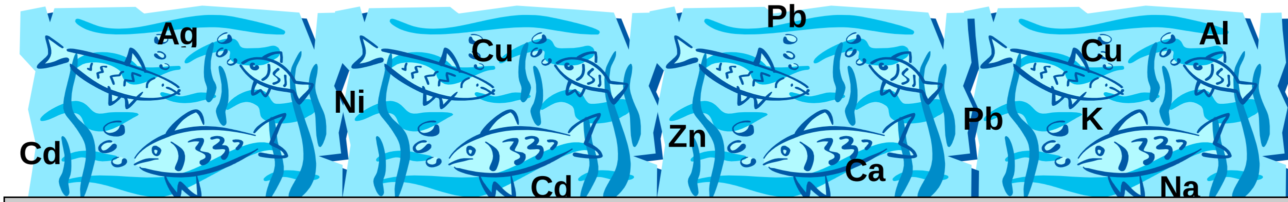
NSERC – Industry Project on Metal Bioavailability
Research Newsletter



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NEWS

The aim of this newsletter is to report on people and the projects they are working on. The star & scholar of this newsletter edition is none other than **my good self**. I shall be treating you to a main course of Ni toxicity to mysids, with a side of DOC concentration and source effects. Please over indulge and come back for seconds.

Comings and goings (Nov. 2012 – Nov. 2013):

In the Smith lab, **Mona Ashammari** has started her M.Sc. working on silver ion selective electrode titrations of natural organic matter. **Rabia Nasir** has now completed her M.Sc. (Congrats!!) and has officially joined the dark side. For the summer, Rabi's going to study the transformation of silver nanoparticles in simulated wastewater. **James Mori** (B.Sc.) will be in Smith lab this summer doing Ni titrations and measuring binding by changes in the intrinsic fluorescence of natural organic matter. **Catherine Lambert** (B.Sc.) will be volunteering in the lab to do *Mytilus* toxicity tests with Pb, Zn and Ni in the presence of organic matter from variable sources. **Kelly Livingston** will be finishing 6 months of work in Smith's lab on AGNES method to determine Zn speciation as well as Zn hydra toxicity testing. **Gaganprit Gill** has finished her B.Sc. honours thesis on making Ni ion selective electrodes for use in salt water.

In the McGeer lab studies in Cu, Ni and Zn impacts in estuarine environments include **Rabia**'s work with mysids (M.Sc. completed, as mentioned above) and **James Duncan**'s B.Sc. project developing short term tests (48-h) with the hyroid *Eudendrium carneum*. James' B.Sc. focused on salinity and DOC influences on Cu toxicity and he will be continuing his work this coming summer with the able assistance of **Emily Newman**. **Alyssa Verdin** (B.Sc.), **Che Lu** (M.Sc.) and **Oliver Vukov** (M.Sc.) are working on different aspects of rare earth metal toxicity (Ce, Sm and Dy) using biotic ligand approaches to study the influence of toxicity modifying factors and they have been joined by **Alex Loveridge** who will tackle mixture effects (Ce and Tm). DOM and the variability within invert species are incorporated in the work. **Prachi Deshpande** (M.Sc.) is continuing her investigations of Cu and Ni mixture effect in daphnia and amphipods. The context of her work is the recovery metal impacted ecosystems in Sudbury (collaboration with Gunn and Yan at Laurentian and York U respectively) and new additions this spring include **Stephanie Kaye** & **Craig Johnson** (summer RAs) and **Dr. Nadine Taylor** (Postdoc). Nadine comes from Mark Viant's lab at the U. of Birmingham and will be developing metabolomic approaches to characterize the effects of Cu and Ni mixtures in Daphnia. On the fish side of metals research **Wes Truong** is studying the chronic sublethal effects (subcellular distribution, ROS & swim capacity) of Cu and Ag mixtures to trout.

Across yonder In the Wood lab, **Michael Lim** (B.Sc.) who was mentored by our Ph.D. student, **Alex Zimmer**, has finished his 4th year thesis project in April, entitled "Investigating Rhesus proteins as a possible target for waterborne copper toxicity in rainbow trout". Good job! **Margaret Tellis**, our research assistant completed her multi-metal project at the end of April and is actively looking for opportunities in the environmental sector. Her project characterized interactions of metal mixtures in juvenile rainbow trout by using multi-metal toxicity modeling. Specifically, she has looked at effects of different metals on the uptake curves of Cd, Zn, Ni, Pb, Cu and Ag at the gills. It is a tremendous amount of work and time commitment, thanks Margaret for your great contribution on the metal research! **Tania Ng**, a former postdoctoral fellow on metal research, working as the research and administrative assistant for Chris Wood since 2013, has taken a research position in the Department of Family Medicine, McMaster University from mid-May. She completed the second part of the dietary Pb project originally started by Derek Alsop. This investigated the interaction of waterborne and dietborne Pb toxicity in rainbow trout. She has decided to explore new career opportunities in clinical research. Good luck! **Tamzin Blewett**, Ph.D. student of Chris received a travel fellowship from the Journal of Experimental Biology valued at $2,500 (British pounds), as well as $19,700 (New Zealand dollars) from the Brian Mason Trust Grant, to conduct research in New Zealand from January to May. Congratulations! She worked on the effect of Ni toxicity and DOC on the embryonic development of the sea urchin, kina (*Evechinus chloroticus*) in the lab of **Dr. Chris Glover** at University of Canterbury, New Zealand. Her second project in New Zealand examined the mechanisms of Ni uptake and toxicity in the euryhaline fish, inanga (*Galaxias maculatus*) which is declining in New Zealand; one theory is that this is due to metal pollution in streams. Chris Wood and new Ph.D. student **Marina Giacomin** recently returned from a 1-month visit to the Amazon Research Institute (INPA), Manaus, Brazil, where they started a new research project on dietary metal toxicity to a model tropical teleost, the tambaqui, in collaboration with Ph.D. student **Gisele Cortez** and INPA Director **Adalberto Val**.

In the McClelland lab, **Sheridan Baker** successfully completed his undergraduate project "The effects of 96-hour aquatic copper exposure on the acute ventilatory drive in freshwater acclimated killifish (*Fundulus heteroclitus*)". Sheridan will be starting a M.Sc. project in the McClelland lab in May to follow up in this work. **Adam Kulesza** joins the lab as a summer student funded by the NSERC USRA program to study the effects of Cu on whole animal and cellular respiration. He will stay to do an undergraduate thesis in the fall.

Smith and McGeer are planning an intensive DOC sampling effort this summer. Samples (as RO concentrates as well as ‘grab samples’) will be collected from a variety of sources in Southern Ontario (autochthonous, terrigenous, sewage). These samples will be utilized for characterization of speciation and toxicity mitigation for projects on rare earth elements as well as Cu, Zn, Ni, Pb and Cd. Logistics are currently being worked out for sampling plans in the North West Territories as well to address questions around potential differences between ‘Northern’ and ‘Southern’ DOC.

New Funding:

- Unilever Canada is supporting a two year project in **Smith's lab** investigating Ag binding to dissolved organic matter with an emphasis on trying to understand the role of reduced sulfur. This project is in collaboration with WCA environment (Graham Merrington, Adam Peters, Peter Simpson, Iain Wilson) as well as Steve Lofts (NERC).
- **McClelland's** NSERC Discovery grant has been renewed for another 5 years. He also received an Accelerator Supplement award for the next 3 years.
- **McClelland** also received a CFI-JELP grant to construct "A Facility for Multi-stressor Biology on Aquatic Organisms". This will greatly increase McMaster's capacity for studying environmental physiology by providing the ability to control multiple water quality variables at the tank level.

