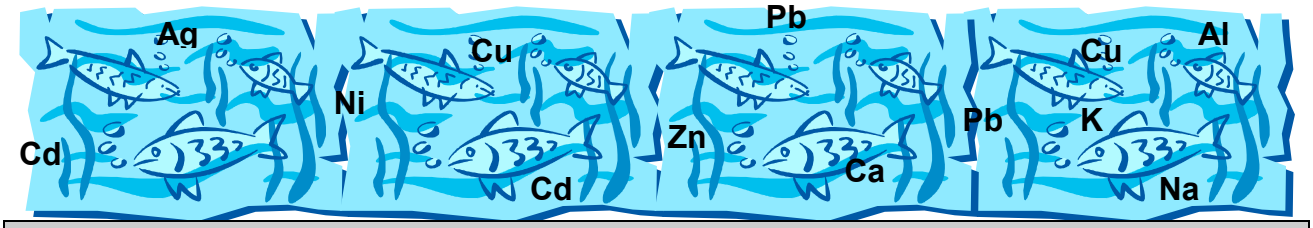
NSERC – Industry Project on Metal Bioavailability
Research Newsletter



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News

NSERC Strategic Grant (2008-2010)

The NSERC Strategic Grant project, with industrial co-funding from Rio Tinto Alcan and in kind support from Environment Canada, awarded to Dr. Chris Wood (McMaster University) and Dr. Jim McGeer (Wilfrid Laurier University) has officially started in September. The project is titled "Development of a Tissue Residue Approach for Risk Assessment of Metals in the Canadian Freshwater Environment. This project focuses on three important areas: (1) assessing the bioaccumulation patterns and toxic responses of three economically important but environmentally concerning metals (Cu, Cd, Ni) in two fish and five invertebrate species; (2) pin-pointing, at a subcellular level, the critical tissue metal residues that elicit toxicity; and (3) developing quantitative models to predict toxicity based on tissue residues.

The McMaster University research team includes Dr. Chris Wood (Principal Investigator), Dr. Tania Ng (Research Associate), Erin Leonard (Ph.D. student) and Nish Pais (4th year undergraduate student). Erin studied her Master degree under the supervision of Dr. Michael O'Donnell at McMaster University and she successfully defended her M.Sc. Thesis "Investigations into Cd tolerance in *Chironomus riparius*: spatial patterns of Cd transport and sequestration" in August. Erin was also previously involved in the NSERC CRD Grant Project, examining gastrointestinal Ni uptake in the rainbow trout. She has started her Ph.D. recently, under the supervision of Chris Wood. The Wilfrid Laurier University research team includes Dr. Jim McGeer (Co- Investigator), Matt Clifford (M.Sc. student), Tony Straus (M.Sc. student), Amanda Mancini (M.Sc. student) and Emily-Jane Costa (Directed studies). Matt was previously involved in the NSERC CRD Grant, developing softwater BLMs of Cd and Zn for *Daphnia pulex*.

The research teams from both universities had a meeting at McMaster University, discussing the roles of each member on 28 August. Dr. Tania Ng will examine acute and chronic effects of Cu on invertebrates (Chironomids, the worm *Lumbriculus variegatus*, Daphnia and Hydra) and fish (rainbow trout and round goby). She will also train the team on subcellular fractionation analysis of metals. Erin will examine Ni effects on all the invertebrate and fish species whereas Nish will focus on accumulation and toxicity of Cu to the snail *Lymnaea stagnalis*. Matt will continue to investigate his expertise area: Cd effects on Hydra and Daphnia, and train Emily-Jane for studying chronic Cd effects on Daphnia since he will finish his degree next

year. Tony will investigate the Cd effects on Chironomids, *Lumbriculus* and snails. Amanda will mainly work on chronic Cu exposures on trout.

The renewed collaboration between the two academic labs will certainly bring more insights. We look forward to this new and exciting chapter of metal research.

