AROMATIC-RICH NATURAL ORGANIC MATTER INCREASES GROWTH AND
REDUCES NICKEL TOXICITY IN A WILD *DAPHNIA* HYBRID

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Abstract

Natural organic matter (NOM) is on the rise across the Shield. Its heterogeneous, amalgamate nature makes each NOM source potentially unique. Relatively little is known about the direct effects distinct NOM sources have on daphniids, or the varying protection NOM provides against metal toxicity –specifically Ni. I show that NOM from different natural sources increases growth rates and decreases time to maturation of *Daphnia* to various degrees. These changes relate to aromatic content of NOM isolates, the mechanism most likely being a hormonal effect or mild oxidative stress. Aromatic content (representing phenolic groups) is also positively related to Ni mitigation for those isolates with specific UV absorbance below 19.3 cm^2mg^{-1}. Incorporating NOM aromatic content into a predictive model improves the relationship between predicted and measured LC_{50}s by 5%. I demonstrate that structural differences between NOM isolates relating to aromatic content play a major role in their effects on *Daphnia*.

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Introduction

Daphnia are planktonic crustaceans found in lakes (Tatarazako and Oda 2007), swamps and ephemeral ponds (Hanski and Ranta, 1983) on all continents (Benzie, 2005). They are an important species in aquatic food webs (Dodson and Hanazato 1995) and are a preferred model for use in assorted research fields. *Daphnia* are sensitive to changes in their environment, including changes in temperature (Goss and Bunting, 1980); food quantity (Tessier et al. 1983; Lampert 1987) and quality (Boersma and Kreutzer 2002; von Elert 2004), and chemical contaminants (Adema, 1978). Many of these changes in aquatic environments are known to be influenced or altered by natural organic matter (NOM).

Natural organic matter (NOM) is found in all water bodies (e.g. streams, lakes, oceans) (Ertel et al., 1984). It is a polydisperse macromolecule (Baker and Khalili 2003) made up of decayed plant, animal (Nebbioso and Piccolo 2013), bacterial and Archaea tissue, as well as viruses. It can be fractioned and defined using various measures: physical state, as either dissolved (passes through a 0.45 µm filter) or particulate (removed by a 0.45 µm filter) (Perdue 2009); origin, as either autochthonous (from within the water body) or allochthonous (terrigenous in origin then washed into the water) (McKnight et al., 2001); and solubility, which determines NOM's hydrophobic acids to be either humic acid (HA) (soluble at pH greater than 2) or fulvic acid (FA) (soluble at all pH levels) (Perdue 2009).

NOM influences aquatic biota in several ways it: provides protection from UV-B radiation (Molot et al., 2004); complexes metals (Playle et al., 1993) and organic contaminants (Haitzer et al., 1999) influencing metal and contaminant transport and bioavailability; acts as a food source for food webs (Pace et al., 2004); and influences light attenuation and thermal structure of small lakes (Fee et al., 1996). The complex mixtures of polyelectrolytes, and the

wide range of functional moieties and molecular weight distribution of NOM (Celo et al., 2001) make it impossible to give NOM a specific chemical formula (Meinelt et al., 2007). While the general way in which NOM influences aquatic biota is understood, its heterogeneity within each source makes each NOM isolate in effect, unique. It is this variation of NOM's structure between sites which interests the author. How does this structural disparity affect its accepted roles in aquatic ecology? Can general patterns in NOM structure be related to these altered affects? Much research is needed to address such questions.

Zooplankton consumes NOM via two major routes. The first is by ingesting bacteria which use low molecular weight fractions of dissolved organic matter (DOM) for growth (Grey et al. 2001; Karlsson et al. 2003; Jansson et al. 2007; Berggren et al. 2010). The second is direct ingestion of particulate organic matter (POM) as a food source (Pace et al., 2004; Cole et al., 2011 and Wenzel et al., 2012). In small nutrient deficient lakes 20-50% of carbon consumed by *Daphnia* originates terrestrially—outside of their home water bodies (Pace et al., 2004)—though this may be more out of need than preference.

NOM is a poor quality food for daphniids. Brett et al. (2009) compared the quality of several daphniid diets. The first diet was ground red alder (*Alnus rubra*) leaves, representing an exclusive NOM diet. The second diet was a cyanobacterium (*Anabaena),* and the third, a green algae *(Scenedesmus).* They found that growth and time to primiparity were dramatically reduced on the alder and bacterial diet, and reproductive output was reduced by 90%. Masclaux et al. (2011) observed similar results when *Daphnia longispina* was fed a diet of pollen (a component of NOM). Wenzel et al. (2012) observed that *Daphnia galeata* fed ground peat could not survive beyond two days unless 20% of the diet was supplemented with the cryptophyte algae, *Rhodomonas lacustris*, which fostered both survival and reproduction.

NOM's nutritional deficiencies across these studies were due to difficulties with digestion (pollen) (Masclaus et al., 2011), and low mineral content and a higher C:P ratio (red alder, ground peat) (Wenzel et al., 2012) than *Daphnia* require (Andersen and Hessen 1991). Allochthonous NOM also appears to be short of essential biochemicals such as polyunsaturated fatty acids (PUFA)—specifically eicosapentaenoic acid (EPA) and α-linolenic acid (ALA) (Wenzel et al., 2012)—which are essential for zooplankton growth and reproduction (Brett and Muller-Navarra 1997; Wacker and von Elert 2001). However, while uptake of NOM may not lead to an improved diet, there do appear to be other direct effects of NOM on zooplankton and other aquatic invertebrates.

In amphipods, NOM exposure increases expression of stress hormones such as Hsp70 (Bedulin et al., 2010), induces the reaction of the multixenobiotic resistance system (MSR) (Timofeyev et al., 2007), and causes oxidative stress as demonstrated by the accumulation of hydrogen peroxide and lipid peroxidation (Timofeyev et al., 2006a; Timofeyev et al., 2006b). Humic substances (HS) have also been shown to alter membrane permeability in *Daphnia magna*, which enhances sodium influx (Glover and Wood 2005; Glover et al., 2005b). Additionally, HSs induce the production of male offspring, decrease female lifespan while increasing male lifespan of *D. magna* (Euent et al., 2008), and diminish cumulative offspring numbers (Bouchnak and Steinberg 2010). In multiple stress situations, NOM alleviates the stress of a poor quality diet of yeast; increases offspring counts and lessens ephippia production with *D. magna* (Bouchnak and Steinber 2010); eases salt-induced reductions of somatic growth, boosting body volume (Suhett et al., 2011); and increases reproduction and lifespan in the cladoceran *Moina macrocopa* (Engert et al., 2012). The composition of NOM varies with source, with the amount of allochthonously-based NOM making its way into the water body, and

with the chemical composition of the surrounding foliage adding to the terrigenously-based NOM. And so the question presents itself, how does the composition of NOM relate to its direct effects on an aquatic organism such as *Daphnia*?

Glover et al (2005b) noted that both Suwanne River NOM (SRNOM) and commercial Aldrich humic acid increased the capacity for sodium passage while simultaneously decreasing the affinity for sodium uptake, but only SRNOM increased sodium loss. However, the amphipod, *Gammarus pulex*, increased peroxidase activity and stress protein expression of sHSPs and HSP70 when exposed to three different NOM sources, including two brown-water lakes in Germany, and one isolate from the black layer of a Brazilian sandbar soil (Bedulina et al., 2010). *M. macrocopa* lived longer with exposure to an NOM isolate from Brazil and the synthetic humic acid, HuminFeed® to varying degrees, but also had contrasting changes in life time reproductive output. While the NOM isolate improved reproduction, HuminFeed® curtailed lifetime neonate production (Steinberg et al., 2010.). Hofmann et al. (2012) also noted differences in life history traits when *M. macrocopa* was exposed to four different leaf litter leachates. Three of the four leaf leaches enhanced body size and extended lifespan and the two *Picea* species increased reproduction. The structural heterogeneity of NOM would appear to be the cause of these differences. So what is it about the structure of NOM that induces these changes? By using spectroscopic techniques the general structure, relative fractions and origins of five NOM isolates were determined and compared to the changes in life history traits of a wild *Daphnia pulex/pulicaria* clone in chapter one of this thesis.

The characterization of NOM has been aided by modern, sophisticated technologies in absorbance and fluorescence (Fellman et al., 2010). The process is now both affordable and dependable (Jaffe et al., 2008), while at the same time being a straightforward and unobtrusive

(Senesi et al. 1990) method of analysis. Absorbance measures such as the specific absorbance coefficient (SAC) and the specific UV absorbance (SUVA) were rendered to gauge the differences in colour and aromatic content of the NOM isolates (Curtis and Schindler 1997; Schwartz et al., 2004; Weishaar et al., 2003). The ratio of the absorbance of NOM at 254 nm and 365 nm was used as a proxy inversely related to the molecular weight of the isolates (Dahlen et al., 1996). I also employed fluorescence spectroscopic techniques, which are considered more sensitive (Borisover et al., 2009) and discriminating as compared to absorbance measures (Luider et al., 2004). The Fluorescence Index (FI) was used to determine the origin of the FA portion of the NOM as either mostly allochthonous or autochtonous (McKnight et al., 2001). In addition, Emission and Excitation Matrices (EEMs) in combination with Parallel factor analysis (PARAFAC) were used to determine the relative proportions of humic acids (HA) and fulvic acids (FA) (Nadella et al., 2009). These spectroscopic methods of characterizing NOMs have been helpful in identifying features of organic matter related to metal complexation and reductions in toxicity. NOMs that are dark in colour, high in aromatic content, larger in molecular weight, allochthonous in origin and/or containing higher concentrations of HA have been found to alleviate acute Cu (Al-Reasi et al., 2012), Pb, and to a lesser degree, Cd toxicity (Al-Reasi et al., 2011). Steinberg et al. (2003) suggested that these functional groups associated with toxicity reduction may also play a role in the biological interactions described above. For example, Meinelt et al. (2007) found that reductions in vegetative growth of the water mold *Saprolegnia parasitica* were related to aromatic content (measured using SUVA) and molecular weight of 10 NOM isolates, the synthetic HS1500, HuminFeed® humic acid, and a Na-Humate. This present study is the first in which these techniques of NOM characterization (in concurrence

with life history bioassays) are performed to determine how the specific characteristics of NOM directly affect *Daphnia*.

