, R. L., Smith, D. S. 2010.** The effects of salinity, pH, and dissolved organic matter on acute copper toxicity to the rotifer, *Brachionus plicatilis* (L strain). *Archives of Environ. Contam. Toxicol.*. 59 pp: 225-234.

- **Gheorghiu, C., Hanna, J., Smith, J. W., Smith, D. S., Wilkie, M. P. 2010.** Encapsulation and physiological effects of pit tags in brown trout (*Salmo trutta* L.). *Aquaculture* 298 pp: 350-353.

- **Arnold, W. R., Cotsifas, J. S., DePalma, S. G., Smith, D. S. 2010.** A comparison of the copper sensitivity of *Mytilus galloprovincialis, Crassostrea virginica, Dendraster excentricus,* and *Strongylocentrotus purpuratus* in ambient saltwater of varying dissolved organic matter concentrations. *Environ. Toxicol. & Chem*. 29 pp: 311-319.

- **Gillis, P.L., Mackie, G.L. McGeer, J.C., Wilkie, M.P., Ackerman, J.D. 2010.** The effect of natural dissolved organic carbon on the sensitivity of larval freshwater mussels (*glochidia*) to acute copper exposure. *Environ. Toxicol. Chem.* In Press.

- **McGeer, J.C., Smith, D.S., Brix K.V., Adams, W.J. 2010.** The importance of metal speciation and its application in biotic ligand modelling to understand effects in aquatic biota. *in* Environmental Radioactivity and Ecotoxicology of Radioactive Substances, Ed. Bird, G. Vol XX of Encyclopaedia of Sustainability Science and Technology. Springer Science. In Press.

- **McGeer, J.C., Clifford, M., Janssen, C.R., De Schamphelaere, K.A.C. 2010.** Modeling the toxicity of metals to aquatic biota using the biotic ligand approach. pg 205-231 *In*. Essential Reviews in Experimental Biology Vol. 2. Surface Chemistry, Bioavailability and Metal Homeostasis in Aquatic Organisms: an Integrated Approach. Ed. Bury, N.R. and R.D. Handy. SEB Press. London. UK.

- **Clifford, M., McGeer, J.C. 2010.** Development of a biotic ligand model to predict the acute toxicity of cadmium to *Daphnia pulex*. *Aquat. Toxicol.* 98: 1-7.

- **Diamond, M., Adams, W., Atherton, J., Bhavsar, S., Bulle, C., Campbell, P., Dubreuil, A., Fairbrother, A., Farley, K., Gandhi, N., Green, A., Guinee, J., Hauschild, M., Humbert, S., Jensen, K., Jolliet, O., Margni, M., McGeer, J., Peijnenburg, W., Rosenbaum, J., Van de Meent, D., Vijver, M. 2010.** The Clearwater Consensus: the estimation of metal hazard in fresh water. *Internat. J. Life Cycle Assess.* 15: 143-147.

- **Arnold, W. R., Cotsifas, J. S., Smith, D. S., LePage, S., Gruenthal, K. M. 2009**. A comparison of the copper sensitivity of two economically important saltwater mussel species and a review of previously reported copper toxicity data for mussels: important implications for determining future ambient copper saltwater criteria in the USA. *Environ. Toxicol*. 24 pp: 618-628.

- **Craig, P.M., Hogstrand, C., Wood, C.M., McClelland, G.B. 2009.** Gene expression endpoints following chronic waterborne copper exposure in a genomic model organism, the zebrafish, *Danio rerio*. *Physiol Genomics.* 40 pp: 23-33

- **Klinck, J.S., Tania Ng,T. Y.-T, Wood, C.M. 2009.** Cadmium accumulation and in vitro analysis of calcium and cadmium transport functions in the gastro-intestinal tract of trout following chronic dietary cadmium and calcium feeding. *Comp. Biochem. Physiol. C.* 150 pp: 349-360.

- **Leonard, E.M., Nadella, S.R., Bucking, C., Wood, C.M. 2009.** Characterization of dietary Ni uptake in the rainbow trout, *Oncorhynchus mykiss*. *Aquat. Toxicol.* 95 pp: 205-216.