New member in the lab

Hassan Ali Al-Reasi from Oman, recently joined the lab as a Ph.D. candidate. He received his M.Sc. degree in 2005 at the University of Ottawa under the supervision of Dr. David Lean. In his M.Sc. thesis, he studied mercury and methlymercury in fish in the Gulf of Oman. After graduation, he then returned to Oman and worked as a lecturer at Sultan Qaboos University, Oman for three years. Now, he is fully sponsored by the government of Oman to pursue his Ph.D. degree at McMaster University, under the guidance of Dr. Chris Wood. He is also co-supervised by Dr. Scott Smith at Wilfrid Laurier University. He will quantify the effects of DOM on crustacean and fish: both the direct influence on the gills with respect to physiochemical properties of DOM, and its interactive effects with metals. Welcome back to Canada, Hassan!

Conference presentations

The following papers will be presented by the Metals Bioavailability Group at the SETAC North America 29th Annual Meeting, Tampa, Florida, USA. Nov 16-20, 2008.

- **Ng, T. Y.-T., Klinck, J. S., Wood, C. M. (2008)**. Importance of testing low metal concentrations in a dietary toxicity study – toxicity of dietary Cd and protection by elevated dietary Ca in the rainbow trout.

- **Wood, C.M., Smith, D.S., Donini, A., Playle, R.C., O'Donell, Glover, C., Galvez, F. (2008)**. Rethinking DOC.

- **Klinck, J. S., Ng, T. Y.-T., Wood, C. M. (2008)**. The protective effects of calcium against gastro-intestinal uptake of cadmium in rainbow trout, an *in vivo* and *in vitro* approach.

- **Leonard, E. M., Nadella, S. R., Bucking, C., Wood, C. M. (2008)**. Characterization of dietary nickel uptake in the rainbow trout, (*Oncorhynchus mykiss*).

- **Craig, P. M., Hogstrand, C., Wood, C. M., McClelland, G. B. (2008)**. Gene expression endpoints of chronic copper toxicity in zebrafish.

- **Wilkie, M. P., Birceanu, O., Gillis, P. L., Chowdhury, M. J., McGeer, J. C., Wood, C. M. (2008)**. Synergistic effects of Pb plus Cd mixtures on gill function in the rainbow trout (*Oncorhynchus mykiss*).

- **Chowdhury M. J., Ng, T.Y-T., Smith, S., Wood, C. M. (2008)**. Water quality parameters and binding constants for the development of a chronic biotic ligand model for copper toxicity to rainbow trout.

- **Niyogi, S., Kent, R., Wood, C. M. (2008).** Influence of water chemistry on gill accumulation and acute toxicity of cadmium in rainbow trout (*Oncorhynchus mykiss*): development of an acute cadmium biotic ligand model (BLM).

- **Clifford, M. S., McGeer, J. (2008)**. Development and comparison of acute cadmium toxicity prediction models in soft water for *Daphnia pulex* and *Hydra Attenuata*.

The following peer reviewed paper was published by the Metals Bioavailability Group in September - October 2008

- **Gillis, P. L. and Wood, C.M**. **(2008)**. Investigating a potential mechanism of Cd resistance in *Chironomus riparius* larvae using kinetic analysis of calcium and cadmium uptake. Aquat. Toxicol. 89: 180-187.

The following peer reviewed papers by the Metals Bioavailability Group are in press in September – October 2008:

- **Galvez, F., Donini, A., Smith, S., O'Donnell, M., and Wood, C.M.** (2008). A matter of potential concern: Natural organic matter alters the electrical properties of fish gills. Env. Sci. Tech. In Press.

- **Ng, T., Klinck, J. and Wood, C.M. (2008)**. Does dietary Ca protect against toxicity of a low dietborne Cd exposure to the rainbow trout? Aquat. Toxicol. In press.

- **Mirza, R.S., Green,W.R., Connor, S., Weeks, A.C., Wood, C.M., and Pyle, G.G. (2008)**. Do you smell what I smell? Olfactory impairment in wild yellow perch from metal-contaminated waters. Ecotoxicol.and Environ. Safety. In press.