The ability of NOM and its fractions (HA and FA) to decrease metal toxicity has long been recognized (Zitko et al., 1973). Trace metals complex with the functional groups within NOM, decreasing the abundance of the most toxic form of metals (the free metal ion), reducing their bioavailability to aquatic organisms and thus, creating a protective effect (Baken et al. 2011, Al-Reasi et al. 2012, Ryan et al. 2004,Richards et al. 2001 and De Schamphelaere et al. 2004). This relationship is known to be concentration dependent (Playle et al., 1993; Erickson et al., 1996). With the wide heterogeneity between geographical sites demonstrated in differing proportions of functional group makeup of NOM, it follows that these protective effects would vary by source. When trying to construct a metal toxicity model with inorganic Hg and fish gills, Playle (1998) found it necessary to alter Hg affinity for DOM to compensate for a ten-fold difference in toxicity which was due to differences in NOM source. Richards et al. (2001) supported this finding that source influenced the protective effects provided by NOM with a six-metal mixture and three different sources of NOM. Investigations with single-metal solutions identified how NOM source alters the toxicity of Pb (MacDonald et al., 2002; Schwartz et al., 2004), Ag (VanGenderen et al., 2003; Glover et al., 2005 - heterogeneity), Cd (Schwartz et al., 2004) and Cu (De Schamphelaere et al., 2004; Ryan et al., 2004; Schwartz et al., 2004). Several of these investigators observed that darker, more aromatic and allocthonous NOM provided a greater amount of protection (Richards et al., 2004); this was particularly true for Pb (MacDonald et al., 2002; Schwartz et al., 2004) and Cu (De Schamphelaere et al., 2004; Schwartz et al., 2004). Ryan et al. (2004) also noted that HA content had a positive relationship with alleviation to metal toxicity. Based on these observations, Al-Reasi et al. (2011) reviewed

the influence of NOM source on metal toxicity mitigation from the existing literature (in which NOM isolates had been collected by reverse osmosis) and compared metal toxicity parameters to NOM characteristics. Correlations between spectroscopic-determined NOM characteristics and metal toxicity were found for all of the above mentioned metals except Ag. After observing the differences in metal toxicity mitigation, most authors took the next step and tried to incorporate these disparities into models capable of predicting metal toxicity. The state of the science model in this field is the Biotic Ligand Model (BLM).

The BLM identifies the binding site of toxic action on an organism as equal to any other ligand, and defines it as a biotic ligand (BL) (De Schamphelaere and Janssen 2004, Di Toro et al. 2001 and Paquin et al. 2002). A BL is made up of a subset of negatively charged proteins on an organism (e.g. on a gill) which bind as readily with metals and various cations as other similarly charged ligands in the water (Paquin et al. 2002). More specifically, the BL has multiple transport sites for essential cations (Ca^{2+}, Na^{2+}). These sites can also transport ionoregulatory toxicants such as free ions of Cu, Cd and Ni, which disrupt the ion balance in the organism and cause toxic effects (Di Toro et al. 2001 and Niyogi and Wood 2004).

Reductions in free metal ion concentration, due to complexation with inorganic and organic ligands in solution, are also essential to the BLM. Stability constants (or log K values which describe the attractions between an ion and a ligand in solution) are given to gill-metal, gill-essential cations, as well as metal-inorganic and metal-organic interactions (Paquin et al. 2002).

The BLM takes into account the competition between cations (Ca^{2+}, Na^{2+}, Mg^{2+}) and pH (H^+ ions) with free metal ions for dissolved organic carbon (DOC), synthetic ligands and gill binding sites. Both cations and free metal ions operate in terms of concentration and affinity as

rated by stability constants; the higher the concentration and stability constant, the greater competitive success (Di Toro et al., 2001).

To describe the interactions between DOC and metal binding in the BLM, the Windermere Humic Aqueous Model (WHAM) by Tipping (1994) is utilized. It incorporates proton and metal binding, with carboxyl and phenolic sites of humic acid (Di Toro et al., 2001). It is based on a large data set of acid-base titrations; binding affinity is based on the median binding and spread around the median for both carboxyl and phenolic sites. Competition between protons, calcium and metal ions for DOC binding sites is also taken into consideration (Di Toro et al., 2001; Paquin et al., 2002; Tipping 1994). While two different binding sites are considered and the data set on which WHAM is large, the heterogeneity between NOM sources does not appear to be optimally accounted for in the BLM. Several authors have attempted to accommodate the disparity in NOM source structure by using the spectroscopic techniques used to characterize NOM.

Richards et al. (2001) created the quality factor (F) to incorporate NOM structure into a BLM. The quality factor uses NOM colour and aromaticity based on the results of SAC to alter the number of NOM binding sites. This parameter improved predictions of a Pb-BLM (McDonald et al., 2002), and Schwartz et al. (2004) also found improvements to calculate gill Cu and Pb burdens using the quality factor. De Schamphelaere et al. (2004) used the % active fulvic acid (%AFA) to describe the complexing capacity of each DOM they employed with great success for an acute Cu-BLM. Al-Reasi et al. (2012) indicated that using EEMs and PARAFAC to modify HA% to a relative HA% also improved an acute Cu-BLM, as did HA% based on the relationship between SAC and relative HA%. Two key questions remain. Do these differences

in metal toxicity with various-sourced NOMs apply to other metals not previously studied, and which of these methods used to improve the BLM produce the best results?

One important metal to investigate is nickel. This is particularly true for the Canadian environment as Canada is one of the largest nickel producing countries in the world (Mandal et al., 1999a). The environmental effects brought about by 130 years of this industry's operation remain today. Areas such as Sudbury, Ontario, are still coping with lakes containing nickel concentrations exceeding Ontario's Provincial Water Quality Objectives (25 μg L^{-1}) and Ni levels are falling more slowly than other metals in the lakes (Norman Yan personal communication). The zooplankton communities (i.e. planktonic cladocerans and copepods) of Sudbury's urban lakes have been slow to recover due to elevated metal concentrations (Valois et al., 2011) and underscore the importance of understanding nickel's potential toxic interactions with zooplankton. However, Ni's low binding affinity to NOM and high concentrations required for acute toxicity have led several authors to believe that NOM did not play a vital role in metal mitigation (Wu et al., 2003), only recently have the interactions between Ni and organic matter been considered in this matter. Kozlova et al. (2009) noted an increase in Ni LC$_{50}$s with *Daphnia pulex* when NOM was added to solution in a concentration dependent manner and also observed that two NOM isolates protected to differing degrees. In this study, I will show that NOM does play a vital role in the alleviation of Ni toxicity; that its ability to protect zooplankton from Ni toxicity is dependent on NOM source; and that it is specifically the aromatic content of NOM which plays a major role in reducing metal toxicity. Based on the relevance of Ni to the Canadian environment and the lack of information regarding Ni-NOM interaction, Ni was my metal of choice for exploring how the variability in NOM structural make-up influences metal toxicity. In chapter two of this thesis, I explore how thirteen different NOM isolates protect

Daphnia pulex/pulicaria from acute Ni toxicity, and how the differences in Ni mitigation relate to NOM structural heterogeneity.

In summary, my objectives in this thesis were to: (1) identify life history traits of a *Daphnia pulex/pulicaria* clone which are altered when exposed to NOM; (2) determine if NOM from various sources produced different results; (3) describe which measurable characteristics of NOM relate to observed life history changes; (4) establish if 7 mg $C \cdot L^{-1}$ of NOM protected a *Daphnia* hybrid from acute Ni toxicity; (5) determine if protection varied with NOM source; (6) identify NOM characteristics which correlated with amelioration of Ni toxicity; (7) ascertain if a Ni BLM can be improved with inclusion of NOM characteristics; and (8) identify which inclusion method performed best.

References (Introduction)

Adema, D. M. M. 1978. "Daphnia-Magna as a Test Animal in Acute and Chronic Toxicity Tests." *Hydrobiologia* 59 (2): 125-134.

Al-Reasi, Hassan A., D. Scott Smith, and Chris M. Wood. 2012. "Evaluating the Ameliorative Effect of Natural Dissolved Organic Matter (DOM) Quality on Copper Toxicity to *Daphnia magna*: Improving the BLM." *Ecotoxicology* 21 (2): 524-537.

Al-Reasi, Hassan A., Chris M. Wood, and D. Scott Smith. 2011. "Physicochemical and Spectroscopic Properties of Natural Organic Matter (NOM) from various Sources and Implications for Ameliorative Effects on Metal Toxicity to Aquatic Biota." *Aquatic Toxicology (Amsterdam)* 103 (3-4): 179-190.

Andersen, T., and D.O. Hessen. 1991. "Carbon, Nitrogen, and Phosphorus Content of Freshwater Zooplankton. Limnology and Oceanography 36 (4): 807-814.

Baken, Stijn, Fien Degryse, Liesbeth Verheyen, Roel Merckx, and Erik Smolders. 2011. "Metal Complexation Properties of Freshwater Dissolved Organic Matter are Explained by its Aromaticity and by Anthropogenic Ligands." *Environmental Science & Technology* 45 (7): 2584-2590.

Baker, H. and F. Khahli. 2003. "Comparative Study of Binding Strengths and Thermodynamic Aspects of Cu(II) and Ni(II) with Humic Acid by Schubert's Ion-Exchange Method." *Analytica Chimica Acta* 497 (1-2): 235-248.

Bedulina, Darya S., Maxim A. Timofeyev, Martin Zimmer, Elke Zwirnmann, Ralph Menzel, and Christian E. W. Steinberg. 2010. "Different Natural Organic Matter Isolates Cause Similar Stress Response Patterns in the Freshwater Amphipod, *Gammarus pulex*." *Environmental Science and Pollution Research International* 17 (2): 261-269.