- **McGeer** will receive funding for a subproject within the project "A watershed approach to monitoring cumulative impacts of landscape change" which his funded through the NWT Cummulative Impacts Monitoring Program (CIMP). The focus of the project is the impact of suspended solids on fish in northern rivers, associated with thermokarst "mega-slump" activity induced by climate change (M.Sc. student Tyler Weinhardt).

Upcoming presentations at meetings:

- Wood, C. M., McGeer, J. C., Smith, D. S. Brix, K., Cooper, C. A. and Blewett, T. A. will be presenting at the Aquatic Toxicity Symposium (ATS) meeting, June 2014, Fort Worden State Park, Washington. In sessions on physiology, marine metals and metal mixtures. ATS is sponsored by ICA, IZA, NiPERA, MWH, and Rio Tinto.

- Merrington, G., Peters, A., Smith, S., Lofts, S., van Egmond, R. and Alsham-mari, M. Understanding the chemical speciation of silver from the use of personal care products in aquatic freshwater systems. SETAC Asia Pacific, September 2014. Adelaide, South Australia (poster).

- Settimio, L., McLaughlin, M., Kirby, J., Langdon, K. and Smith, D. S. A multidisciplinary approach to determining complexed Ag in soil water extracts. SETAC Asia/Pacific, September 2014. Adelaide, South Australia.

- Blewett, T., Glover, C., Niyogi, S. and Wood, C. M. Epithelial transport of trace metals in pacific hagfish. International Congress on the Biology of Fish, August 2014, Edinburgh, Scotland, the United Kingdom.

- Blewett, T., Ransberry, V., McClelland, G. and Wood, C. M. The effect of salinity on the mechanisms of Ni toxicity in the euryhaline Atlantic killifish. International Congress on the Biology of Fish, August 2014, Edinburgh, Scotland, the United Kingdom (poster).

The following peer reviewed papers and book chapters were published by the Metals Bioavailability Group (Nov. 2013 – May 2014):

- Birceanu, O., Sorensen, L., Henry, M., McClelland, G.B., Yang, Y.S. and Wilkie, M.P. (2014). The effects of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) on fuel stores and ion balance in a non-target fish, the Rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. [C] 160:30-41.

- Cooper, C.A., Tait, T., Gray, H., Cimprich, G., Santore, R., McGeer, J.C., Wood, C.M. and Smith, D.S. (2014). Influence of salinity and dissolved organic carbon on acute Cu toxicity to the rotifer *Brachionus plicatilis*. Environ. Sci. Technol. 48:1213-1221.

- Giacomin, M., Gillis, P.L., Bianchini, A. and Wood, C.M. (2013). Interactive effects of copper and dissolved organic matter on sodium uptake, copper bioaccumulation, and oxidative stress in juvenile freshwater mussels (*Lampsilis siliquoidea*). Aquat. Toxicol. 144-145:105-115.

- Khan, F.R. and McGeer J.C. (2013). Zn-stimulated mucus secretion in the rainbow trout (*Oncorhynchus mykiss*) intestine inhibits Cd accumulation and Cd-induced lipid peroxidation. Aquat. Toxicol. 142:17-25.

- Leonard, E.M., Banerjee, U., D'Silva J.J. and Wood, C.M. (2014). Chronic nickel bioaccumulation and sub-cellular fractionation in two freshwater teleosts, the round goby and the rainbow trout, exposed simultaneously to waterborne and dietborne nickel. Aquat. Toxicol. In Press.
- Leonard, E.M., Marentette, J.R., Balshine, S. and Wood, C.M. (2014). Critical body residues, Michaelis-Menten analysis of bioaccumulation, lethality and behaviour as endpoints of waterborne Ni toxicity in two teleosts. Ecotoxicol. 23:147-162.
- Loro V.L., Nogueira L., Nadella S.R., and Wood C.M. (2014). Zinc bioaccumulation and ionoregulatory impacts in *Fundulus heteroclitus* exposed to sublethal waterborne zinc at different salinities. Comp. Biochem. Physiol. [C] (In Press).
- McClelland, G.B. and Scott, G.R. (2013). Muscle Plasticity. In: Evans, D.H., Claiborne, J.B., and Currie, S (eds.). The Physiology of Fishes, 4th ed. CRC press, Baton Raton, Fl.
- Ransberry, V.E., Blewett, T. and McClelland, G.B. (2014). The oxidative stress response in freshwater-adapted killifish, *Fundulus heteroclitus* to acute copper and hypoxia exposure. Comp. Biochem. Physiol. [C] (In Press).
- Sandhu, N., McGeer, J.C. and Vijayan, M. (2014). Exposure to environmental levels of waterborne cadmium impacts corticosteroidogenic and metabolic capacities, and compromises secondary stressor performance in rainbow trout. Aquat. Toxicol. 146:20-27.
- Tellis M.S., Lauer M.M., Nadella S.R., Bianchini A. and Wood C.M. (2014). The effects of Cu and Ni on the early embryonic life stages of development in the purple sea urchin (*Strongylocentrotus purpuratus*). Arch. Environ. Contam. Toxicol. (In Press).
- Tellis, M.S., Lauer, M.M., Nadella, S., Bianchini, A. and Wood, C.M. (2013). Sublethal mechanisms of Pb and Zn toxicity to the purple sea urchin (*Strongylocentrotus purpuratus*) during early development. Aquat. Toxicol. 146:220-229.
- Zimmer, A.M., Brauner, C.J. and Wood, C.M. (2014). Exposure to waterborne Cu inhibits cutaneous Na^+ uptake in post-hatch larval rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 150:151-158.

The following platforms and posters were presented by the Metals Bioavailability Group (Nov. 2013 – May 2014):

- Blewett, T.A., Wood, C.M. and Glover, C. What's up DOC? Protective effects of dissolved organic carbon against nickel toxicity to sea urchin early life-stages. Canadian Society of Zoologists Annual Meeting, May 2014, Montreal, Quebec, Canada (oral).
- Chen, W., Guéguen, C., Smith, D.S., Galceren, J., Puy, J. and Company, E. Study of Zn speciation in presence of dissolved organic matter (DOM) using combination of Absence of Gradient and Nernstian Equilibrium (AGNES), potentiometry, and fluorometry with application of Non-ideal Competitive Adsorption Model (NICA) and Conditional Affinity Spectrum (CAS). Environ. Analysis Conference, September 2013. Toronto, Ontario, Canada (oral).
- Clement, A. and McGeer. J.C. The influence of chronic copper exposure on the uptake and effects of silver in rainbow trout (*Oncorhynchus mykiss*). Ontario Biology Day, March 2014, U of Toronto, Mississauga, Canada (oral).

- Cooper, C.A., Nasir, R., McGeer, J.C. and Smith, D.S. Influence of DOC concentration and source on chronic Ni toxicity to the mysid *Americamysiss bahia*. Environmental Science and Engineering Conference. Feb. 2014. Gananoque, Ontario, Canada (poster).

- Cooper, C.A., Nasir, R., McGeer, J.C. and Smith, D.S. Influence of DOC concentrations and source on chronic Ni toxicity to the mysid *Americamysis bahia*. SETAC N. Am., November 2013. Nashville, Tennessee, USA (oral).

- Costa, E-J. and McGeer J.C. The acute and chronic toxicity of three forms of nanoparticle silver and silver nitrate to *Daphnia pul*ex: is uptake and toxicity related to particle dissolution? 40th Annual Aquatic Toxicity Workshop. October 2013. Moncton, New Brunswick, Canada. (Oral presentation awarded the Dr. Rick Playle Award for Outstanding MSc Thesis in Aquatic Toxicology).