- **Nadella, S., Fitzpatrick, J.L, Franklin, N., Bucking, C.P., Smith, S., and Wood, C.M. (2008)**. 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. In press.

- **Birceanu, O., McGeer, J.C., Chowdury, M.J., Gillis, P., Wood, C.M. and Wilkie, M. (2008)**. Modes of metal toxicity and impaired branchial ionoregulation in rainbow trout exposed to mixtures of Pb and Cd in soft water. Aquat. Toxicol. In press.

- **Ojo A.A. and Wood C.M. (2008).** *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. In press.

- **Bechard, K., Gillis, P.L. and Wood, C.M.** (2008). Trophic transfer of larval chironomids (*Chironomous riparius*) exposed via sediment or waterborne routes, to zebrafish (*Danio rerio*): tissue specific and subcellular comparisons. Aquat. Toxicol. In Press.

- **Niyogi, S., Kent, R., Wood, C.M. (2008).** Effects of water chemistry variables on gill binding and acute toxicity of cadmium in rainbow trout (*Oncorhynchus mykiss*): a biotic ligand model (BLM) approach. Comp. Biochem. Physiol. C. In press.

- **Clifford, M. and McGeer, J. C. (2008).** Influence of water chemistry on the toxicity of Zn to *Daphnia pulex* in soft waters. Aquat. Toxicol. In press.

Research Highlights

This issue will highlight research conducted by Dr. Fernando Galvez (McMaster University and Louisiana State University, USA), Andrew Donini (McMaster University and York University), Dr. Richard C. Playle (Wilfrid Laurier University), Dr. D. Scott Smith (Wilfrid Laurier University), Dr. Michael J. O'Donnell (McMaster University) & Dr. Chris M. Wood (McMaster University). Dr. Playle passed away while this work was being done, and we remember him fondly. This paper has now been accepted for publication in Environmental Science and Technology.

A matter of potential concern: Natural organic matter alters the electrical properties of fish gills

Fernando Galvez, Andrew Donini, Richard C. Playle, D. Scott Smith, Michael J. O'Donnell and Chris M. Wood

Natural organic matter is an important constituent of aquatic environments; however its influence on aquatic biota remains poorly studied. Natural organic matter (NOM, commonly known as dissolved organic carbon, DOC) is an important regulator of biogeochemical processes such as global nutrient and carbon cycling, metal redox reactions and cation complexation (McKnight et al., 2001). This functional diversity is largely associated with its high degree of chemical heterogeneity. NOM can be subdivided into two major types. Allochthonous NOMs consist almost exclusively of terrestrially-derived lignin-degradation products, containing high levels of aromatic humic and fulvic substances enriched in carboxylic and phenolic groups. This high aromatic content imparts significant ultraviolet light absorptivity and makes allochthonous NOMs darkly colored (Curtis and Schindler, 1997; McKnight et al., 2001). In comparison, autochthonous NOMs are formed endogenously in watersheds from photosynthetic activity or bacterial degradation of allochthonous NOM (Curtis and Schindler, 1997). Chemically, these NOMs are enriched in carbohydrate and nitrogen functional groups, with low aromaticity, diminished UV absorptivity and are pale in color (Curtis and Schindler, 1997; McKnight et al., 2001).

The ability of NOM to affect aquatic biota indirectly by exerting strong control on