Benzie, John A. H. 2005. *The Genus Daphnia (Including Daphniopsis) : (Anomopoda, Daphniidae)*. Guides to the Identification of the Microinvertebrates of the Continental Waters of the World,. Ghent: Kenobi Productions.

Berggren, Martin, Hjalmar Laudon, Mahsa Haei, Lena Strom, and Mats Jansson. 2010. "Efficient Aquatic Bacterial Metabolism of Dissolved Low-Molecular-Weight Compounds from Terrestrial Sources." *Isme Journal* 4 (3): 408-416.

Boersma, M. and C. Kreutzer. 2002. "Life at the Edge: Is Food Quality really of Minor Importance at Low Quantities?" *Ecology* 83 (9): 2552-2561.

Borisover, Mikhail, Yael Laor, Arkady Parparov, Nadezhda Bukhanovsky, and Marcos Lado. 2009. "Spatial and Seasonal Patterns of Fluorescent Organic Matter in Lake Kinneret (Sea of Galilee) and its Catchment Basin." *Water Research* 43 (12): 3104-3116.

Bouchnak, Rihab and Christian E. W. Steinberg. 2010. "Modulation of Longevity in *Daphnia magna* by Food Quality and Simultaneous Exposure to Dissolved Humic Substances." *Limnologica* 40 (2, Sp. Iss. SI): 86-91.

Brett, Michael T., Martin J. Kainz, Sami J. Taipale, and Hari Seshan. 2009. "Phytoplankton, Not Allochthonous Carbon, Sustains Herbivorous Zooplankton Production." *Proceedings of the National Academy of Sciences of the United States of America* 106 (50): 21197-21201.

Brett, Michael T. and Dorthe C. Mueller-Navarra. 1997. "The Role of Highly Unsaturated Fatty Acids in Aquatic Foodweb Processes." *Freshwater Biology* 38 (3): 483-499.

Celo, V., J. Murimboh, M. S. A. Salam, and C. L. Chakrabarti. 2001. "A Kinetic Study of Nickel Complexation in Model Systems by Adsorptive Cathodic Stripping Voltammetry." *Environmental Science & Technology* 35 (6): 1084-1089.

Cole, Jonathan J., Stephen R. Carpenter, Jim Kitchell, Michael L. Pace, Christopher T. Solomon, and Brian Weidel. 2011. "Strong Evidence for Terrestrial Support of Zooplankton in Small Lakes Based on Stable Isotopes of Carbon, Nitrogen, and Hydrogen." *Proceedings of the National Academy of Sciences of the United States of America* 108 (5): 1975-1980.

Curtis, P. Jefferson and David W. Schindler. 1997. "Hydrologic Control of Dissolved Organic Matter in Low-Order Precambrian Shield Lakes." *Biogeochemistry (Dordrecht)* 36 (1): 125-138.

Dahlen, J., S. Bertilsson, and C. Pettersson. 1996. "Effects of UV-A Irradiation on Dissolved Organic Matter in Humic Surface Waters." *Environment International* 22 (5): 501-506.

De Schamphelaere, K. A. C. and C. R. Janssen. 2004. "Effects of Dissolved Organic Carbon Concentration and Source, pH, and Water Hardness on Chronic Toxicity of Copper to *Daphnia magna*." *Environmental Toxicology and Chemistry* 23 (5): 1115-1122.

De Schamphelaere, K. A. C., F. M. Vasconcelos, F. M. G. Tack, H. E. Allen, and C. R. Janssen. 2004. "Effect of Dissolved Organic Matter Source on Acute Copper Toxicity to Daphnia magna." *Environmental Toxicology and Chemistry* 23 (5): 1248-1255.

Di Toro, D. M., H. E. Allen, H. L. Bergman, J. S. Meyer, P. R. Paquin, and R. C. Santore. 2001. "Biotic Ligand Model of the Acute Toxicity of Metals. 1. Technical Basis." *Environmental Toxicology and Chemistry* 20 (10): 2383-2396.

Dodson, Stanley I. and Takayuki Hanazato. 1995. "Commentary on Effects of Anthropogenic and Natural Organic Chemicals on Development, Swimming Behavior, and Reproduction of *Daphnia*, a Key Member of Aquatic Ecosystems." *Environmental Health Perspectives* 103 (SUPPL. 4): 7-11.

Engert, A., S. Chakrabarti, N. Saul, M. Bittner, R. Menzel, and C. E. Steinberg. 2013. "Interaction of Temperature and an Environmental Stressor: *Moina macrocopa* Responds

with Increased Body Size, Increased Lifespan, and Increased Offspring Numbers Slightly Above its Temperature Optimum." *Chemosphere* 90 (7): 2136-2141.

Erickson, R. J., D. A. Benoit, V. R. Mattson, H. P. Nelson, and E. N. Leonard. 1996. "The Effects of Water Chemistry on the Toxicity of Copper to Fathead Minnows." *Environmental Toxicology and Chemistry* 15 (2): 181-193.

Ertel, J. R., J. I. Hedges, and E. M. Perdue. 1984. "Lignin Signature of Aquatic Humic Substances." *Science (Washington D C)* 223 (4635): 485-487.

Euent, Sandra, Ralph Menzel, and Christian E. W. Steinberg. 2008. "Gender-Specific Lifespan Modulation in *Daphnia magna* by a Dissolved Humic Substances Preparation." *Annals of Environmental Science*: 7-10.

Fee, E. J., R. E. Hecky, S. E. M. Kasian, and D. R. Cruikshank. 1996. "Effects of Lake Size, Water Clarity, and Climatic Variability on Mixing Depths in Canadian Shield Lakes." *Limnology and Oceanography* 41 (5): 912-920.

Fellman, Jason B., Eran Hood, and Robert G. M. Spencer. 2010. "Fluorescence Spectroscopy Opens New Windows into Dissolved Organic Matter Dynamics in Freshwater Ecosystems: A Review." *Limnology and Oceanography* 55 (6): 2452-2462.

Glover, C. N., S. K. Sharma, and C. M. Wood. 2005a. "Heterogeneity in Physicochemical Properties Explains Differences in Silver Toxicity Amelioration by Natural Organic Matter to *Daphnia magna*." *Environmental Toxicology and Chemistry* 24 (11): 2941-2947.

Glover, Chris N., Eric F. Pane, and Chris M. Wood. 2005b. "Humic Substances Influence Sodium Metabolism in the Freshwater Crustacean *Daphnia magna*." *Physiological and Biochemical Zoology* 78 (3): 405-416.

Glover, Chris N. and Chris M. Wood. 2005. "The Disruption of *Daphnia magna* Sodium Metabolism by Humic Substances: Mechanism of Action and Effect of Humic Substance Source." *Physiological and Biochemical Zoology* 78 (6): 1005-1016.

Goss, L. B. and D. L. Bunting. 1980. "Temperature Effects on Zooplankton Respiration." *Comparative Biochemistry and Physiology A-Physiology* 66 (4): 651-658.

Grey, J., R. I. Jones, and D. Sleep. 2001. "Seasonal Changes in the Importance of the Source of Organic Matter to the Diet of Zooplankton in Loch Ness, as Indicated by Stable Isotope Analysis." *Limnology and Oceanography* 46 (3): 505-513.

Haitzer, Markus, Gudrun Abbt-Braun, Walter Traunspurger, and Christian E. W. Steinberg. 1999. "Effects of Humic Substances on the Bioconcentration of Polycyclic Aromatic Hydrocarbons: Correlations with Spectroscopic and Chemical Properties of Humic Substances." *Environmental Toxicology and Chemistry* 18 (12): 2782-2788.

Hanski, I. and E. Ranta. 1983. "Coexistence in a Patchy Environment 3 Species of *Daphnia* in Rock Pools." *Journal of Animal Ecology* 52 (1): 263-280.

Hofmann, Sylva, Maxim A. Timofeyev, Anke Putschew, Nadine Saul, Ralph Menzel, and Christian E. W. Steinberg. 2012. "Leaf Litter Leachates have the Potential to Increase Lifespan, Body Size, and Offspring Numbers in a Clone of *Moina macrocopa*." *Chemosphere* 86 (9): 883-890.

Jaffe, R., D. McKnight, N. Maie, R. Cory, W. H. McDowell, and J. L. Campbell. 2008. "Spatial and Temporal Variations in DOM Composition in Ecosystems: The Importance of Long-Term Monitoring of Optical Properties." *Journal of Geophysical Research-Biogeosciences* 113 (G4): G04032.

Jansson, Mats, Lennart Persson, Andre M. De Roos, Roger I. Jones, and Lars J. Tranvik. 2007. "Terrestrial Carbon and Intraspecific Size-Variation Shape Lake Ecosystems." *Trends in Ecology & Evolution* 22 (6): 316-322.

Karlsson, J., A. Jonsson, M. Meili, and M. Jansson. 2003. "Control of Zooplankton Dependence on Allochthonous Organic Carbon in Humic and Clear-Water Lakes in Northern Sweden." *Limnology and Oceanography* 48 (1): 269-276.

Kozlova, Tatiaria, Chris M. Wood, and James C. McGeer. 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." *Aquatic Toxicology* 91 (3): 221-228.

Lampert, W. 1978. "Field Study on the Dependence of the Fecundity of *Daphnia* Species on Food Concentration." *Oecologia* 36: 363-369.

Luider, Chad D., John Crusius, Richard C. Playle, and P. Jeff Curtis. 2004. "Influence of Natural Organic Matter Source on Copper Speciation as Demonstrated by Cu Binding to Fish Gills, by Ion Selective Electrode, and by DGT Gel Sampler." *Environmental Science & Technology* 38 (10): 2865-2872.

Macdonald, A., L. Silk, M. Schwartz, and R. C. Playle. 2002. "A Lead-Gill Binding Model to Predict Acute Lead Toxicity to Rainbow Trout (*Oncorhynchus mykiss*)." *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology* 133 (1-2): 227-242.

McKnight, Diane M., Elizabeth W. Boyer, Paul K. Westerhoff, Peter T. Doran, Thomas Kulbe, and Dale T. Andersen. 2001. "Spectrofluorometric Characterization of Dissolved Organic Matter for Indication of Precursor Organic Material and Aromaticity." *Limnology and Oceanography* 46 (1): 38-48.