- Deshpande, P. and McGeer, J.C. Nickel and copper mixture toxicity to Daphnia pulex-pulicaria in soft water. Environmental Science and Engineering Conference. Feb. 2014. Gananoque, Ontario, Canada (oral).

- Deshpande, P. and McGeer, J.C. Copper and nickel mixture toxicity to sensitive aquatic invertebrates. Canadian Society of Zoologists Annual Meeting, May 2014, Montreal, Quebec, Canada (oral).

- Duncan, J. and McGeer, J.C. The effects of salinity and dissolved organic matter on Cu toxicity to a euryhaline hyroid (*Eudendrium carneum*). Ontario Biology Day, March 2014, U of Toronto, Mississauga, Canada (oral).

- Lim, M., Zimmer, A. and Wood, C.M. Waterborne copper alongside high external ammonia exposure suggest sub-lethal copper induced inhibition of ammonia transport via Rhesus proteins in rainbow trout (*Oncorhynchus mykiss*). 23rd Annual Comparative Physiology and Biochemistry Workshop, Feb. 2014. Elmhirst's Resort, Keene, Ontario, Canada (oral).

- Livingstone, K., Smith, D.S. and McGeer J.C. Does ecosystem disturbance alter the capacity of dissolved organic matter to mitigate the toxicity of Cu to *Hyalella azteca*? 40th Annual Aquatic Toxicity Workshop. October 2013. Moncton, New Brunswick, Canada (oral).

- McGeer, J.C., Nasir, R., Duncan, J., Cunningham, J. and Smith, D.S. Salinity and dissolved organic matter mitigation of Cu toxicity in estuarine environments. SETAC N. Am., November 2013. Nashville, Tennessee, USA (poster).

- Nasir, R. and McGeer. J.C. Cu toxicity to *Americamysis bahia* in estuarine environments: effects on growth, survival and sexual maturation. Environmental Science and Engineering Conference. Feb. 2014. Gananoque, Ontario, Canada (oral).

- Nasir, R., Cunningham, J., Smith, D.S. and McGeer, J.C. Mitigating effects of dissolved organic matter on Cu toxicity to *Americamysis bahia* in estuarine waters. SETAC N. Am., November 2013. Nashville, Tennessee, USA (poster).

- Peters, A., Merrington, P., Simpson, A., Smith, D.S., Lofts, S. and van Egmond, R. Understanding the chemical speciation of silver in aquatic freshwater systems. SETAC Europe, May 2014. Basel, Switzerland (poster).

- Smith, D.S., Tait, T., Ashoka, M. and McGeer, J.C. Utility of fluorescence quenching to determine metal (Cu, Zn, Pb, Ni) speciation for saltwater solutions containing dissolved organic matter. SETAC N. Am., November 2013. Nashville, Tennessee, USA (poster).

- Smith, D.S., Tait, T., Cooper, C.A., Cimprich, G., Chen, W., Guéguen, C. and McGeer, J.C. Free ion (Cu^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}) measurements for predicting metal bioavailability to saltwater invertebrates at various DOC and salinity conditions. SETAC N. Am., November 2013. Nashville, Tennessee, USA (poster).

- Smith, D.S., Vukov, O., Nasir, R., Cunningham, J., Chowdury, J. and McGeer, J.C. Impacts of waterborne Pb to *Americamysis bahia* in a chronic 30 day flow-through exposure including a detailed investigation of Pb analytical methods for saltwater samples. SETAC N. Am., November 2013. Nashville, Tennessee, USA (oral).

- Verdin, A.J. and McGeer, J.C. The effects of toxicity modifying factors on the acute toxicity of samarium to *Hyalella azteca*. Ontario Biology Day, March 2014, U of Toronto, Mississauga, Canada (Winner, Best Oral Presentation).

- Vukov, O., Lu, C., Smith, D.S., Dixon, G. and McGeer, J.C. The effect of cationic composition on dysprosium toxicity to sensitive invertebrates. World Water Day University of Waterloo, March 2014. Waterloo, Ontario, Canada (poster).

- Wood, C.M. Regulation and toxicity of metals in aquatic ecosystems. Keynote address. Congresso Brasileiro de Toxicologia Aquatica, November 2013. Rio Grande, Brazil (oral).

- Wood C.M., Chowdhury J., Ng T.T. and Scott D.S. BLM approaches to predicting chronic copper toxicity to rainbow trout in very soft water. Canadian Society of Zoologists Annual Meeting, May 2014, Montreal, Quebec, Canada (oral).

- Zimmer, A. L. and Wood, C. M. Exposure to waterborne copper inhibits sodium uptake by the skin and gills of larval rainbow trout. Canadian Society of Zoologists Annual Meeting, May 2014, Montreal, Quebec, Canada (oral).

Research Highlight:

Influence of dissolved organic carbon concentration and source on chronic 7-day Ni toxicity to the mysid, *Americamysis bahia*

Christopher A Cooper, Rabia Nasir, James C McGeer, D Scott Smith.

Abstract

The biotic ligand model (BLM) has been successfully used to predict metal toxicity in freshwater environments. However, due the different water chemistries, metal toxicity in marine and estuarine environments can differ considerably when compared to freshwater. In addition, there is also a growing body of evidence that shows that Dissolved Organic Carbon (DOC) concentration can have a protective effect against metal toxicity and that this effect can be DOC source dependent. Therefore, chronic 7-d Ni toxicity tests were performed using sea water acclimated mysid shrimp, *Americamysis bahia*, exposed to differing [DOC] and sources (EPA approved toxicity test methods). Increasing [DOC] up to 8 mg L^{-1} elicited no significant increase in LC50 (~200 μg Ni L^{-1}) or EC10 values (30 μg Ni L^{-1} for sexual maturation). However, a [DOC] of 20 mg L^{-1} resulted in a significant increase in these chronic Ni toxicity values (LC50 of 416 μg Ni L^{-1}, and a sexual maturation EC10 of 189 μg Ni L^{-1}). There were also DOC source dependent effects. PARAFAC analysis showed that DOC sources rich in tyrosine, indicative of autochthonous origin (synthesised by biological activity within the water column), offered the greatest protection against Ni toxicity. Whereas, DOC sources rich in humic acid, indicative of allochthonous origin (terrigenously derived from the breakdown of plant material), offered the least protection against Ni toxicity. For example, the LC50 for a tyrosine rich DOC was 443 μg Ni L^{-1} compared to a humic rich DOC source LC50 value of 194 μg Ni L^{-1}. This is contrary to what is found in the literature with regards to Cu toxicity, where higher concentrations of humic acid generally elicit the highest protective effect against toxicity. Data from this research will be used in the development of a marine and estuarine BLM, and ultimately criteria for the protection of organisms in these environments.

Introduction

Nickel (Ni) is the 22nd most abundant element and is ubiquitous in marine and freshwater ecosystems. Nickel concentrations increase in aquatic systems that receive inputs from urban and industrial effluents (Pyle and Couture, 2012). At pH values common to most marine and estuarine systems, Ni speciation is dominated by Ni^{2+} with increasing hydroxide complexation with increasing pH. Under oxic conditions, most Ni is either adsorbed onto insoluble Fe or Mn oxyhydroxides, or more commonly, bound to dissolved organic matter (DOM) (Pyle and Couture, 2012).