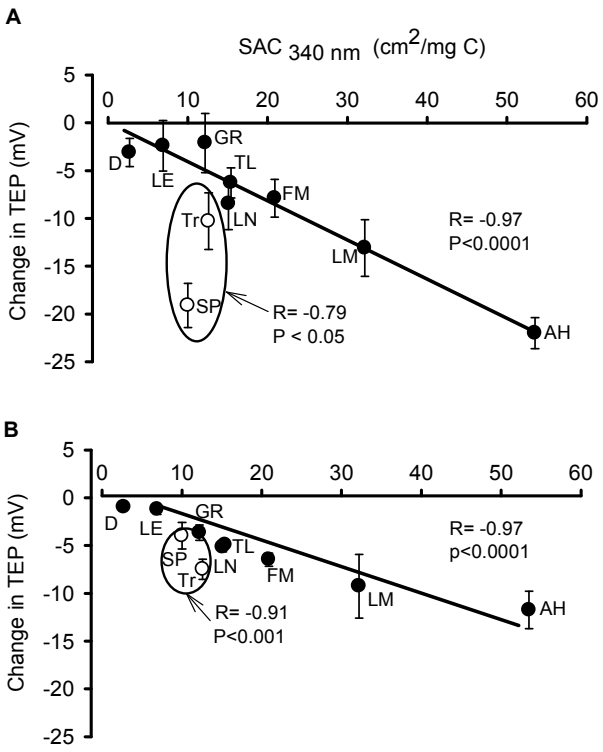
the surrounding biogeochemical conditions is well established. In contrast, the potential direct interaction of these amphiphilic compounds on the exchange surfaces of aquatic animals has been largely overlooked.

The present study incorporated whole-animal and cell culture-based approaches to study the impact of different sources of NOM on the electrophysiology of the fish gill. NOMs were isolated from nine different sites in Southern Ontario, ranging from autochthonous to highly allochthonous. A commercially-available humic substance with strong allochthonous characteristics was used as a positive control. The molar absorptivity at 340 nm was used as an index of aromaticity (McKnight et al., 2001).

In this study, cultured gill epithelia were kept under asymmetrical conditions with either control (Lake Ontario) or NOM-enriched water on the apical side, and a blood serum substitute (L-15 medium) on the basolateral side. Under this arrangement, gill epithelia were monitored for TEP (transepithelial potential) and TER (transepithelial resistance) to assess the effects of NOM on the electrophysiological parameters of the gill epithelium. Replacing the control water with NOM-enriched water resulted in a hyperpolarization of cultured gill epithelia (Fig. 1A). The magnitude of this hyperpolarization at a standard concentration of 10 mg C/L was strongly correlated with the $SAC_{340\ nm}$ (specific absorption coefficient) of the particular NOM, suggesting that the potency for hyperpolarization is a function of aromaticity of the molecules.

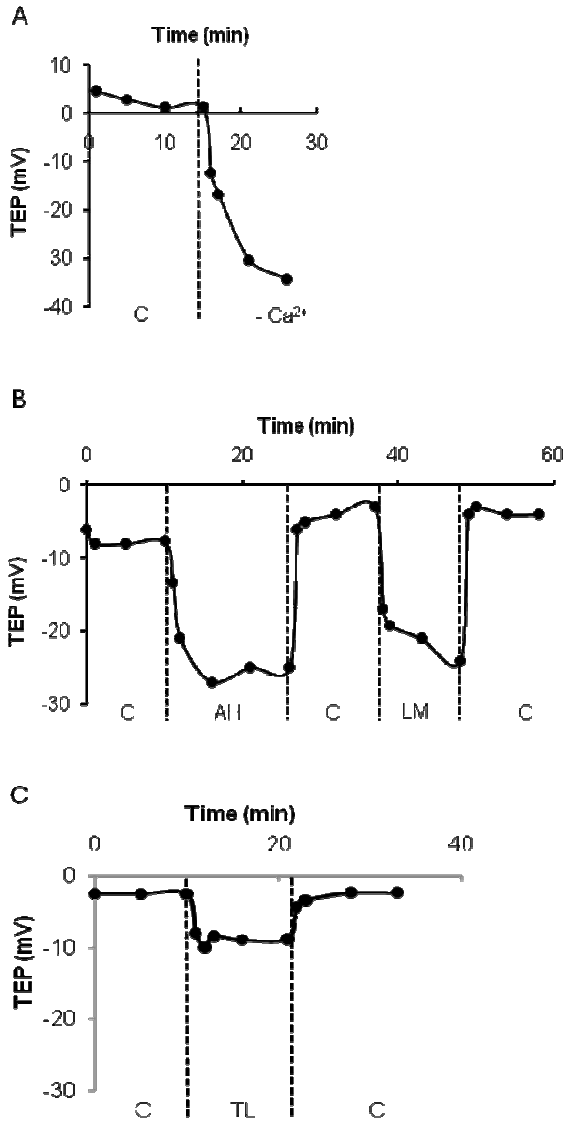
TEP *in vivo* measured in cannulated trout showed a similar response, with the magnitude of the hyperpolarization again strongly correlated with the $SAC_{340\ nm}$ (Fig. 1B).