Meinelt, Thomas, Andrea Paul, Thuy My Phan, Elke Zwirnmann, Angela Krueger, Andreas Wienke, and Christian E. W. Steinberg. 2007. "Reduction in Vegetative Growth of the Water Mold *Saprolegnia parasitica* (Coker) by Humic Substance of Different Qualities." *Aquatic Toxicology (Amsterdam)* 83 (2): 93-103.

Molot, L. A., W. Keller, P. R. Leavitt, R. D. Robarts, M. J. Waiser, M. T. Arts, T. A. Clair, et al. 2004. "Risk Analysis of Dissolved Organic Matter-Mediated Ultraviolet B Exposure in Canadian Inland Waters." *Canadian Journal of Fisheries and Aquatic Sciences* 61 (12): 2511-2521.

Nadella, Sunita R., John L. Fitzpatrick, Natasha Franklin, Carol Bucking, Scott Smith, and Chris M. Wood. 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." *Comparative Biochemistry and Physiology Part C Toxicology & Pharmacology* 149 (3): 340-348.

Nebbioso, Antonio and Alessandro Piccolo. 2013. "Molecular Characterization of Dissolved Organic Matter (DOM): A Critical Review." *Analytical and Bioanalytical Chemistry* 405 (1): 109-124.

Niyogi, S. and C. M. Wood. 2004. "Biotic Ligand Model, a Flexible Tool for Developing Site-Specific Water Quality Guidelines for Metals." *Environmental Science & Technology* 38 (23): 6177-6192.

Pace, Michael L., Jonathan J. Cole, Stephen R. Carpenter, James F. Kitchell, James R. Hodgson, Matthew C. Van do Bogert, Darren L. Bade, Emma S. Kritzberg, and David Bastviken. 2004. "Whole-Lake Carbon-13 Additions Reveal Terrestrial Support of Aquatic Food Webs." *Nature (London)* 427 (6971): 240-243.

Paquin, P. R., J. W. Gorsuch, S. Apte, G. E. Batley, K. C. Bowles, P. G. C. Campbell, C. G. Delos, et al. 2002. "The Biotic Ligand Model: A Historical Overview." *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology* 133 (1-2): 3-35.

Perdue, E.M. 2009. "Natural Organic Matter" in G.E. Likens, Encyclopedia of Inland Waters 2: 806-819. Oxford:Elsevier

Playle, R. C. 1998. "Modelling Metal Interactions at Fish Gills." *Science of the Total Environment* 219 (2-3): 147-163.

Playle, R. C., D. G. Dixon, and K. Burnison. 1993. "Copper and Cadmium-Binding to Fish Gills - Modification by Dissolved Organic-Carbon and Synthetic Ligands." *Canadian Journal of Fisheries and Aquatic Sciences* 50 (12): 2667-2677.

Richards, J. G., P. J. Curtis, B. K. Burnison, and R. C. Playle. 2001. "Effects of Natural Organic Matter Source on Reducing Metal Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) and on Metal Binding to their Gills." *Environmental Toxicology and Chemistry* 20 (6): 1159-1166.

Ryan, A. C., E. J. Van Genderen, J. R. Tomasso, and S. J. Klaine. 2004. "Influence of Natural Organic Matter Source on Copper Toxicity to Larval Fathead Minnows (*Pimephales*

promelas): Implications for the Biotic Ligand Model." *Environmental Toxicology and Chemistry* 23 (6): 1567-1574.

Schwartz, Melissa L., P. Jefferson Curtis, and Richard C. Playle. 2004. "Influence of Natural Organic Matter Source on Acute Copper, Lead, and Cadmium Toxicity to Rainbow Trout (*Oncorhynchus mykiss*)." *Environmental Toxicology and Chemistry* 23 (12): 2889-2899.

Steinberg, C. E. W., A. Paul, S. Pflugmacher, T. Meinelt, R. Klocking, and C. Wiegand. 2003. "Pure Humic Substances have the Potential to Act as Xenobiotic Chemicals - A Review." *Fresenius Environmental Bulletin* 12 (5): 391-401.

Steinberg, Christian E. W., Sheku Kamara, Valeria Yu Prokhotskaya, Levonas Manusadzianas, Tatyana A. Karasyova, Maxim A. Timofeyev, Zhang Jie, et al. 2006. "Dissolved Humic Substances - Ecological Driving Forces from the Individual to the Ecosystem Level?" *Freshwater Biology* 51 (7): 1189-1210.

Steinberg, Christian E. W., Laura Vicentic, Ramona Rauch, Rihab Bouchnak, Albert L. Suhett, and Ralph Menzel. 2010. "Exposure to Humic Material Modulates Life History Traits of the Cladocerans *Moina macrocopa* and *Moina micrura*." *Chemistry and Ecology* 26 (Suppl. 2): 135-143.

Suhett, Albert L., Christian E. W. Steinberg, Jayme M. Santangelo, Reinaldo L. Bozelli, and Vinicius F. Farjalla. 2011. "Natural Dissolved Humic Substances Increase the Lifespan and Promote Transgenerational Resistance to Salt Stress in the Cladoceran *Moina macrocopa*." *Environmental Science and Pollution Research International* 18 (6): 1004-1014.

Tatarazako, Norihisa and Shigeto Oda. 2007. "The Water Flea *Daphnia magna* (Crustacea, Cladocera) as a Test Species for Screening and Evaluation of Chemicals with Endocrine Disrupting Effects on Crustaceans." *Ecotoxicology* 16 (1): 197-203.

Tessier, A. J., L. L. Henry, C. E. Goulden, and M. W. Durand. 1983. "Starvation in *Daphnia* Energy Reserves and Reproductive Allocation." *Limnology and Oceanography* 28 (4): 667-676.

Timofeyev, M. A., Z. M. Shatilina, D. S. Bedulina, R. Menzel, and C. E. W. Steinberg. 2007. "Natural Organic Matter (NOM) has the Potential to Modify the Multixenobiotic Resistance (MXR) Activity in Freshwater Amphipods *Eulimnogammarus cyaneus* and *E.verrucosus*." *Comparative Biochemistry and Physiology Part B Biochemistry & Molecular Biology* 146 (4): 496-503.

Timofeyev, Maxim A., Zhanna M. Shatilina, Aleksey V. Kolesnichenko, Darya S. Bedulina, Viktoria V. Kolesnichenko, Stephan Pflugmacher, and Christian E. W. Steinberg. 2006a. "Natural Organic Matter (NOM) Induces Oxidative Stress in Freshwater Amphipods *Gammarus lacustris* Sars and *Gammarus tigrinus* (Sexton)." *Science of the Total Environment* 366 (2-3): 673-681.

Timofeyev, Maxim A., Zhanna M. Shatilina, Aleksey V. Kolesnichenko, Viktoria V. Kolesnichenko, and Christian E. W. Steinberg. 2006b. "Specific Antioxidant Reactions to Oxidative Stress Promoted by Natural Organic Matter in Two Amphipod Species from Lake Baikal." *Environmental Toxicology* 21 (2): 104-110.

Tipping, E. 1994. "Wham - a Chemical-Equilibrium Model and Computer Code for Waters, Sediments, and Soils Incorporating a Discrete Site Electrostatic Model of Ion-Binding by Humic Substances." *Computers & Geosciences* 20 (6): 973-1023.

Valois, Amanda E., W. Bill Keller, and Charles W. Ramcharan. 2011. "Recovery in a Multiple Stressor Environment: Using the Reference Condition Approach to Examine Zooplankton Community Change Along Opposing Gradients." *Journal of Plankton Research* 33 (9): 1417-1429.

VanGenderen, E. J., A. C. Ryan, J. R. Tomasso, and S. J. Klaine. 2003. "Influence of Dissolved Organic Matter Source on Silver Toxicity to *Pimephales promelas*." *Environmental Toxicology and Chemistry* 22 (11): 2746-2751.

von Elert, E. 2004. "Food Quality Constraints in Daphnia: Interspecific Differences in the Response to the Absence of a Long Chain Polyunsaturated Fatty Acid in the Food Source." *Hydrobiologia* 526 (1): 187-196.

Wacker, Alexander and Eric von Elert. 2001. "Polyunsaturated Fatty Acids: Evidence for Non-Substitutable Biochemical Resources in *Daphnia galeata*." *Ecology (Washington D C)* 82 (9): 2507-2520.

Weishaar, J. L., G. R. Aiken, B. A. Bergamaschi, M. S. Fram, R. Fujii, and K. Mopper. 2003. "Evaluation of Specific Ultraviolet Absorbance as an Indicator of the Chemical Composition and Reactivity of Dissolved Organic Carbon." *Environmental Science & Technology* 37 (20): 4702-4708.

Wenzel, Anja, Ann-Kristin Bergstrom, Mats Jansson, and Tobias Vrede. 2012. "Poor Direct Exploitation of Terrestrial Particulate Organic Material from Peat Layers *by Daphnia galeata*." *Canadian Journal of Fisheries and Aquatic Sciences* 69 (11): 1870-1880.

Zitko, P., W. V. Carson, and W. G. Carson. 1973. "Prediction of Incipient Lethal Levels of Copper to Juvenile Atlantic Salmon in Presence of Humic Acid by Cupric Electrode." *Bulletin of Environmental Contamination and Toxicology* 10 (5): 265-271.