Dissolved organic matter is quantified as dissolved organic carbon (DOC), which makes up generally about 50% by mass of these heterogeneous molecules (Wood et al., 2011). There is now a substantial body of evidence in the literature that supports the notion that DOC plays a key role in ameliorating the aquatic toxicity of many metals. Metals binds to DOC, the resulting complex is less bioavailable to organisms, hence reduced toxicity. In fact, DOC concentration is now incorporated into Biotic Ligand Models (BLM's) used to predict the site-specific toxicity of metals or to derive site-specific ambient water quality criteria (Di Toro et al., 2001; Santore et al., 2001; Paquin et al., 2002; Niyogi and Wood, 2004; Wood et al., 2011).

Natural DOCs are generally divided into two classes, allo- and autochthonous. Allochthonous DOCs are mainly humic acid based, derived from the breakdown of plant material; they tend to be optically darker and are composed of larger molecules with more aromatic rings (i.e., more phenolic groups). Whereas, autochthonous DOCs are mainly fulvic acid based, produced within lakes and rivers by algae or by microbial activity and/or photodegradation of allochthonous DOM. They are usually optically lighter and composed of smaller molecules with a lower content of aromatic ring structures (Wood et al., 2011).

The quality of DOC is commonly determined spectroscopically (Chen et al., 2002; Senesi et al., 1991; Al-Reasi et al., 2012). The UV–visible absorption properties have been utilized to detect the presence of chromophores (i.e. light absorbing moieties) of the heterogeneous DOCs. For example, the 'SAC340' (the specific absorbance coefficient at 340 nm) is considered to be a measure of aromaticity, with higher numbers relating to a higher humic content (Curtis and Schindler, 1997; Al-Reasi et al., 2012). Fluorescence spectroscopy can also reveal a number of useful measures. The 'FI' (fluorescence index: ratio of the emission intensity at a wavelength of 450 nm to that at 500 nm, both obtained at an excitation wavelength of 370 nm) can distinguish source or origin of various DOMs (McKnight et al., 2001; Al-Reasi et al., 2012). In addition, three dimensional counter plots can be generated, which are known as excitation–emission matrices (EEMs). Recent advances in data analysis of EEMs by parallel factor analysis (PARAFAC) have improved identification of the fluorescent components, and estimation of their abundance and the contribution of each one to the total fluorescence (Stedmon and Bro, 2008; Al-Reasi et al., 2012; Ishii and Boyer, 2012).

In sea water, Ni is less toxic than in fresh water, which is most likely due to the protection offered by Na^{+}, Ca^{2+}, and particularly Mg^{2+} competing for physiologically sensitive binding sites at the biological surface and the reduced bioavailability of Ni in the presence of these cations (Hall and Anderson, 1995; Pyle and Couture, 2012). However, there are relatively few sea water Ni toxicity studies. In addition, the relative effects of differing DOC concentrations, and DOC of different compositions, on Ni toxicity in marine environments is unknown. To address this, mysids (*Americamysis bahia*) were exposed to artificial seawater containing a DOC of varying concentrations, or DOCs from a variety of sources, for 7 days. *Americamysis bahia* was chosen for this study because they tolerate a wide range of salinities and are sensitive to metals (De Lisle et al., 1987; Lussier et al., 1988).

A bahia is an established indicator organism and there are standardised protocols for culturing (EPA-505/8-0-006b) and testing (EPA 712-C-96-136; EPA 1007.0) (USEPA, 1990). While many studies have looked at acute (96-h) metal toxicity to *A. bahia* (Lussier et al., 1985; Toussaint et al., 1995; Lussier et al., 1999; Hunt et al., 2002), there is a lack of data on chronic Ni toxicity. Standardised tests for assessing chronic (lethal an sub-lethal) effects on *A. bahia* include the 28-d life-cycle toxicity test (Lussier et al., 1985; Hunt et al., 2002; Ward et al., 2002) and also the 7-d growth and survival (EPA 1007.0). The latter was used in this study as it has been shown to be comparable to the longer test (Lussier et al., 1999), and there is the obvious advantage of the shorter test of being able to increase productivity over time.

Methodology

Test organism:

Test organisms (*A. bahia*, 4 d old) were obtained from Aquatic Research Organisms (Hampton, New Hampshire, USA) and acclimated to lab conditions following standard EPA method (EPA-505/8-0-006b) for 3 d in reconstituted salt water using synthetic sea salt (Kent Marine Reef Salt Mix, Big Als Canada Inc, Kitchener ON). Salinity and temperature were monitored daily using a handheld conductivity meter (YSI 30, YSI Inc., Yellow Springs) and kept constant at 25 ppt and 26 ± 0.5 ^{o}C. The mysids were fed *Artemia nauplii* (Brine Shrimp Direct, Ogden, UT) at a density of 150 artemia/day/neonate.

Toxicity method:

Seven day short-term toxicity tests were carried out to assess the short-term chronic toxicity of

Ni following standard EPA method (EPA-821/R-02-014; 1007.0). All test solutions for the DOC concentration dependent study were adjusted to 30 ppt using Kent Marine Reef Salt Mix 24 h before the test to allow solutions to reach equilibrium. For the source dependent study, the grab samples were used and salted up to 30 ppt using Kent sea salt.

Toxicity tests were static with daily renewals. The tests were carried out in crystallizing dishes (Pyrex, Fisher Scientific, Ottawa, ON) with 250 mL of test solution. Test solutions were prepared to allow for a daily test solution renewal using synthetic Kent sea salt mixed with appropriate Atomic Absorption Spectroscopy (AAS) standard Ni stock solution (TraceCERT, Sigma-Aldrich Co., Oakville, ON). Salinity, pH and temperature were monitored and recorded on a daily basis. Each test consisted of four replicates with 10 neonates per replicate and included unexposed controls.

The end points were chosen to be mortality, growth as well as a new end-point sexual maturity. Any dead individuals were removed daily. The final number of surviving mysids was used to calculate mortality LC50 values. Biomass was calculated by individually weighing the survivors on a Sartorius SE2 Ultra Microbalance (Sartorius Mechanatronics Corp., Bohemia, NY). Images of surviving mysids were taken using an inverted microscope to determine sex of the organism as well as determine a sexual maturation score. Sexual maturity in females was quantified using a brood-sac scoring system that identifies the presence of clearly distinguished gonads, testis or brood-sac. The score ranges from 0 to 5, 0 being not sexually mature and 5 representing a fully mature female with eggs in the brood pouch.

Dissolved Organic Carbon (DOC) sources, storage and quantification:

The DOC concentrate was obtained from a bog area in Kouchibouguac National Park, New Brunswick (Table 1). The DOC sample was obtained using a portable reverse osmosis unit (for details on reverse osmosis, see Sun et al. 1995). To remove potential metal contamination, the concentrates were passed through a cation exchange resin (Amberlite IR-118H, Sigma), acidified to pH 2, before being stored in the dark at 4 °C, in 4 L polyethylene bottles until used (Schwartz et al,. 2004; Winter et al., 2007).

DOC grab samples (~ 50 L) were obtained from 4 sites located along the St. Lawrence River between Québec city and Gaspé, in the province of Quebec (Table 1). As the aim was to obtain water samples that contained different types of DOC (e.g. refer to Fig. 1), sites were chosen based on a visual assessment of the local geography and on the general catchment area in which they were located (using ‘Google Maps’ ™). The idea was to obtain water samples from areas where a fresh water tributary source flowed into the St. Lawrence River channel, and the fresh water source was geographically separate from the other samples along the river channel. The [DOC] in the water samples (50 mL) were measured as stated in Cooper et al. (2014).