- **Mirza, R. S., Green, W.R., Connor,S., Weeks,A.C., Wood, C.M., Pyle, G.G. 2009.** Do you smell what I smell? Olfactory impairment in wild yellow perch from metal-contaminated waters. *Ecotoxicol.and Environ. Safety.* 72 pp: 677-683.

- **Leonard, E. M., Pierce, L. M., Gillis, P. L., Wood, C.M., O'Donnell, M. J. 2009.** Cadmium transport by the gut and Malpighian tubules of *Chironomus riparius*. *Aquat. Toxicol.* 92 pp: 179-186.

- **Kozlova, T., Wood, C.M., McGeer, J.C. 2009.** The effect of water chemistry on the acute toxicity of nickel to the cladoceran *Daphnia pulex* and the development of a Biotic Ligand Model. *Aquat.Toxicol.* 91 pp: 221-228.

- **Craig, P.M., Galus,M., Wood,C.M., McClelland, G.B. 2009.** Dietary iron alters waterborne-copper induced gene expression in softwater-acclimated zebrafish (*Danio rerio*). *Am. J. Physiol. R.* 296 pp: R362-R373.

- **Ng, T., Klinck, J., Wood, C.M. 2009.** Does dietary Ca protect against toxicity of a low dietborne Cd exposure to the rainbow trout? *Aquat. Toxicol.* 91 pp: 75-86.

- **Galvez, F., Donini, A., Smith, S., O'Donnell, M., Wood, C.M. 2009.** A matter of potential concern: Natural organic matter alters the electrical properties of fish gills. *Env. Sci. Tech.* 42 pp: 9385-9390.

- **Nadella, S. R., Fitzpatrick, J.L, Franklin, N., Bucking, C.P., Smith, S., Wood, C.M. 2009.** Toxicity of dissolved Cu, Zn, Ni and Cd to developing embryos of the blue mussel (*Mytilus trossolus*) and the protective effect of dissolved organic carbon. *Comp. Biochem. Physiol. C.* 149 pp: 340-348.

- **Ojo A.A., Nadella, S.R., Wood C.M. 2009.** *In vitro* examination of interactions between copper and zinc uptake via the gastro-intestinal tract of the rainbow trout (*Oncorhynchus mykiss*). *Arch. Environ. Contam. Toxicol.* 56 pp: 244-252.

- **Bury, N., McGeer, J.C. 2009.** The effect of inorganic contaminants and cyanobacterial toxins on cation homeostasis in freshwater fish. pg.181-203. *in* Essential Reviews in Experimental Biology Vol 1. Osmoregulation and ion transport: integrating physiology, molecular and environmental biology and molecular aspects. *Ed*. Handy, R., N. Bury and G. Flik. SEB Press. London. UK.

- **Clifford, M., McGeer, J.C. 2009.** Development of a biotic ligand model for the acute toxicity of zinc to *Daphnia pulex* in soft water. *Aquat Toxicol.* 91: 26-32.

# ***Research Highlights***

*This issue will highlight research conducted by Erin Leonard (Ph.D. student at McMaster University under the supervision of Chris M Wood) during a visit to the laboratory of Dr. Adalto Bianchini at the Federal University of Rio Grande, Brazil . This work is currently being written up for publication..*

## Evaluating the effects of salinity on acute and chronic Ni toxicity and bioaccumulation in the euryhaline crustacean *Litopenaeus vannamei*

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### Introduction

Current nickel (Ni) toxicity data are less extensive than for other divalent cationic metals (Wu et al., 2003), especially in marine environments. Anthropogenic Ni entry into the marine aquatic environment can occur via coastal mining effluent, sewage disposal, atmospheric deposition and marine mining and drilling (Bryan, 1984). Ni levels can reach 0.04 µmol $l^{-1}$ in the open ocean and as high as 1.4 µmol $l^{-1}$ in coastal and estuarine environments where the confined space causes greater Ni accumulation (Boyden, 1975).