Chapter 1

Direct Effects of Natural Organic Matter (NOM) Isolates from Canadian Shield waters on laboratory performance of a native *Daphnia*

Abstract

Many indirect effects of Natural Organic Matter (NOM) on biota are well understood, e.g. binding of toxic metals, absorbance of damaging UV radiation, and sustenance of bacteria. In contrast, it is unclear if NOM interacts directly with herbivores, including *Daphnia*, and this knowledge gap is concerning given that NOM concentrations are currently on the rise in north temperate lakes. In this study, five NOM isolates were obtained from Canadian Shield sources including lakes, a stream and a wetland , and added at 6 mg C·L^{-1} to a synthetic soft-water medium to determine the NOM effects on a well fed local *Daphnia pulex/pulicaria* hybrid in the laboratory. Four out of the five isolates raised the intrinsic rate of natural increase, specific growth rate, and mean clutch size of the *Daphnia* while decreasing mean generation time and time to primiparity. Changes in *Daphnia* performance metrics were not correlated with bacterial densities, or with algal feeding rate. They were, however, associated with two absorbance metrics which are used to distinguish the structural differences of NOM: specific absorbance coefficient (SAC), an index of NOM colour and aromaticity; and the specific UV absorbance (SUVA), another proxy for aromaticity. The observed positive effects of the NOM on *Daphnia* performance were likely due either to a xenobiotic hormone characteristic of the NOM, with irradiation of NOM by fluorescent light in the chamber increasing its estrogenic activity; or to mild oxidative stress, which created an overall biological benefit. The results indicate that environmentally-realistic concentrations of NOM can be directly beneficial to *Daphnia*, increasing both growth and reproductive output. Therefore, in addition to the known indirect benefits of rising NOM for aquatic biota, direct benefits are now worthy of consideration.

Introduction

Natural organic matter (NOM) is a heterogeneous (Ertel et al. 1984; Guthrie et al. 2005), ubiquitous (Hudson et al., 2007), organic substance present in shallow ground and surface waters. Composed mainly of deteriorated woody plant debris (Ertel et al., 1984), decayed animals, and microbes (Al-Reasi et al. 2011, Timofeyev et al. 2004, Chen et al. 2012), 50 to 70% of this material is made up of humic substances (HSs) (Timofeyev et al., 2004) which further segregate into humic acids (HA) and fulvic acids (FA) (Thurman 1985; Al-Reasi et al. 2011; Schindler et al. 1992; Sekaly et al., 2003; Guthrie et al., 2005). The remaining material within NOM is carbohydrates, protein, peptides and amino acids (Al-Reasi et al., 2011, Fellman et al., 2010, Jaffé et al., 2008, Hudson et al., 2007, Baker and Khalili 2003).

NOM levels are currently rising in several parts of the world, including northeastern North America, and northern and central Europe (Monteith et al., 2007). The many hypotheses that may be proposed to explain this phenomenon (Evans et al., 2006) include:
 i) reduced NOM adsorption to mineral soils, resulting in a greater loss of NOM to surface waters (Yan et al., 1996; Keller et al., 2008; Kerr and Eimers 2012), accompanying falling acidity in watersheds that followed reduced SO_2 emissions (Keller et al., 2008,Evans et al., 2006; Monteith et al., 2007); ii) reduced binding of NOM to Al in soils, as porewater pH rises (Yan et al., 1996); iii) decreased, UV-induced photo-oxidation of NOM associated with rising lake water pH (Gennings et al., 2001); iv) decreased retention of negatively charged NOM in soils associated with base cation depletion (Kerr and Eimers 2012) linked to acidification and logging/afforestation cycles; v) enhanced decomposition of organic matter in soils augmenting delivery of NOM into lakes as the climate warms (Freeman et al., 2001; Keller et al., 2008) (Keller et al., 2008); vi) elevated primary productivity of wetland plants at higher carbon dioxide

concentrations levels leading to increased root exudation of NOM and more carbon leaching into water courses (Freeman et al., 2004; Evans et al., 2006); and finally, vii) greater NOM export from agricultural soils linked to increased nitrogen deposition. While the mechanism for this last hypothesis is unclear, N does decrease oxidative enzymes, leading to reduced decomposition of phenolic compounds in the bulk NOM pool, contributing potentially to greater export of NOM to surface waters (Findlay 2005). Many of the underlying drivers of these proposed causes of NOM rise are also rising, suggesting that NOM concentrations will continue to increase (Monteith et al., 2007). To understand the potential impacts, if any, of increasing NOM concentrations on biota, we need to consider both its indirect and direct effects.

NOM plays many fundamental roles in aquatic ecosystems (Prairie 2008). As a predominant determinant of lake transparency, it impacts the thermal structure of small lakes by increasing the vertical attenuation of light (Fee et al., 1996). It is the principle factor responsible for absorption of UV-B radiation, naturally protecting aquatic organisms (Molot et al., 2004). It complexes metals (Playle et al., 1993) and organic contaminants (Haitzer et al., 1999), determining the biotransport of essential metals, and the bioavailability of toxic metals. Furthermore, it has long been recognized that NOM serves as an energy source for aquatic food webs as a major food source for bacteria (Jones 1992 and references within) and a possible component in the diets of aquatic organisms (e.g. zooplankton and fish) at higher trophic levels (Pace et al., 2004). Via its effects on underwater light climate, coloured NOM may also affect ecological and ethological interactions in lakes, e.g. mate selection in fish (Sabbah et al. 2010), and vulnerability of pelagic macroinvertebrates to their fish predators (Wissel et al. 2003b)

NOM clearly has major indirect effects on aquatic metazoa. Might it also have direct effects? It has traditionally been assumed that NOM is too large and inert to cause any direct

effects on aquatic biota, but recent research is shifting this paradigm. It is now believed that NOM can cause molecular and biochemical changes in organisms (Steinberg et al., 2010b), eliciting direct physiological effects.   For example, NOM isolates from various sources were found to increase reproduction of the nematode *Caenorhabditis elegans* (Höss et al., 2001; Steinberg et al., 2002), and create oxidative stress in four freshwater amphipods (Timofeyev et al., 2004; 2006a; 2006b).

While NOM and its HS components have been found to induce physiological changes in aquatic organisms, the majority of the few studies in this field have employed commercial, synthetic HSs, as the NOM source. HS1500, a synthetic HS, stimulated metabolism in swordtail fish (*Xiphophorus helleri*) and initiated a hormone-like effect by altering their sex ratio (Meinelt et al., 2004). Hormone-like effects also followed exposure in the South African clawed frog (*Xenopus laevis*) by increasing expression of the estrogenic biomarker Er-mRNA as well as enhancing the expression of the thyroid-stimulating hormone, TSHβ-mRNA (Lutz et al., 2005). Another commercial HS preparation, HuminFeed®, elicited a hormone-like effect in *Daphnia magna*, altering the sex ratio of offspring by inducing the production of males (Euent et al., 2008).

Glover and Wood (2005) cautioned against using these synthetic HSs in their study of sodium metabolism in *Daphnia magna*. They reported that commercially available Aldrich humic acid disrupted the sodium metabolism of the *Daphnia,* while an NOM isolate from a marsh in Ontario, Canada, did not produce the same metabolic disruption. While use of synthetic HSs provides numerous benefits, such as ready access, consistency and lower cost, or, say, the reduced risk of contamination by xeno or phytoestrogens (Steinberg et al., 2004), too few studies have compared natural NOM isolates with synthetic HSs to establish if synthetic HSs

produce environmentally relevant responses. There is reason to question the value of work based on synthetic HSs, as they lack various features often associated with natural NOM (Meinelt et al., 2007; Hofmann et al., 2012).

How might natural NOM directly elicit physiological responses in aquatic biota? Menzel et al., (2012) found that phenolic and quinonoid moieties of HSs may be responsible for extended lifespan, increased tolerance to thermal stress, and stronger pumping of the pharynx in the nematode, *Caenorhabditis elegans*. Meinelt et al., (2007) established that HSs characterized by high molecular weights and high aromaticity inhibited the growth of the fungal fish pathogen *Saprolegnia parasitica*, to the benefit of the fish.   Little else is currently known, because the characteristics of natural NOMs have so rarely been linked with biological effects, including growth and reproduction. However, steady progress in fluorescence and absorbance technologies (Fellman et al. 2010) has provided means for characterizing NOM in an easy, non-invasive (Senesi et al. 1990), affordable and dependable manner (Jaffé et al. 2008). Metrics developed from fluorescence and absorbance spectra can distinguish molecular differences between NOMs from natural sources, and between their fulvic and humic acid fractions (Senesi et al., 1991). Such techniques have been useful in understanding the protective effects of NOM on metal toxicity (Al-Reasi et al., 2011). I hypothesized that these methods might be of similar assistance in explaining which features of natural NOM have a direct influence on the life history traits of a common cladoceran herbivore, *Daphnia*, and might also distinguish NOMs from different sources which might differ in their magnitude of impact on life history parameters.

Daphnia, is a key species recommended for ecotoxicological research and monitoring by the ASTM (ASTM 2012), OECD (2004, 2012), US-EPA (2002), and Environment Canada (1996, 2007). The sensitivity of daphniids to chemicals (Adema, 1978), their wide geographic

range (they can be found on all continents) (Benzie, 2005), their ubiquity within most permanent (Tatarazako and Oda 2007) and transient lakes and ponds (Hanski and Ranta, 1983), their key role in aquatic food webs as food for fish and invertebrate predators, as well as their role in maintaining water quality by consuming algae (Dodson and Hanazato 1995) all make *Daphnia* a good choice for studying the physiological changes potentially induced by the xenobiotic (Steinberg et al., 2003) characteristics of NOM.

Several studies on the direct effects of commercial HSs have used *Daphnia magna* as their test subject (Glover and Wood, 2005; Euent et al., 2008; Bouchnak and Steinberg 2010 and Steinberg et al., 2010b). As *Daphnia magna* is not present in the soft water lakes of the Canadian Shield, a local *Daphnia pulex/pulicaria* hybrid isolated from the Sudbury region of Ontario, Canada, was chosen for this study. Exposures of *D. magna* to HSs have produced conflicting results. Euent et al., (2008) did not find a quantitative change in reproductive output, but did observe a decrease in female lifespan; conversely, Bouchnak and Steinberg (2010) and Steinberg et al. (2010b) noted a decrease in reproductive output and an overall increase in lifespan. Therefore, I hypothesized that the *Daphnia pulex/pulicaria* used in this study would experience a change in reproduction when exposed to NOM; however, the current knowledge makes it difficult to ascertain whether it will be positive or negative. Furthermore, only a single study (by Glover and Wood, 2005) used more than one humic substance. While the two substances produced dissimilar results, it is difficult to identify whether the differences were due to the structural makeup of the HS, or attributed to their disparate origins (natural and commercial). Thus it is difficult to surmise whether the source of the NOM will impact the life history traits of *Daphnia*. With the lack of studies on the effects of NOM structure on *Daphnia*, I made no specific hypothesis with regards to how NOM structure would affect *Daphnia* health.