Dissolved Organic Carbon (DOC) absorbance and fluorescence:

All DOC samples were prepared as described previously (Al-Reasi et al., 2012; 2013). The aromatic composition of DOCs was estimated by SAC340 according to Curtis and Schindler (1997). The fluorescence index (FI) was used as an indicator of DOC origin as suggested by McKnight et al. (2001). In addition, the DOC samples were characterised via PARAFAC (Stedmon and Bro 2008) using the methods presented in DePalma et al. (2011).

Dissolved Ni measurements – voltammetry:

Water samples (10 mL) were collected on days 1, 3, 5 and 7 for Ni analysis. Samples were filtered (0.45 µm; Acrodisc HT tuffryn membranes, Pall Corp., Ann Arbor, MI) and acidified (few drops of 5 N HCl). Dissolved Ni concentrations used in the toxicity tests were determined by differential pulse anodic stripping voltammetry (DPASV) using manufacturer recommended settings from the manual (Metrohm Application Bulletin No. 231/2e). Voltammetric measurements were made with a computer controlled Autolab potentiostat/galvanostat (Eco Chemie, Metrohm) and a Metrohm 663 VA stand (Metrohm). The working electrode was a Static Mercury Drop Electrode (SMDE) and the counter electrode was made of a platinum rod (Metrohm). Analysis of peaks was done using Nova 1.7 software (Eco Chemie, Metrohm).

Statistical analyses:

The 7-d LC50 values were determined using Probit analysis and the EC10 Values were calculated with CETIS, as prescribed by the US EPA (EPA-821/R-02-014; 1007.0). If the 95% confidence limits (CL) did not overlap, the LC50 and EC10 values were considered significantly different (Fig. 3A-C) (Arnold et al., 2010). Linear regression analysis was carried to compare molecular spectroscopy with toxicity data (significant correlation if $P<0.05$; Figs. 4-7). All figures and calculations were performed using SigmaPlot version 11, Matlab, SigmaStat version 3.1 and Microsoft Excel.

DOC source	Co-ordinates	SAC_{340}	FI
Kouchibouguac	46° 49' 13" -64° 55' 5"	27.9	1.07
Rivière-au-Renard-Ouest	48° 59' 45" -64° 23' 27"	3.2	1.38
Gros-Morne	46° 15' 8" -65° 32' 1"	20.9	1.41
Rimouski	48° 26' 39" -68° 32' 27"	14.5	1.31
Rivière-du-Loup	47° 50' 50" -69° 33' 57"	56.0	1.24

Table 1. Characteristics of Kouchibouguac DOC isolate and grab samples used in the chronic toxicity tests to *A. bahia*. The concentrate was obtained via reverse osmosis, whereas the grab samples involved taking approximately 50 L from each site. All grab samples contained, on average 2.2 mg C L^{-1}, whereas, the Kouchibouguac concentrate [DOC] used in this analysis was 5.3 mg C L^{-1}. The specific absorbance coefficient (SAC340) = (2.303 X absorbance at 340 nm)/[DOC] = $(cm^2\ mg^{-1})^d$ (Curtis and Schindler 1997). The fluorescence index (FI) = emission intensity of 450 nm/emission intensity of 500 nm, both taken at excitation at 370 nm (McKnight et al. 2001).

Fluorophore (%)	Slope	Intercept	R^2	P value
Trp	-0.001	4.5	0.006	0.970
HA	-0.132	71.8	0.46	0.207
Tyr	0.148	-28.2	0.83	0.031*
FA	-0.016	51.9	0.01	0.870

Table 2. Linear regression parameters when plotting percent fluorophore [tryptophan (Trp), humic acid (HA), tyrosine (Tyr) and fulvic acid (FA)] against Ni LC50 values. An asterisk indicates a significant correlation between tyrosine and the LC50 values (see Fig. 4).

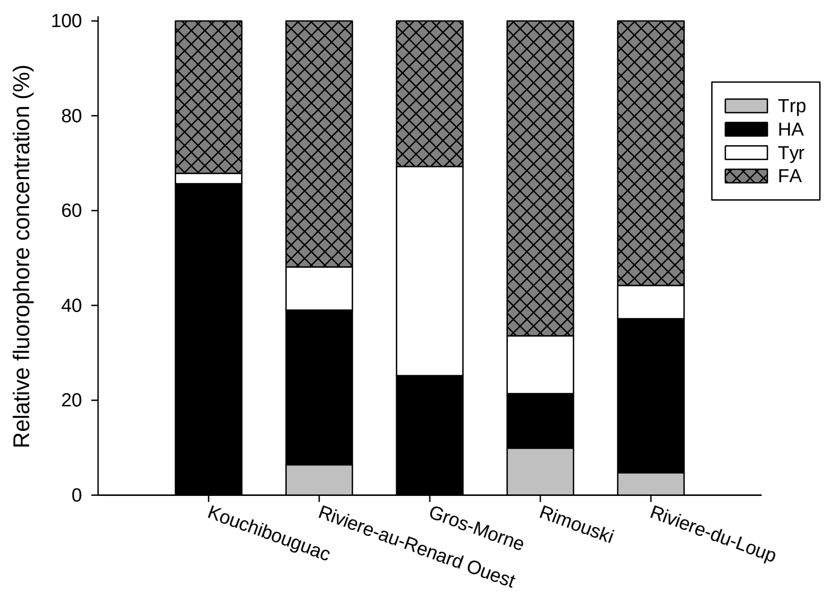

Figure 1. PARAFAC analysis of the DOC concentrate (Kouchibouguac) and grab samples. Relative percentage of tryptophan (Trp; light grey bars), humic acid (HA; black bars), tyrosine (Tyr; white bars) and fulvic acid (FA; dark grey hatched bars).

Results

Characterisation of these DOC samples via PARAFAC revealed that 66% of the Kouchibouguac DOC consisted of humic acid. Gros-Morne and Rimouski contained relatively high amounts of tyrosine (44% and 12%, respectively; Fig. 1), with the latter also rich in fulvic acid (66%; Fig. 1). Both Rivière-au-Renard-Ouest and Rivière-du-Loup DOCs looked very similar, both with relatively high levels of humic and fulvic acid, and low levels of tyrosine and tryptophan (Fig. 1).

In the DOC concentration dependent study, the 7-d chronic Ni LC50 values for *A. bahia* were not significantly different from zero added DOC to 8.2 mg L^{-1} DOC, with an average of 214 μg Ni L^{-1} (Fig. 2Ai). However, at high [DOC] (20 mg L^{-1}) the LC50 value was almost double, 416 μg Ni L^{-1} (95% C.L. 340-541 μg Ni L^{-1}) (Fig. 2Ai). A very similar, yet exaggerated trend was observed for the chronic Sexual maturity EC10 (i.e. brood sac development score; Fig. 2Bi). The sexual maturity EC10 values averaged 32 μg Ni L^{-1} for all DOC concentrations under 10 mg L^{-1}, but this average increased more than four-fold to 189 μg Ni L^{-1} (95% C.L. 98-251 μg Ni L^{-1}) with the addition of 20 mg L^{-1} DOC (Fig. 2Bi). Almost identical chronic EC10 values, from zero added DOC up to 20 mg L^{-1}, were also found for biomass (i.e. combined weight of the surviving individual *A. bahia*; Fig. 2Ci).