Figure 1. The effects of 10 mg C/L NOM on the TEP of (A) cultured gill epithelia derived from adult rainbow trout (N=10 per NOM), or (B) cannulated adult rainbow trout (N=6 per NOM). TEPs are expressed relative to the apical side of membranes and are corrected for junction potentials. The Pearson's co-efficient of regression (r) and the p-values are reported with the inclusion of all data or all the data excluding two NOM sources (Trout Lake and Sanctuary Pond: circled) which were excluded due to their ability to greatly lower Ca^{2+} activity. D- Dundas Sewage Treatment Plant; FM- Four Mile Lake; GR- Grand River; LE- Lake Erie; LM- Luther Marsh; LN- Lake Nippissing; SP-Sanctuary Pond; TL-Talon Lake; Tr-Trout Lake; AH- Aldrich humic acid. Data are expressed as the mean ± SEM (N).



However, the extent of the hyperpolarization was slightly attenuated to that observed *in vitro*. The effects *in vivo* were shown to be completely reversible, since transfer back to control water after NOM exposure led to a typical control TEP of approximately -5 mV (relative to the water side) (Fig. 2B-C). These results demonstrated the ability of allochthonous NOM to strongly influence TEP (Fig. 2B), in comparison to autochthonous NOM (Fig. 2C).

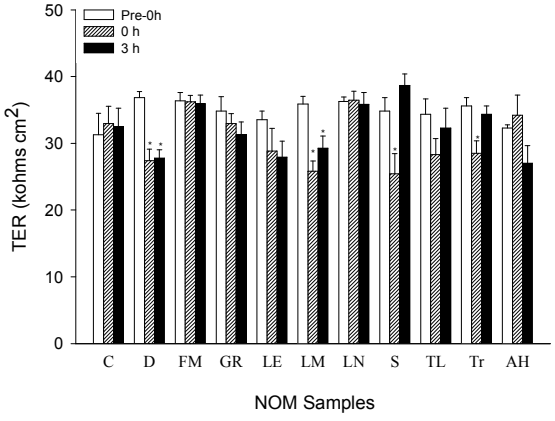
Figure 2. Representative profiles of transepithelial potential (in mV) in vivo in cannulated trout exposed to (A) trout exposed to pure distilled water (- Ca^{2+}) as a positive control, (B) control Lake Ontario water – C; Aldrich humic acid- AH, or LM- Luther Marsh over time, or (C) control Lake Ontario water – C or Talon Lake- TL over time.



The TEP in freshwater fish is traditionally interpreted as a diffusion potential predominantly regulated by the relative paracellular permeability to positively- (mainly Na^+) and negatively- (mainly Cl^-) charged ions (Potts and Eddy, 1973; Wood et al. 1998; Potts 1984). There are two possible explanations for the somewhat attenuated response of NOM on *in vivo* TEP. First, NOM may simply have had a reduced capacity to influence the paracellular permeability to ions in whole animals relative to cultured epithelia. However, an alternate explanation is that NOM may have initiated an electrogenic uptake of ions *in vivo* that tended to counteract the effect on diffusive permeability (Potts 1984), thereby making the TEP less negative. Current models of Na^+ uptake in fish suggest that active Na^+ uptake is driven by an electrogenic H^+ pump (Marshall and Grosell 2005). Zientara (1983) provided evidence that humic substances may stimulate the H^+ pump in the giant alga *Nitellopsis obtusa*. Certainly, there is evidence that allochthonous sources of NOM lead to enhanced Na^+ influx in *Daphnia* (Glover and Wood 2005; Glover et al. 2005) and fish (Qiao and Farrell 2002; Steinberg et al. 2006). Also worth noting is that, although the gill epithelial cell cultures mimic the passive properties of the fish gill *in vivo* (Wood et al. 2002), they exhibit only limited capacity to actively take up ions under asymmetrical conditions (Fletcher et al. 2000; Zhou et al. 2003).