My aims were to determine if: i) NOM influences the demographics of a native *Daphnia*
(i.e. growth, survival, time to primiparity, fecundity); ii) NOM from various sources affect the
Daphnia in different ways; and iii) structural differences among NOM isolates are correlated
with performance changes in NOM-exposed *Daphnia*.

Materials and Methods

NOM Collection:

NOM was collected from five sites in Sudbury, Ontario, Canada. The Sudbury region is
in a state of recovery from over a century of the regional mining and smelting of metal rich ores,
exacerbated by deforestation from the mass harvesting of timber to fuel early smelters (Gunn,
1995). Airborne contaminants dramatically elevated the sulfur and metal concentrations of the
lakes, bringing about acidification, metal contamination and biotic depletion in the mid-twentieth
century. Changes in SO_2 and metal emission regulations beginning in the 1970s enabled the start
of a slow recovery in the region (Keller and Yan 1990). I selected five sample sites as part of a
larger project known as Terrestrial Aquatic Linkages for Ecosystem Recovery (TALER), which
has the main objective of determining the regulators and effects of cation and carbon exports on
aquatic systems in the early stages of recovery. My sample sites included lakes, a wetland and a
stream. A portable reverse osmosis (RO) apparatus was used to collect the samples. Collecting
NOM by RO yields the highest quantities of NOM, and it is fast and chemically gentle (Perdue
2009). After collection, the pH of the NOM concentrate was lowered to approximately two by
treating it with a cation exchange resin (Resin USF C-211 (H) Cation, Siemens) which had been
activated with 4 N HCl (Schwartz et al., 2004) with intent to remove all major and minor cations.
It was then stored, un-illuminated, at 4℃ refrigeration.

Culturing:

The *Daphnia pulex/pulicaria* hybrid I employed was obtained from McFarlane Lake in Sudbury, Ontario, Canada, and cultured for 3 years at the Field Laboratory for the Assessment of Multiple Ecological Stressors (FLAMES) in Dorset, Ontario. This pond (*pulex*)/lake(*pulicaria*) hybrid is quite common in Sudbury-area lakes and is one of the first Daphnia to inhabit lakes as they recover from acidification and high metal concentrations (Yan unpub. Data). Animals were held in Conviron E7/2 growth chambers, at 20 °C with a diurnal split of 16 light hours at 100 µmol m^{-2} s^{-1}, and 8 dark hours with 20 minute transitioning periods simulating dawn and dusk. The animals were cultured in 1 L glass beakers with approximately 800 ml of the synthetic soft water FLAMES medium (Celis-Salgado et al. 2008). The medium was made up at least 24 hours in advance of use and aerated to allow chemical equilibrium and stabilization. *D. pulex/pulicaria* were fed a combination of *Pseudokirchneriella subcapitata* and *Ankistrodesmus falcatus* at a particulate C level of 1 mg/L. Grown in batch cultures, algae were derived from pure stocks originally acquired from the University of Toronto Culture Collection (currently the Canadian Phycological Culture Centre). Each beaker contained thirty *Daphnia*, algae were dispensed into the beakers three times a week, the soft water medium was changed twice a week. Broods three to eight were placed individually in 45 mL glass vials with the same feeding regime, the changing of soft water medium was increased to three times weekly to obtain the maternal lines used for neonate production for experiments.

Bioassay and Growth:

An 18-day, partial life-cycle, static-renewal bioassay was initiated with 3rd to 8th brood neonates less than 24-h old (Environment Canada, 1996). FLAMES, a synthetic soft-water

medium, was modified by replacing the EDTA with the collected NOM. FLAMES was inoculated with 6 mg $C·L^{-1}$ of NOM from five various sources. This concentration was chosen as boreal shield lakes in Ontario often have NOM concentrations of 6 mg $C·L^{-1}$ or below (Keller et al., 2008). Before inoculation, the DOC concentrations of FLAMES ranged from 0.6 to 1.3 mg $C·L^{-1}$, attributed mostly to the vitamin solution in the medium. Prior to adding the NOM, solutions were brought up to a pH of 6.4 with 0.1 M NaOH (this is the pH we normally employ in our soft-water assays). There were ten replicates of the control and NOM-inclusive solutions. Neonates were rinsed twice with FLAMES-without-EDTA to minimize transfer of EDTA and algae from the medium in which they were cultured. During the bioassay, individuals were held in vials within 45 mL of solution with media changes occurring every other day. Each day, animals were fed 1 mg particulate $C·L^{-1}$ of non-axenic algae to ensure *Daphnia* growth was not food-limited (Lampert and Schober 1990) with a combination of *Pseudokirchneriella subcapitata* and *Ankistrodesmus falcatus*. Animal survival and neonate production were scored every day. Offspring were removed from solutions when the medium was changed. Before each use, all containers were base, then acid washed to remove all organic materials and rinsed 7 times in RO water. Test solutions were prepared a minimum of 24-h in advance (to ensure they reached equilibrium) and were not aerated. To quantify daphniid weights at the initiation of the experiment, 24 randomly chosen neonates were rinsed with de-ionized water and transferred to Teflon® strips placed within a large plastic Petri tray. Teflon® strips allowed for easy removal of dried *Daphnia*. Three neonates were placed on each Teflon® strip. Animals were dried for 48-h at 65℃ (Shapiera et al., 2011) then left to stand over silica gel in a desiccator for 20 minutes to allow animals and trays to cool. Animals were removed from the Teflon® inserts using paint brushes with plastic bristles and weighed to the nearest µg on a Cahn 29 Automatic

Electrobalance (Cahn Instruments Inc., Cerritos, California). All plates, inserts and brushes were base, then acid washed prior to use. At the completion of the bioassay, six *Daphnia* from each treatment were dried and weighed in accordance with the above protocol; however, these larger animals were weighed individually.

Chemical analyses:

The pH, dissolved oxygen and temperature of each test solution was measured at the start of the experiment; day 8; and at test finish. At test initiation and completion, solutions were also analysed for alkalinity, colour, conductivity, ammonia, phosphorous, dissolved inorganic carbon, sulphate and chloride, calcium, magnesium, potassium and sodium. The pH of solutions was measured with an Accumet® Basic AB15 pH Meter (Fisher Scientific), and pHC2001-8 electrode (Radiometer Analytical, France) calibrated with buffer solutions of pH 4, 7 and 10. Dissolved oxygen and temperature were measured with a ProODO® digital professional series YSI handheld optical dissolved oxygen meter. Alkalinity, colour, conductivity, ammonia, phosphorous, dissolved inorganic carbon, sulphate and chloride, calcium, magnesium, potassium, sodium and dissolved organic carbon (DOC) were measured by chemists of the Ontario Ministry of the Environment, using their standard protocols (MOE 1983)

NOM Characterization:

For absorbance measures, concentrated NOM solutions were diluted to 10 mg C·L^{-1} using RO water with a normal conductivity of 18.2 MΩ. Solution pH was adjusted to 7.0±0.1 with a 0.1 M NaOH solution, then filtered with a 3-mL syringe (Luer-LOK Tip Franklin Lakes, NJ, USA) fitted with a 0.45-μm Acrodisc® syringe filter (Pall Corporation, Ann Arbor, MI, USA). Absorbances were measured at 254, 340 and 365 nm in a 1 cm quartz spectrophotometer

cuvet (VWR, West Chester, PA, USA) with a UV-Vis spectrophotometer (Thermo Scientific

Genesys, G 10s UV-Vis, Madison, WI, USA)(Al-Reasi et al. 2012). A specific absorbance

coefficient (SAC), used as a colour and aromatic index, was obtained using the equation from

Curtis and Schindler (1997) SAC = ((2.303* absorbance at 340 nm)/pathlength))/ ([DOC]/1000).

A second measure of aromaticity, known as the specific UV absorbance (SUVA), was obtained

following Weishaar et al. (2003), as:

$$SUVA = Abs_{254} \, (m^{-1})/[DOC \, (mg \cdot L^{-1})]$$

To obtain the approximate molecular weight of the NOM molecules, absorbance at 254 nm was

divided by the absorbance at 365 nm (Dahlen et al. 1996). All absorbance measurements were

made in triplicate for each NOM sample. The average variance among triplicates was <1%.

Fluorescence values for each NOM source were obtained from K. Livingstone (Wilfred Laurier

University, unpublished). Emission intensity was measured at 450 nm and 500 nm; the

excitation wavelength of 370 nm was used to determine the fluorescence index (FI) as

established by McKnight et al. (2001). This was designated to indicate if the source of NOM

was allochthonous (terrestrial in origin and washed into water bodies from the surrounding

catchment) or autochthonous (produced within the water column from macrophytes, algae,

bacteria, and/or microbes; or photodegradation of terrestrially derived NOM (Leenheer and

Coure, 2003; Curtis and Schindler, 1997; McKnight et al., 2001; Al Reasi et al., 2011)). The

stream and wetland were expected to produce NOM that was more allochthonous in origin and

the lakes more autochthonous. FI values closer to 1.4 and below indicate that NOM is

allochthonous in nature while those closer to 1.9 and above indicate more autochthonous NOM.

Emissions and Excitation Matrices (EEMs) provide a unique fingerprint for NOM (Luider et al.,

2004). EEMs, in combination with parallel factor analysis (PARAFAC), designate the main components of NOM and establish relative component concentration, thereby allowing NOM isolate composition to be compared within a data set to reveal trends between NOM composition and biological function (Nadella et al. 2009).

Statistics

The five parameters used to measure daphniid health included the intrinsic rate of natural increase (r); mean generation time (T_bar); specific growth rate (g); time to primiparity; and mean clutch size. The two life history parameters (T_bar and r) were calculated using the PopTools add-in for Microsoft Excel (http://www.ese.csiro.au/poptools). T_bar designates the average amount of time separating births of one generation from the next; and r incorporates age, age-specific survival, and age-specific fecundity (Carey, 1993) to determine the immediate per capita increase rate in a population with established age distribution.