It is important to note, that in the DOC source dependent study, all [DOC] concentrations in the grab samples were very similar (2.2 ± 0.2 mg L^{-1} DOC) and all samples were salted up to 30 ppt using Kent Marine Reef Salt mix. The 7-d chronic Ni LC50 values were very similar when both Rivière-au-Renard-Ouest DOC and Rivière-du-Loup DOC were used, 235 and 234 μg Ni L^{-1}, respectively (Fig. 2Aii). When using Rimouski DOC, the LC50 value was higher (351 μg Ni L^{-1}), but it was not significant (compared to aforementioned sources). However, the use of Gros-Morne DOC increased the chronic Ni LC50 value to 443 μg Ni L^{-1} (95% C.L. 388-513 μg Ni L^{-1}), which was significantly higher than both Rivière-au-Renard-Ouest DOC and Rivière-du-Loup DOC, but not Rimouski DOC (Fig. 2Aii). The Sexual maturity EC10 values for both Gros-Morne DOC and Rimouski DOC were both similar, and both were significantly higher than Rivière-au-Renard-Ouest DOC and Rivière-du-Loup DOC, which were also both very similar (90 and 73 vs 7 and 17 μg Ni L^{-1}, respectively; Fig. 2Bii). Although a similar trend was observed for the biomass EC10 values, the use of Gros-Morne DOC and Rimouski DOC only yielded EC10 values that were significantly higher than Rivière-au-Renard-Ouest, not Rivière-du-Loup, which was not significantly different from the other sources (Fig. 2Cii).

The dashed line that runs across Figure 2A (mortality) is the respective LC50 value obtained by Lussier et al. (1985) for the 36-d chronic Ni *A. bahia* exposure. The dashed lines across both Figure 2B and 2C (Reproduction and Sexual maturity, respectively) is the EC10 value for reproduction from the 36-d chronic Ni *A. bahia* exposure (adapted from Lussier et al., 1985 by DeForest and Schlekat, 2013). The purpose of these dashed lines is to compare the 7-d short-term chronic test with the longer-term 36-d test, as is discussed in more detail in the 'Discussion' below.

Figure 3A shows the influence of aromaticity (SAC340, displayed in Table 1) on Ni toxicity. For 3 DOC sources (Rivière-au-Renard-Ouest [B], Rimouski [D], Gros-Morne [C]), an increase SAC340 from 3.2 to 20.9 $(cm^2\ mg^{-1})^d$ correlated to an increase in LC50 values, from 235 to 443 μg Ni L^{-1}. The other 2 DOC sources (Kouchibouguac [A], Rivière-du-Loup [E] did not follow this trend, with relatively high SAC340 values (up to $56(cm^2\ mg^{-1})^d$) not having a significant effect on mortality (Fig. 3A). The correlations between the DOC source fluorescence index (FI, Table 1) and toxicity are displayed in Figure 3B. The general trend was that as the FI increased from 1.07 to 1.41, as did the LC50 values, the only outlier appeared to be Rivière-au-Renard-Ouest [B], which had a relatively high FI of 1.38, but a comparatively low LC50 value of 235 μg Ni L^{-1} (Fig. 3B).

Linear regression analysis of the relative percent of each fluorophore (calculated by PARAFAC, Fig. 1) versus the LC50 values from each DOC source revealed one significant correlation (Fig. 4), although all parameters for each fluorophore are presented in Table 2. As the relative percent of tyrosine increased from 2.2 to 44.1%, the LC50 values increased (R^2 = 0.833; P = 0.031; Fig. 4 and Table 2).

Discussion

Ni is far less toxic in seawater than it is in freshwater, probably because of the protection conferred by Na^+, Ca^{2+}, and particularly Mg^{2+} competing for physiologically sensitive binding sites on the fish and the reduced bioavailability of Ni in the presence of these cations (Hall and Anderson, 1995). However, sea water acclimated *A. bahia* was very sensitive to Ni toxicity, which is in agreement with other literature (Lussier et al., 1985; Deforest and Schlekat, 2013). The 7-d chronic Ni LC50 values, and in particular the EC10 values (under control conditions, no added DOC), were very comparable to those found by Lussier et al. (1985). This is very encouraging, as the toxicity data from Lussier et al. (1985) was derived from a 36-d chronic test, while the present study used a 7-d chronic test.

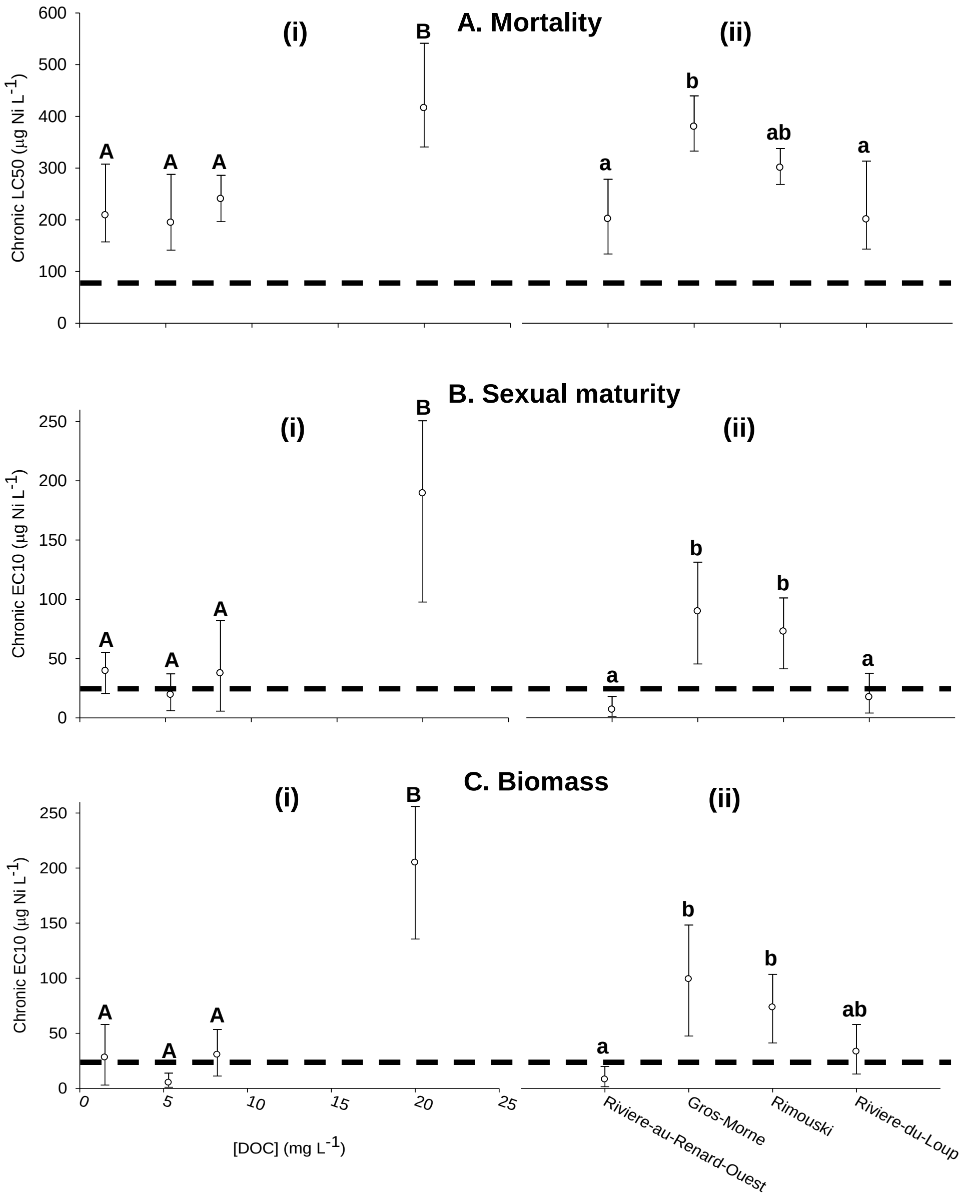

Figure 2. Chronic 7-d Ni LC50 values for Mortality (A), and EC10 values (all including 95% Confidence Limits) for Sexual maturity (B) and Biomass (C), from *A. bahia* exposed to different DOC concentrations (i) and DOC sources (ii). Different capital letters denote a significant difference in the DOC concentration dependent study (Fig. 3Ai, Bi and Ci); whereas lower case letters signify differences in the DOC source study (Fig. 3Aii, Bii and Cii) (determined by the 95% CL not overlapping). The dashed lines are the respective LC50 or EC10 (for reproduction) obtained from the 36-d chronic Ni toxicity *A. bahia* study (adapted from Lussier et al., 1985 by DeForest and Schlekat, 2013).