In contrast to these marked effects on TEP, the TER of the gill epithelium, which could only be measured *in vitro*, was relatively unresponsive to the various NOMs (Fig. 3).

Figure 3 TER (in Kohms · cm^2) in cultured gill epithelia under asymmetrical conditions before exposure to NOM (pre-0 h), or after 0-3 h after transfer to 10 mg C/L NOM. See Figure 1 legend for additional information and legend for NOM source abbreviations. Data are expressed as the mean ± SEM (N=10 per NOM).



TER is a general indicator of epithelial membrane integrity (Wood et al. 2002), suggesting that this is not affected by these compounds at the concentrations tested. In some cases, there was an initial drop in TER (at 0h) upon first exposure, but this is a commonly seen disturbance effect of handling. Only in two instances (Luther Marsh and Dundas Sewage Treatment Plant), did the fall in TER persist at 3h. Note that these two have different $SAC_{340\ nm}$ characteristics, so the response was not related to aromaticity.

What mechanism then explains the NOM-induced hyperpolarization in fish epithelia? Glover and Wood (2005) and Glover et al. (2005) suggested that NOM could lead to indirect biological effects by complexing biologically-active ions such as free Ca^{2+} from the water. Ca^{2+} is an important constituent of epithelial tight junctions, and reductions in this ion to very low levels are known to cause a general increase in diffusive ion losses, as well as selectively enhancing the permeability of the gills to Na^+ relative to Cl^-. The resulting hyperpolarization of the gills (more negative TEP) has been documented in a variety of species *in vivo* (Potts and Eddy 1973; Wood et al. 1998; Potts 1984), including the rainbow trout (Perry and Wood 1985), as well as in cultured trout gill epithelia *in vitro* (Kelly and Wood 2008). To test whether allochthonous NOMs were exerting their effects on fish epithelia by complexing Ca^{2+}, ion-selective micro-electrodes were used to measure free Ca^{2+} activities at 10 mg/L NOM (Fig. 4).

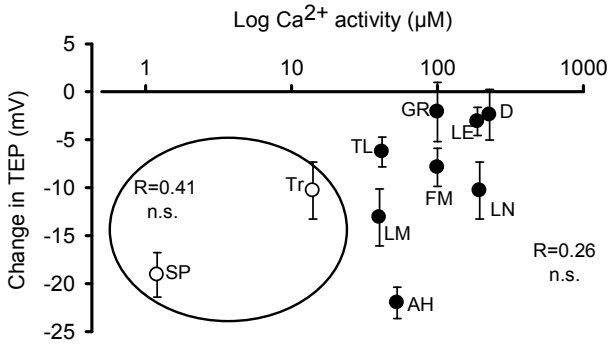


Figure 4. The relationship between the free Ca^{2+} activities in each of the sources of water containing 10 mg C/L NOM and the absolute change in TEP (in mV) across epithelia. Pearson's co-efficient of regression (r) are reported with the inclusion of all data or all the data excluding two NOM sources (Trout Lake and Sanctuary Pond:circled). n.s. represents no statistical significance. See Figure 1 legend for NOM source abbreviations. Data are expressed as the mean ± SEM (N).