In general, salinity is thought to act protectively against the toxicity of nickel and other metals (Eisler, 1998). Explanations include the increased presence of complexing anions such as $Cl^-$ and $OH^-$ (Sadiq, 1989) as well increased competition at the biotic ligand by cations such as $Na^+$, $Mg^{2+}$ and $Ca^{2+}$ (Paquin et al., 2000; Bianchini et al., 2002; Janssen et al., 2003). Euryhaline species are good model organisms to assess the influence of salinity on Ni toxicity. The euryhaline crustacean, the white shrimp, *Litopenaeus vannamei*, lives in tropical and subtropical estuaries, salt marshes and open oceans in the eastern Pacific, from Sonora in Mexico to northern Peru. This species is required to iono- and osmo-regulate in environments where salinity fluctuates (Sowers et al., 2006; Holthuis, 1980). Specifically, *L. vannamei* is an osmoconformer at the isosmotic concentration between 20 and 30 ppt, and hyperosmoregulates at salinities <20 ppt (Lin et al., 2000). In general, organisms are more sensitive to metal stress when they hyperosmoregulating rather than when they are closer to their isomotic point (Henry and Cameron, 1982).

Our research aimed to identify the relationship between salinity and Ni toxicity (both acute and chronic) in the euryhaline crustacean, *L. vannamei*. More specifically: (i) What are the acute and chronic LC50 values for Ni at different salinities (5 and 25 ppt). (ii) Can differences in LC50 be correlated to Ni speciation within the water column? (iii) Can we relate Ni bioaccumulation to toxicity looking at acute (96-h) and chronic (30-day) patterns, and define a Critical Tissue Residue value for Ni in this species in marine and estuarine environments? (iv) What can bioaccumulation factors (BCFs) tell us about Ni regulation? (v)

How does acute and chronic Ni exposure affect essential ion homeostasis?

*Table 1 (A) Acute (96-h) and (B) Chronic (30-d) LC50 values for waterborne Ni toxicity in L. vannamei (n=7 per treatment, with 3 replicates) acclimated to 5 ppt and 25 ppt. 95% confidence intervals are presented in brackets in µmol $l^{-1}$. No significant difference in LC50 values when assessing the nominal, total, dissolved and ionic fractions of Ni. *Indicates a significant difference in LC50 values between the two salinities.*

A

| Salinity | Dissolved | Free ion | Activity |
|---|---|---|---|
| 5 ppt | 41<br>(25-66) | 40<br>(24-65) | 15<br>(9.1-25) |
| 25 ppt | 362*<br>(254-508) | 314*<br>(220-441) | 135*<br>(95-186) |

B

| Salinity | Dissolved | Free ion | Activity |
|---|---|---|---|
| 5 ppt | 2.7<br>(1.7-4.0) | 2.7<br>(1.7-3.9) | 1.0<br>(0.7-1.5) |
| 25 ppt | 7.6<br>(1.7-25) | 6.5<br>(1.8-19) | 1.9<br>(0.5-5.4) |

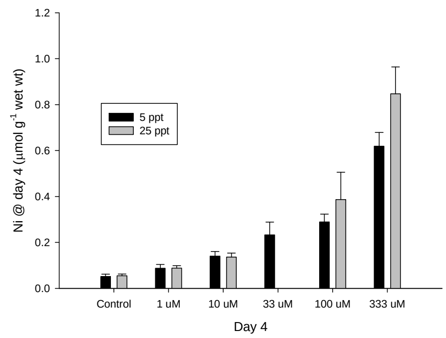
## Results

Acute (96-h) LC50 values were approximately 9 x greater in 25 ppt vs. 5 ppt (362 µmol $l^{-1}$ and 41 µmol $l^{-1}$ as dissolved Ni respectively; Table 1). This trend remained consistent when considering the nominal, total, dissolved, free ion and active fractions of the metal (Tables 1). In contrast, chronic (30-d) LC50 values were not significantly different between 25 ppt (7.6 µmol $l^{-1}$) and 5 ppt (2.7 µmol $l^{-1}$), and this consistency was independent of the Ni fraction being examined (Tables 1). Note that chronic LC50 values were 15 x and 50 x lower than acute LC50 values at 5 and 25 ppt, respectively (Table 1).