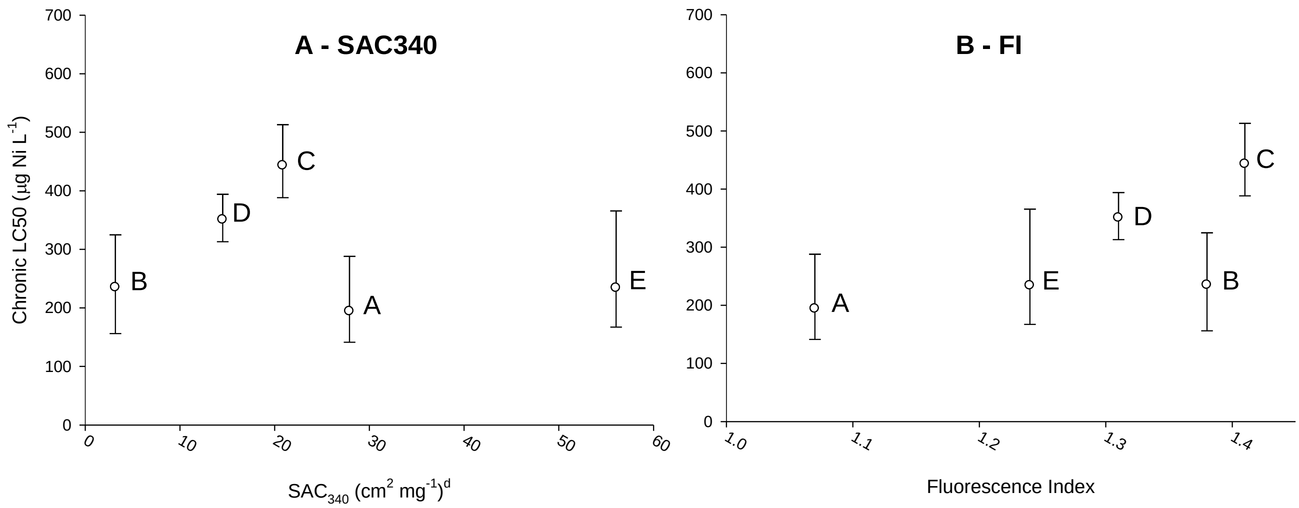

Figure 3. SAC340 (A) and FI (B) for all samples plotted against mortality. All grab samples contained, on average 2.2 mg C L^{-1}, whereas, the Kouchibouguac concentrate [DOC] used in this analysis was 5.3 mg C L^{-1}. Each letter refers to a different DOC source: Kouchibouguac [A], Rivière-au-Renard-Ouest [B], Gros-Morne [C], Rimouski [D] and Rivière-du-Loup [E]. The specific absorbance coefficient (SAC340) = (2.303 X absorbance at 340 nm)/[DOC] (Curtis and Schindler 1997); whereas the fluorescence index (FI) = emission intensity of 450 nm/emission intensity of 500 nm, both taken at excitation at 370 nm (McKnight et al. 2001).

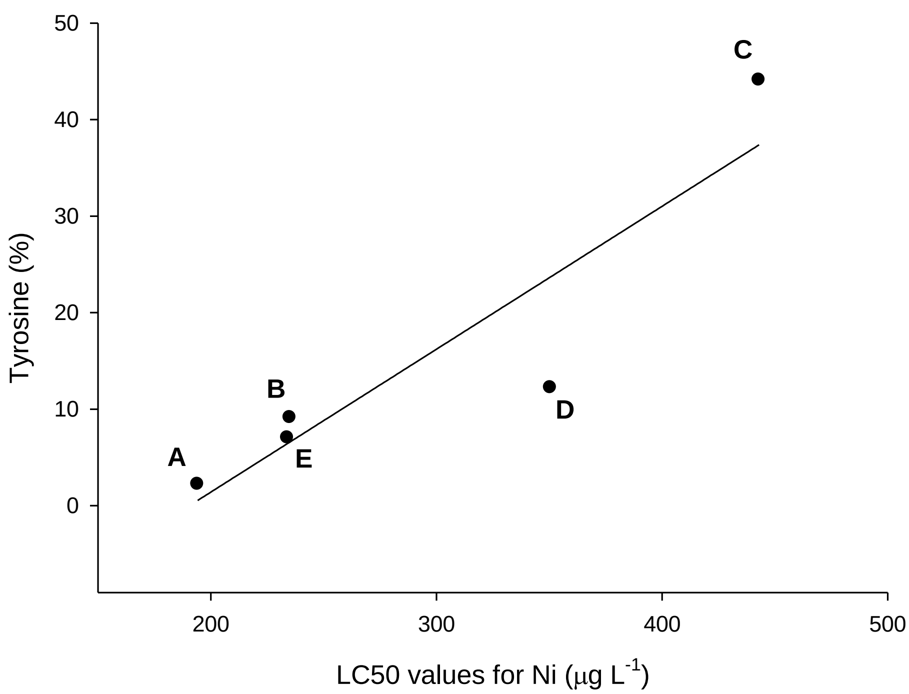

Figure 4. A significant positive relationship between tyrosine (%) and the LC50 values for Ni. Includes Kouchibouguac concentrate and all 4 grab samples ($P = 0.031$; $R^2 = 0.833$). For the linear regression parameters, refer to Table 3. Each letter refers to a different DOC source: Kouchibouguac [A], Rivière-au-Renard-Ouest [B], Gros-Morne [C], Rimouski [D] and Rivière-du-Loup [E].

The addition of a DOC concentrate, Kouchibouguac, (up to 8 mg L^{-1}) did not have a significant effect on any of the parameters tested (mortality, sexual maturation and biomass). A significant decrease in Ni toxicity was only observed when very high concentrations of DOC were added (20 mg L^{-1}). Interestingly, there was a strong DOC source dependent effect, with DOC rich in tyrosine offering the greatest protection against Ni toxicity. Accordingly to the literature, allochthonous DOC (high in humic-like substances) usually offers the greatest protective effect against metal toxicity, particularly in freshwater studies (Wood et al., 2011; Al-Reasi et al., 2012). However, this may not be the case for marine environments, where autochthonous DOC (bacterially derived DOC) may have more of an effect on metal toxicity.