Although all of the NOMs tended to complex Ca^{2+} to a certain degree, only the samples from Trout Lake and Sanctuary Pond had free Ca^{2+} activities (14.0 and 1.2 μM) approaching that of distilled water. Eddy (1975) found that total waterborne Ca^{2+} levels below 0.5 mM lead to a hyperpolarization of TEP *in vivo* in goldfish, with the most pronounced effect occurring below 0.1 mM. In the current study, if NOM from Trout Lake and Sanctuary Pond were excluded from the analyses described in Fig. 1A and 1B, the relationships between TEP hyperpolarization and $SAC_{340\ nm}$ were greatly improved (Fig. 1A: from an R = 0.79 up to 0.97; Fig. 1B: from an R = 0.91 up to 0.97). Ca^{2+} complexation alone could not explain the TEP response (Fig. 4). Whether the data from Trout Lake and Sanctuary Pond are included in the analyses or not, there was no significant relationship between waterborne Ca^{2+} activities and TEP hyperpolarization (Fig. 4).

The results therefore suggest that NOM, rather than acting to reduce Ca^{2+} activity, in the water (an indirect effect), in some way acts directly on the gills in an analogous fashion to low Ca^{2+}. The time course of the effect is not instantaneous as would occur if NOM had an artifactual effect on the reference electrode, but rather asymptotic over several minutes (Fig. 2B,C), similar to the well-documented effect of varying environmental Ca^{2+} on TEP across the gills (e.g. Fig. 2A). This could be an action on the paracellular/tight junction pathway to increase the relative permeability ratio of Na^+ versus Cl^-, with aromatic,

allochthonous molecules being most effective in this regard. Direct evidence that NOM can accumulate on the surfaces of fish gill cells, as well as algae and microbial cells, has been provided (Visser 1985; Munster 1985; Campbell et al. 1997), as well as evidence that NOM can integrate into algal and model lipid membranes, thereby changing their permeability (Vigneault et al. 2000). In both studies, the effects were pH-dependent, with greater effectiveness in acidic environments. This suggests that in future investigations, the interactive effects of NOM and pH on gill function should be studied at a range of acidic pH. In this regard, it is interesting that NOM reduced ion losses at fish gills at moderately low pH (4.0), similar to the action of Ca^{2+} (*39*) but this did not occur at circumneutral pH (Wood et al. 2003).

The TEP is a key component determining the electrochemical gradient through which the active and passive transport of all ions must occur. Source-dependent effects of NOM on Na^+ flux rates in freshwater *Daphnia* (Glover et al. 2005) and fish (Matsuo et al. 2004) have recently been reported; changes in TEP may provide the explanation. As with the TEP effect, the amelioration of metal toxicity in aquatic animals by NOM also seems to be governed by aromaticity (Schwartz et al. 2004; Glover et al. 2005), so the functional groups and mechanisms involved in the two processes may be similar, providing a guidepost for future research.

To conclude, while NOM effects on the bioavailability of contaminants are well-known, NOM effects on organismal physiology have not been widely recognized by environmental scientists. In the present study, we have demonstrated that NOM may alter the fundamental physiological properties of fish gills. While the influence of water quality characteristics such as salinity, calcium (water hardness), and pH on gill physiology, including ion fluxes and TEP, are universally acknowledged (Potts 1984; Evans et al. 2005; Marshall 2002), these physiological effects of NOM have been curiously overlooked until now. The present study indicates that the effects may be large. Such effects may be of key ecological importance in the real world because of the primary role of the gill epithelium in diverse life-support functions such as gas exchange, acid-base regulation, nitrogenous waste excretion, immunity, and ion transport (Evans et al. 2005; Marshall 2002). The present data add to the growing body of evidence that the nature of ambient NOM, both in terms of concentration and chemical characteristics, can have a major impact on the physiology of aquatic organisms (see *Introduction*). The impact of NOM may be of comparable or greater magnitude than commonly reported for other water quality variables (e.g. hardness, pH, salinity), and therefore of critical importance in ecological understanding and risk assessment. Certainly these matters are of potential concern.

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Tania Ng, 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:taniang@mcmaster.ca