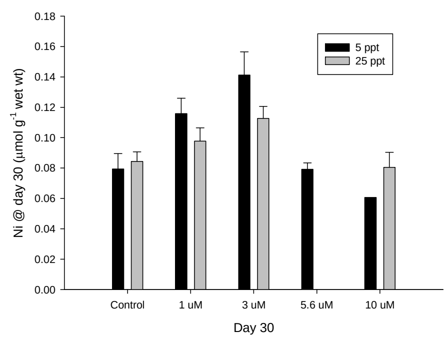
Acute Ni bioaccumulation (96-h whole body burdens) demonstrated that as the concentration of Ni increased in the exposure medium, more Ni accumulated within the organism; however, there were no significant differences in Ni body burden within a concentration between the two salinities (Figure 1 A). In contrast, in surviving animals at 30 days, chronic Ni bioaccumulation was independent of exposure concentration and salinity, averaging approximately 0.1 µmol $g^{-1}$ wet wt, the same value as in non-exposed control animals (Figure 1 B).

*Figure 1 Concentration dependent Ni uptake in L. vannamei over a range of exposure concentrations from 1 to 333 µmol Ni $l^{-1}$ on day 4 (A) and day 30 (B).Note the difference in scales between (A) and (B). Values are means ± S.E.M.; n = 5 per treatment (acute) and n = 7-16 per treatment (chronic). There is no significant difference between 5 and 25 ppt within a concentration.*

A Acute




B Chronic




Acute BCFs were 40 – 60 l kg$^{-1}$ wet wt. at 1 µmol $l^{-1}$, but at 10 µmol $l^{-1}$ significantly decreased by 85% and 80% in 5 ppt and 25 ppt, respectively. Between 10 and 333 µmol $l^{-1}$, acute BCFs did not change significantly, averaging approximately 5.1 l kg$^{-1}$ wet wt., and appeared to be independent of salinity (Figure 2 A). Chronic BCFs in surviving animals at 30-d exhibited similar patterns to acute BCFs, and were again independent of salinity (Figure 2 B).

There were no significant differences in whole body Ca or Na concentrations with increased Ni concentrations at either 5 or 25 ppt (figures not included). However, at 5 ppt, whole body Mg significantly decreased by 60% and 65% in the highest Ni exposure concentrations (333 µmol Ni $l^{-1}$ and 10 µmol Ni $l^{-1}$) on days 4 and 15, respectively (Figure 3 A- only day 4 represented here). At 25 ppt on day 4, there was a significant 70% decrease in whole body Mg in the 10 µmol Ni $l^{-1}$ exposure (Figure 3 B). All significant decreases in Mg can be directly correlated with significant increases in whole body Ni (Figure 3 A and B).

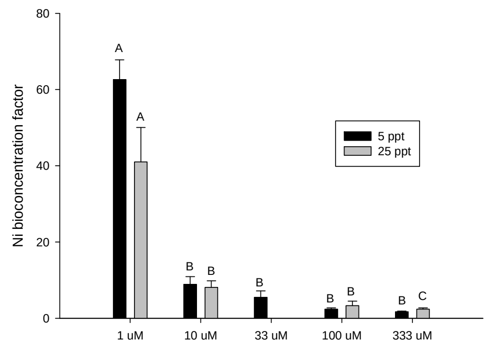
## Discussion

Salinity acts protectively against acute Ni toxicity (Table 1). Speciation analysis demonstrated that the greater presence of anions such as $Cl^-$ and $OH^-$ at 25 ppt vs. 5 ppt had only a small effect in reducing the free $Ni^{2+}$ ion component (Table 1), so complexation was probably of minor influence. However, greater cation competition at 25 ppt (by higher concentrations $Na^+$, $Ca^{2+}$, and particularly $Mg^{2+}$ - see below) could have been a protective factor. However, differences in the physiology of the organism at the two salinities was likely the more important factor. Organisms close to their isosmotic point, like *L. vannamei* at 25 ppt, where they are osmoconforming, are the most tolerant to metal toxicity (Henry and Cameron, 1982; Sprague, 1984). At lower salinities such as 5 ppt, this shrimp must hyper-osmoregulate by active transport processes (Lin et al., 2000) that may be sensitive to metal toxicity.