Specific growth rates (g) (a measure of individual animal mass) were calculated according to Lampert and Trubetskova (1996) as:

$$g = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

W_2 is the dry mass of six individual surviving *Daphnia* from each treatment at test termination. Twenty-four random *Daphnia* were used to determine initial dry mass (W_1). Termination was on day 18 of the test (t_2). Fecundity was assessed as the mean number of neonates released per brood for each experimental *Daphnia*. Time to primiparity in days was also recorded. The mean and standard error for r and T_bar were computed using Microsoft Excel with a Jackknife procedure (Miller, 1974; Efron, 1981) which is recommended for cladoceran populations (Meyer et al., 1986).

I had intended to remove all cations from the NOM with the resinating procedure, but was only partially successful (Table 2). Thus, before linking demographic factors of the daphniids with NOM characteristics, I first had to remove any affects of residual cations in the NOM solutions. Thus I employed stepwise regression to see the degree to which the five demographic factors (T_bar, r, g, mean clutch and time to primiparity) could be predicted from the calcium, magnesium or nickel levels in the NOM solution. There was in fact a significant influence of Ca for four of the metrics, and Ni for specific growth rate (Table 3), although the variance explained was modest. Nonetheless, all subsequent analyses employed the residuals of these linear regression models (Table 3) to ensure we were examining only the NOM effect, not any artifactual effects of differences in Ca or Ni levels among treatments. Means of residuals were compared with a one-way ANOVA, a *post-hoc* Dunnett's procedure ($P < 0.05$) was used to determine differences between the control and the five NOM treatments for each demographic factor and Tukey Honestly Significant Difference (HSD) test ($P < 0.05$) was used to determine differences between each NOM for each demographic factor. To determine if a relationship existed between each *Daphnia* demographic metric in the treatments and the six spectroscopic NOM characteristics, linear regression analysis was employed at $P < 0.05$ for hypothesis testing.

Results

The addition of NOM (6 mg C·L^{-1}) did not alter *Daphnia* survival over 18 days. The controls had 100% survival; three of the five NOM treatments had 100% survival while two NOM treatments, Daisy Lake (DL) and Lake Laurentian (LL) had 90% survival.

At 6 mg C·L^{-1} of NOM, the five demographic factors (T_bar, r, g, mean clutch, and time to primiparity) used to measure *D. pulex/pulicaria* health were affected by NOM addition. The

intrinsic rate of natural increase ranged by almost two fold among NOM treatments, from 0.24 day^{-1} in DL to 0.44 day^{-1} in Clearwater Lake outflow (SSW). The Dunnett's procedure indicated that 3 NOM treatments produced values of r that differed significantly from the control, with DL NOM being lower, while r in all other treatments was higher than the control (Fig. 1B), SSW and Laurentian Wetland (LW) were significantly so. NOM structure appeared to exert a strong influence (mostly positive) on a population-level response of *Daphnia*. Mean generation time was decreased by all of the NOMs, except DL, where it increased to 12.7 days (Fig. 1A). Addition of the four NOMs caused changes in T_bar (generation time) from 11.4 days (control) to 9.3 days (SSW). A similar result was observed for time to primiparity where DL (11.4 days) increased compared to the control (10.4 days) while all other NOMs decreased the juvenile duration, two of the NOMs did so to a significant degree: LW (8.6 days) and SSW (8.1 days) (Fig. 1D). Exposure to most NOMs decreased the period of time *Daphnia* required to reach reproductive maturity, thus decreasing time between consecutive generations. Specific growth rate was increased by all NOM sources, but only the SSW treatment growth rate of 3.3 µg ·day^{-1} was significantly greater than the control rate of 2.3 µg ·day^{-1} (Fig. 1C). Mean clutch size increased with exposure to all five of the NOM isolates, it increased to a significant degree with the addition of NOM from Clearwater Lake (CW) (14.6 eggs·brood^{-1}female^{-1}), SSW (17.4 eggs·brood^{-1} experimental female^{-1}) and LW (16.5 eggs·brood^{-1} experimental female^{-1}) compared to the control (7.9 eggs·brood^{-1} experimental female^{-1}) (Fig. 1E).

The five performance metrics of *D. pulex/pulicaria* were altered to varying degrees by the addition of five different NOM isolates at 6 mg C·L^{-1}. The intrinsic rate of natural increase ranged 3.5-fold with the addition of NOM from the five sources. The r attained with the addition of DL NOM was significantly less than all other NOMs, while r attained with LW and SSW was

significantly greater than all other isolates. Mean generation times amongst the five isolates were all significantly different from each other except between LW and SSW in the following order: LW < SSW < LL < CW< DL, with variation ranging just over two-fold. Time to primiparity also had a two-fold range in values across the five NOM isolates. The order in increasing time was the same as mean generation time; however, CW was not significantly different than either LL or DL, but LL and DL were significantly different from each other. Similar to T_bar, LW and SSW were not significantly different from each other. Specific growth rate ranged by 2.5-fold between the five NOM isolates, however, most of this range was due to LW, with the other four NOMs varying less than 0.5-fold. The LW isolate produced a significantly greater growth rate than CW and DL. Mean clutch was significantly increased by LW compared to the other NOMs (with exception of SSW).

Time to primiparity and mean generation time were significantly negatively related to SAC and SUVA; spectroscopic techniques related to the colour and aromaticity of NOM. There was an almost four-fold range in SAC values, from 9.67 cm^2mg^{-1}(CW) to 38 cm^2mg^{-1}(LW) (Fig 2), and a two-fold range in SUVA (Fig 3). Both specific growth rate and intrinsic rate of natural increase were moderately significant ($P<0.1$) in their positive relationship to SAC (Fig 2). As the colour of the NOM darkened and aromaticity content increased, the time to reach reproductive maturity decreased, days between each generation decreased, animal weight increased and the population increased at a greater rate. This said, none of the other NOM characteristics (i.e. molecular weight, origin, relative percent humic acid and fulvic acid) were related to the five health measures (data not shown). The only performance metric not correlated to any NOM structure was mean clutch size. Over the test period of 18 days, the number of broods produced varied with NOM source. I hypothesised that with decreased time to primiparity

and a greater amount of time to reproduce in the experiment, mean clutch size would increase. There was a significant negative relationship with mean number of clutches and time to primiparity, and a significant positive relationship between mean number of offspring and mean clutch number (Fig 4). NOM appears to have a greater influence over time to reproductive maturity than clutch size. Since r incorporates fecundity and time to primiparity, its positive relationship to SAC had a greater dependence on time to primiparity, i.e. on maturation rate, rather than clutch size.

Discussion

Exposing a *Daphnia* hybrid to four of five NOM sources caused an increase in somatic growth rate and mean clutch size. Increases in r and g were positively correlated to NOM colour and aromaticity while T_bar and time to primiparity diminished with increases in colour and aromatic content. These results are similar to those of Hofmann et al. (2012) who ascertained that when exposed to leaf litter leachates from more aromatic coniferous tree species, the cladoceran *Moina macrocopa* increased both reproductive output and body length when compared with exposure to two deciduous leaf litter leachates. To similar effect, Suhett et al., (2011) found a HS from a Brazilian coastal lagoon increased the body length of *M. macrocopa*. Engert et al., (2012) also witnessed an increase in reproductive output of *M. macrocopa* at 25°C with exposure to the synthetic HuminFeed®. While Steinberg et al., (2010) determined that two concentrations of HuminFeed® increased reproductive output, they also recognised that a HS from Brazil decreased offspring numbers in *M. macrocopa,* and that both HuminFeed® and the Brazilian HS decreased reproduction, body growth and time to primiparity in the clone *M. micrura*. Steinberg et al., (2010b) also described a decrease in reproduction, this time in *Daphnia magna,* when they were exposed to HuminFeed®. The influence that NOM and its

components have on Cladocera appears to depend both on the structural make-up of the NOM as well as the species under investigation.

It has long been recognised that bacteria can act as a food source for Cladocera (Gellis and Clark 1935; Salonen and Hammer 1986; Kankaala 1988: Taipale et al., 2009), and as dissolved organic matter (DOM) is consumed by bacteria as a food source (Murray and Hodson 1984; Pace et al., 2004; Cole et al., 2006), it was reasonable to surmise that the various NOMs used in this study may have contained bacteria that served as an additional food source for the *Daphnia* (which could have caused an increase in growth and reproductive output). To test this hypothesis, an aerobic colony count was performed to determine the number of viable aerobic and anaerobic bacteria per mL of each NOM isolate (except LL), at the concentration of 6 mg $C \cdot L^{-1}$ (this could not be done for LL as too little of the sample remained). This same count was performed using our synthetic soft-water medium (FLAMES) both with and without EDTA—the latter being the medium into which the NOM isolates were diluted. Each solution was incubated for 48 hours at a temperature of 35°C in plate count agar solution (Government of Canada, 2001). Bacterial counts ranged from 66 colony forming units (CFU) to 550 CFU, with the control containing the least and FLAMES-without-EDTA and LW containing the highest counts (Table 4). There were no significant differences in the quantity of bacteria between any of the solutions tested. These counts suggested that differences in *Daphnia* growth and reproduction among NOM isolates were not attributable to differences among isolates in the quantity of bacteria present that could have fed the daphniids.

Furthermore, during the resinating process the pH of each NOM solution was dropped to approximately 2, since the optimal pH range for mesophilic bacteria is narrow (Rousk et al.,

2010) (between five and eight) (Slonczewski et al., 2009) suggests that the number of bacteria surviving at such a low pH would be minimal.