DOC source effect:

There was a strong DOC source dependent effect on Ni toxicity to sea water acclimated *A. bahia*. The DOC from Gros-Morne offered the greatest protectivity against Ni toxicity to mysids, with all 3 end points supporting this (mortality, sexual maturation and biomass). The DOC from Rimouski was similarly protective; however, the DOCs from Rivière-au-Renard-Ouest and Rivière-du-Loup offered the least protection against Ni toxicity. Further analysis of the DOC revealed that the relative concentration of tyrosine was directly proportional to the level of toxicity, with higher levels of tyrosine resulting in less toxicity. Although not significant, the humic acid content was inversely proportional to the level of toxicity. Gros-Morne had by far the greatest relative concentration of tyrosine, which is a proteinaceous material, and a low humic acid content. Rimouski, the next most protective DOC source, had the highest relative concentration of fulvic acid, as well as the lowest humic acid content. From this it can be inferred that Gros-Morne and Rimouski are of autochtonous origin, and that these compounds offer the greatest Ni protectivity. However, this is unusual, as the general consensus is that DOC of allochthonous origin tends to be more protective, in particular humic acid, tends to be more protective against metal toxicity, at least for Cu, Ag, and Pb (Wood et al., 2011). It seems apparent that more studies are required to understand exactly why autochthonous DOC, in particular tyrosine, reduced Ni toxicity to *A. bahia*.

Fluorescence index (FI) is a simple characteristic providing information about the source or origin of DOC isolate (Al-Reasi et al., 2012). In the present study, although not significant ($P > 0.20$ for all 3 endpoints), the trend was that a higher FI correlated to less Ni toxicity. However, if Rivière-au-Renard-Ouest [B] is removed from this data set, this trend becomes more significant for all 3 end points, in particular for *A. bahia* biomass ($P = 0.028$). Typically, autochthonous DOC produces a value of ~1.9, whereas allochthonous DOC generates a value of ~1.4 (McKnight et al. 2001). Nonetheless, if we assume that an increasing FI value means that the DOC is more autochthonous (i.e. humic acid rich Kouchibouguac [A] = 1.07 vs. tyrosine rich Gros-Morne [C] = 1.41), then the data supports the inference that autochthonous DOC is more protective against Ni toxicity to *A. bahia* than allochthonous DOC.

The SAC340 is an index of aromaticity (Curtis and Schindler, 1997), with lower SAC340 values corresponding to autochthonous DOC and higher values to allochthonous DOC, and of course the latter generally offers the greatest protection against metal toxicity (Al-Reasi et al., 2012). Although there appears to be two groups (Rivière-au-Renard-Ouest [B], Rimouski [D], Gros-Morne [C]; and Kouchibouguac [A], Rivière-du-Loup [E]) that supports this notion (i.e. increasing SAC340 resulting in more protectivity); the data do not really make sense, and no clear correlations can be drawn. It is therefore, evident that for the current set of samples, the SAC340 is not an indicator for Ni toxicity.

DOC concentration dependent effect:

In the estuarine and marine environments DOC concentrations typically range from 1 – 15 mg L^{-1} (Wood et al., 2011). When using Kouchibouguac DOC, in this concentration range, there were no significant changes in mortalilty, sexual maturation or biomass, in *A. bahia* exposed to Ni for 7 days. However, very high [DOC] decreased Ni toxicity significantly for all 3 endpoints. It must be noted, that for e.g. *A. bahia* mortality, the LC50 for 20 mg C L^{-1} Kouchibouguac DOC was not significantly different when compared to the LC50 for 2.2 mg C L^{-1} Gros-Morne DOC, again highlighting the importance of tyrosine, not humic acid, in mitigating Ni toxicity to *A. bahia*. Although there is a concentration dependent effect at very high DOC concentrations, 20 mg C L^{-1}, which may be relevant in the sediment, it is not particularly environmentally relevant in estuaries and probably not worth considering for toxicity models such as the biotic ligand model (BLM). Indeed, when plotting all of the known [DOC] and EC10 values (for reproduction) for a large number of species listed in Deforest and Schlekat (2013), there appears to be no correlation between [DOC] and Ni toxicity. More research is required to ascertain DOC concentration dependent effect of Ni in sea water acclimated organisms, with a focus on the different compounds found within different DOC sources.

7-d versus 36-d chronic Ni toxicity test:

A 7-d test measuring survival, growth, and fecundity of *Americamysis bahia* was developed for estimating the chronic toxicity of effluents and associated receiving waters for National Pollutant Discharge Elimination System permits (standard EPA method EPA-821/R-02-014; 1007.0). This test method was used to assess Ni toxicity and it seems apparent that all three endpoints should be used. Although all three endpoints showed the same trends, just focusing on one endpoint may be misleading. For example, Gentile et al. (1982) used the 36-d chronic test and exposed *A. bahia* to Ni. A chronic mortality value of 93 μg Ni $L^{-1}$was calculated (Gentile et al., 1982), which is more than double the control values from this 7-d study (i.e. no added DOC). A 28-d test on another small shrimps, *Mysidopsis intii*, found that these species were even more sensitive with an LC50 of 22 μg L^{-1} (Hunt et al., 2002). However, using 36-d chronic *A. bahia* test data from Lussier et al. (1985) and Gentile et al. (1982), DeForest and Schlekat (2013) determined that the EC10 for reproduction was 17 μg Ni L^{-1} (time to first reproduction or number of young produced). This EC10 value is very comparable to what was observed for both the 7-d sexual maturation and biomass endpoints. Therefore, the use of the mortality data alone could have resulted in an overestimation of the Ni toxicity threshold for *A. bahia*; instead, it appears that the endpoint with the lowest toxicity values maybe more representative when compared to the longer chronic tests. In support of using all three endpoints when assessing toxicity using the 7-d test, Lussier et al. (1999) evaluated this method by examining data from more than one hundred 7-d *A. bahia* tests that were conducted over 5 years while the EPA method was being developed. The authors concluded that the 7-d test estimates the chronic toxicity of effluents most effectively when all three endpoints are used (Lussier et al., 1999).

Not only are the sexual maturity and biomass EC10s from the 7-d test comparable to the reproduction EC10 derived from the 36-d test, these values are also environmentally relevant. According to the marine Ni species sensitivity curve (SSD) published by DeForest and Schlekat (2013), data from the present study would put the *A. bahia* beneath the 20^{th} percentile (i.e. one of the most sensitive species). Furthermore, when using [DOC] under 10 mg L^{-1}, the sexual maturity and biomass EC10s averaged 32 and 21 μg Ni L^{-1}, respectively (with the lowest values recorded for each endpoint being 19.4 and 5.1 μg Ni L^{-1}, respectively). This has environmental relevance when taking into consideration the salt water quality guidelines for Ni, in the US the Ni limit is 8.2 μg Ni L^{-1}; in Australia it's 70 μg Ni L^{-1}; whereas in Europe it is 20 μg Ni L^{-1} (Pyle and Couture, 2012). Due to the large differences in Ni toxicity based on DOC concentration and DOC source, these factors should be taken into account when defining future guidelines.

Conclusion:

The present study revealed that Ni toxicity to *A. bahia* was dependent on where the DOC originated from. There was a positive correlation between Ni protectivity and the relative concentration of tyrosine. This was somewhat unexpected as the general consensus is that toxicity is more dependent on allochthonous DOC, i.e. humic acid content, not autochthonous. There were no DOC concentration dependent effects at environmentally relevant [DOC]; however, at very high levels of DOC, increased protectivity occurred.

Although maybe not as sensitive as the 36-d (for e.g. mortality), the 7-d test generated data that were very comparable, and it also has an obvious time advantage. The 7-d chronic test should definitely be considered when screening multiple toxicity modifying factors, such as DOC concentration and DOC source, as what would take several months to complete with the 36-d test can be done in weeks with the 7-d test.

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Amoeba in a queue: …………

And one that's been squashed: _ (aww)

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