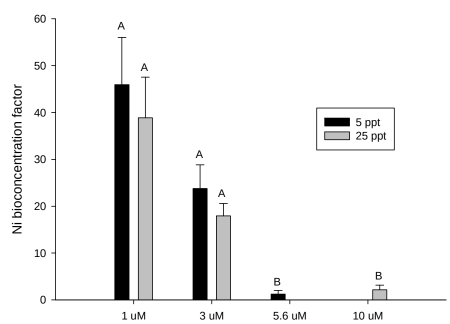
However, after 30-d exposure, salinity no longer affected Ni toxicity (Table 1), suggesting that water chemistry as well as the osmoregulatory strategy of *L. vannamei* at the two different salinities do not influence chronic toxicity. The much lower chronic LC50's, together with very different Ni bioaccumulation patterns, suggest that acute and chronic toxicity mechanisms may be different. In both cases, differences in acute or chronic LC50 values cannot be correlated to Ni speciation within the water column. Therefore, all fractions of the metal equally predict metal bioavailability (Campbell, 1995; Morel et al., 1973).

*Figure 2 Bioconcentration factor (BCF = concentration of Ni in organism expressed in µmol kg$^{-1}$ wet wt/Ni concentration in exposure medium expressed in µmol l$^{-1}$) in L. vannamei at day 4 (A) and 30 (B). Values are means ± S.E.M.; n = 5-16 per treatment. Different letters denote significant differences in bioconcentration factors.*

A Acute




B Chronic



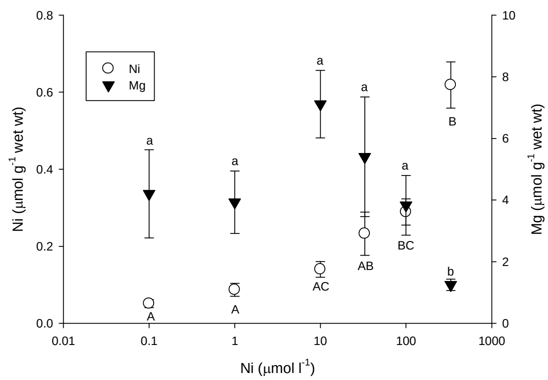

The Criterion Maximum Concentration (CMC – i.e. acute criterion) and Criterion Continuous Concentration (CCC – i.e. chronic criterion) in seawater outlined by the U.S. EPA are 1.3 µmol $l^{-1}$ and 0.14 µmol $l^{-1}$, respectively. The Canadian Water Quality Guideline (chronic) by the CCME is 0.4 µmol Ni $l^{-1}$. Therefore, *L. vannamei* would be protected in both the U.S. and Canada against acute and chronic Ni toxicity (Table 1), though the margin of protection is considerably lower at 5 ppt than at 25 ppt.

The significant difference between acute LC50 values at 5 and 25 ppt (Table 1) cannot be explained by a difference in tissue Ni burden (Figure 1A, 3A,B). However, one possible mechanism of acute toxicity may be the perturbation of the essential ion, Mg. All significant decreases in whole body Mg can be correlated with significant increases in whole body Ni at both salinities. However, the concentration-dependent patterns are rather similar at the two salinities (Figure 3A,B), so again this cannot explain differential toxicity. Historically, Mg is recognized as a specific Ni antagonist in physiological as well as toxicological studies (Pane et al., 2003a,b). The existence of a shared uptake pathway for Mg and Ni is supported by the similar dehydrated ionic radii of Ni and Mg (Weast, 1973). In freshwater systems, chronic Ni exposure is also reported to reduce whole body Mg concentration and unidirectional Mg uptake rate in *Daphnia magna* (Pane et al., 2003a,b), and elevated Mg reduced the unidirectional uptake of Ni across the G.I. tract in *Oncorhynchus mykiss* (Leonard et al., 2009). Additionally, Ni has been implicated as a competitive inhibitor of Mg uptake via three different types of Mg transporters in the prokaryote, *Salmonella typhimurium* (Snavely et al., 1991). Therefore Ni may be replacing Mg at binding sites, thereby contributing to acute Ni toxicity.

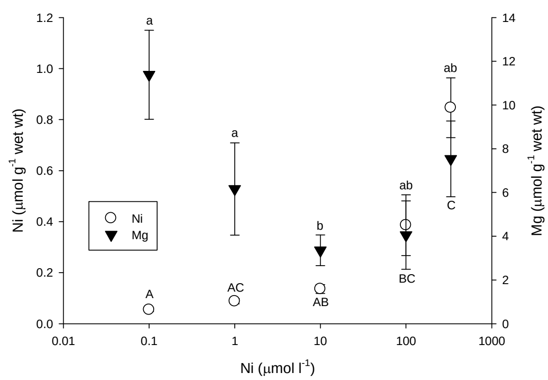
Chronically Ni bioaccumulation appears to be well regulated in that there are only slight variations in Ni whole body burden of the surviving shrimp among concentrations (nominal values: control, 1, 3 and 5.6 µmol $l^{-1}$ for 5 ppt and control, 1, 3 and 10 µmol $l^{-1}$ for 25 ppt; Figure 1B). Total Ni concentrations were in good agreement with previous studies of decapod crustaceans (Mwangi and Alikhan, 1993; Khan and Nugegoda, 2003), including *L. vannamei* (Nunez-Nogueira and Botello, 2007), which shows the capacity to maintain minimal levels of metals in their bodies. This Ni body burden of ~0.1 µmol $g^{-1}$ wet wt. for chronic exposures may define a Critical Tissue Residue Threshold for Ni in this species. Elevations above this level resulted in high mortality over 30 days.

*Figure 3 Whole body Ni and Mg plotted against Ni exposure concentrations on day 4 at (A) 5 ppt and (B) 25 ppt. Values are means ± S.E.M.; N=5 per treatment. Different letters denote significant differences in whole body ion. Capital letters denote Ni and lower case letters denote Mg.*

A




B




Acute and chronic bioconcentration factors followed a similar pattern where there was an inverse relationship between BCFs and water concentrations, which suggests that at lower environmentally relevant exposures, Ni is actively being taken up by the organism to meet metabolic needs (Philips and Rainbow, 1989), but at higher toxic concentrations, internal Ni concentrations are being regulated and therefore are not allowed to increase in proportion to waterborne Ni levels. An earlier study suggested that BCF patterns appear to differ between essential and non-essential metals, where for essential metals, BCFs decrease at higher water concentrations and for non-essential metals, BCFs remain constant over all water concentrations (Radenac et al., 2001). Potentially, this therefore provides further evidence for the essentiality of Ni (Muyssen et al., 2004). However, more recent meta-analyses show that an inverse relationship is characteristic of all metals, essential or non-essential (McGeer et al., 2003; DeForest et al., 2007). Therefore, Ni BCF patterns in *L. vannamei* are following the general pattern for all metals.

## Conclusions

Salinity acts protectively against acute Ni toxicity, at least in part due to the organism's proximity to its isosmotic point. All fractions of the metal appear to equally predict metal bioavailability. Salinity does not affect chronic Ni toxicity, suggesting that water chemistry as well as the osmoregulatory strategy of *L. vannamei* no longer influence toxicity. Chronic LC50's are much lower than acute LC50's and Ni bioaccumulation patterns are very different, indicating that acute and chronic toxicity mechanisms may differ. Assessing chronic (30-d) Ni bioaccumulation patterns, *L. vannamei* regulates Ni at ~ 0.1 µmol $g^{-1}$ wet wt. Body burdens above this concentration eventually lead to death, therefore defining a Critical Tissue Residue threshold value for Ni in this species for marine and estuarine environments. Salinity-dependent differences in acute Ni toxicity cannot be explained by Ni bioaccumulation; however, an acute mechanism of toxicity appears to be interference with Mg regulation. BCF values for Ni in shrimp acclimated to 5 and 25 ppt follow the general pattern for metals as described in McGeer et al. (2003). This species is protected by the U.S. EPA and Canadian Water Quality Criteria/Guidelines.

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