Another possibility is that not the quantity, but the quality of the bacteria which were present that caused the improvements in *Daphnia* life history traits. Several studies have noted differences in *Daphnia* health when fed various bacterial diets. Diets comprised solely of bacteria have noted reduced somatic growth (Brett et al., 2009; Freese and Martin-Creuzburg 2013; Martin Creuzburg et al., 2011; Taipale et al., 2012), increased time to primiparity (Brett et al., 2009) and increased mortality (Freese and Martin-Creuzburg 2013; Martin-Creuzburg et al., 2011; Taipale et al., 2012). These are usually considered to be the result of bacteria lacking sterols and polyunsaturated fatty acids (Martin-Creuzburg et al., 2011).
However, mixed diets of bacteria and algae (where bacteria make up less than 50% of carbon supply) have been found to increase somatic growth compared to a pure algal diet possibly due to improved supply of vitamins (Freese and Martin-Creuzburg 2013). Bacteria-supplemented diets result in reduced fecundity, suggesting the results obtained in this study were not due to variations in the quality of bacteria within NOM treatments.

Particulate carbon in aquatic environments is available for uptake by zooplankton (Hessen et al., 1990); recent stable isotope studies have found that terrestrially derived particulate organic matter may contribute significantly to *Daphnia* diet (Pace et al., 2004). Since NOM contains a fine-particulate carbon portion, it is possible that this fraction of the NOM may have acted as a nutritional supplement. NOM is known to contain small amounts of sterols such as cholesterol (Schmitt-Kopplin et al., 1998; Schulten, 1999; Steinberg et al., 2002). Not only is cholesterol an essential nutrient (Martin-Creuzburg and Von Elert 2004) for growth and reproduction (Wacker and Martin-Creuzburg 2007), it may also be considered the primary sterol

for crustaceans as it is known to be a precursor of steroid hormones as well as a structural component of cell membranes (Goad 1981). De Lange and Arts (1999) found that seston sterol content was a good predictor of Daphnia growth. While NOM from the various sources may be acting as a nutritional supplement, this does not explain the relationship between r, T_bar, time to primaparity, g, and aromatic content of NOM isolates.

I hypothesize that the effects of NOMs with greater colour and higher concentrations of aromatic compounds (which are associated with greater somatic growth and increased population growth) may have been due to an indirect effect of the NOM. Waters of concentrated (darker) colour are often associated with higher densities of *Daphnia* (Wissel et al., 2003a; DeSellas et al., 2008), as well as with longer-bodied *Daphnia* (Yan et al., 2008). These changes in *Daphnia* populations are often associated with transitions in predation pressure from planktivorous fish (visual predators dominant in clearer waters) to *Chaoborus* (a zooplankton which preys upon *Daphnia* in darker waters). Ten of 14 studies in a review by Riessen (1999) indicated that *Daphnia* responded to the presence of *Chaoborus* and *Chaoborus* kairomones (a chemical cue released by predators) with an increase in body size as well as delayed times to reproduction and associated reductions in r. However, there is evidence that contradicts this. A study by Spitze (1991) observed changes in *Daphnia* showing comparable increase in body size, but conversely demonstrated reductions in time to maturity and increased reproduction when in the presence of *Chaoborus*. These findings support what I observed in this present study regarding darker NOMs producing *Daphnia* with greater somatic growth, higher r and decreased times to primiparity.

I hypothesize that *Daphnia* may have an internal mechanism which interprets changes in water colour as a visual cue, indicating a change in predation pressure from fish to *Chaoborus*,

and results in the initiation of morphological changes in size. Visual cues are known to control

Daphnia orientation (Ringelberg 1964) and swimming behaviour (Daan and Ringelberg 1969).

Young et al., (1984) found *D. magna* responded to changes in light distribution by altering their

rate of filter limb beating; as the proportion of light exposed to the side of the *Daphnia*'s head

increased, beat rate decreased. The ratio of top light to sideways scattered light is interpreted by

Daphnia as a measure of suspended particles (food) density. Visual cues have a major influence

on *Daphnia*, from orientation in the water, swimming behaviour and feeding rates. It is not

unreasonable to think that a stimulus-response system (Ringelberg and Flik 1994) is causing the

morphological changes seen in *Daphnia* which dwell in darker waters as a response to changes

in predation.

The uptake of NOM in aquatic organisms (Steinberg et al., 2003) is known to cause mild

oxidative stress in several species. Symptoms include the increasing concentrations of reactive

oxygen species (ROS) in two Baikalian gammarids (Timofeyev et al., 2006), *Gammarus*

lacustris and *Gammarus tigrinus* (Timofeyev et al., 2004), as well as raising concentrations of

the longest lived ROS, hydrogen peroxide, in *Daphnia magna* (Steinberg et al., 2010). These

conditions may bring about an effect known as hormesis, in which mild stress results in

biologically beneficial results (Mushak 2007). Effects include eliciting the formation of a stress

defense system which leads to improved metabolic health and extended lifespan such as

described in the mitohormesis theory (Ristow and Zarse 2010). While mitohormesis (an increase

in the formation of reactive oxygen species in the mitochondria, leading to an increase in stress

resistance) is most often associated with extended lifespan, several authors have suggested other

benefits observed from contact with NOM. Some such benefits involve increased body size and

increased reproduction in the cladoceran species, *Moina macrocopa* (Engert et al., 2012;

Hofmann et al., 2012) while Meinelt et al., (2007) found HSs rich in condensed highly aromatic structures (high SUVA) reduced growth of the water mould *Saprolegnia parasitica*. The authors suggested that materials characterised by high SUVA values were also associated with greater internal oxidative stress. This hormetic effect may be the cause of increased reproduction and reduced time to primiparity in this study.

NOM and some of its components have been known to have hormone-like effects. Male offspring production is increased in cladoceran species such as *D. magna* (Euent et al., 2008) and *Moina macrocopa* (Suhett et al., 2011) in the presence of HSs and NOM. Terpene analogs and juvenile hormones are known to trigger male production in cladocerans (Tatarazako and Oda 2007); this is most likely due to terpenes which are building blocks of HSs (Rostad and Leenheer 2004).

Alkylphenols in HSs are considered to have an estrogenic mode of action for *Xiphophorus helleri,* the swordtail fish which demonstrated dose dependent feminization in the presence of a synthetic HS (Meinelt et al., 2004). An estrogenic mode of action was also postulated for the feminization and increase of the estrogenic biomarker estrogen receptor (ER-mRNA) found in *Xenopus laevis,* the clawed frog, when exposed to the same synthetic HS as *X. helleri* (Lutz et al., 2005). Several studies have found NOM isolates and HSs to modulate reproduction in the nematode *Caenorhabditis elegans* (Höss et al., 2001; Steinberg et al., 2002) which is considered to be due in part to alkylaromatics (Höss et al., 2002) which are key components of HSs (Schmitt-Kopplin et al., 1998; Schulten, 1999).

Estrogenic activities were discovered in mice exposed to the humic acid portion of an isolate of a peat bog using the Allen-Doisy test (Klöcking and Helbig 2005 and references within). While Janosek et al., (2007) determined that eight out of 12 commercially available HSs

(including NOM, HA and FA) elicited anti-estrogenic effects to a human cell line, having lowest effect concentrations ranging from 16 to 150 mg C·L^{-1}. However, Chen et al., (2012) observed that—at low concentrations and after irradiation—three NOM sources significantly increased estrogenic activity of the natural estrogen 17β-estradiol (E2). Solar irradiation of NOM caused a decrease in absorbance (Chen et al., 2012) which was accompanied by release of molecules with lower mean molecular size (Bertilsson and Tranvik 1998). Various functional groups of NOM, such as phenolic hydroxyl, have been found to possess estrogenic activity. Chen et al., (2012) suggested that the molecules (which are released during irradiation) contain some of these active functional groups; alkylaromatics may also be responsible for the estrogenic activity found in these NOM isolates (Chen et al., 2012).

I measured the absorbance of the five NOM solutions prior to exposure to the chamber environment and before the addition of any *Daphnia*. To observe if the NOM was degrading, the absorbance of the same solutions was measured once again, 48-h later (48-h being the standard change cycle for the synthetic soft water medium housing *Daphnia* in this study). SAC values decreased (Table 5), suggesting it was possible that the 32-h of exposure to 100 µmol m^{-2} s^{-1} amount of light was sufficient to increase estrogenic activity. While estrogen has not been observed to affect *Daphnia magna* fecundity, the synthetic hormone 17α-ethinylestradiol (EE) was found to decrease time to primiparity in the cladoceran *Sida crystalline* (Jaser et al., 2003) while the natural hormone 17β-estradiol decreased time to primiparity and increased reproductive output in the cladoceran *Diaphanosoma celebensis* (Marcial and Hagiwara 2007). Similar observations of increased fecundity and decreased primiparity have been observed among other crustaceans such as the copepod *Acartica tonsa* (Andersen et al., 1999), and the amphipod *Gammarus pulex* (Watts et al., 2002) when exposed to the same estrogen hormones as

the two cladocerans. The *Daphnia* hybrid in this study may behave similarly to *S. crystallina* and *D. celebensis* when exposed to estrogen. The aromatic ring within estrogen may explain the link between time to primiparity and SAC.

In this study I have shown that NOM isolates from five different locations affected a native *Daphnia pulex/pulicaria* hybrid to varying degrees. Four out of the five isolates caused an increase in reproduction, intrinsic rate of natural increase, somatic growth, and a decrease in time to primiparity and mean generation time. Except for reproduction, all of these parameters were positively correlated with the aromaticity of the NOM isolates. The four-fold range in intrinsic rate of natural increase found in this study is of greater magnitude than those found by Ashforth and Yan (2008) with a change in calcium concentration from 0.5 to 2 mg·L^{-1} at three different temperatures, as well as an 8°C change (20°-28°C) in temperature. Changes in life history traits may be due to a triggering of a response to water colour, to reflect predator vulnerability, hormesis due to mild oxidative stress, or a hormonal response. Far from inert in nature, NOM's effects in this study have yielded results which are commensurate with those of both calcium declination and seasonal fluctuations in temperature to the life cycles and population growth of *Daphnia*. NOM's recent rising trends make its effects even more salient as various aquatic biota adapt to the transition.

Future studies which may be useful in testing the three hypotheses I have presented may include: assessments of how alterations of light intensity over chronic periods of time to *Daphnia* in darker waters yields changes to life history traits; investigations into which genes are regulated by the presence of NOM and how this relates to NOM characteristics; and explorations into ROS's potential to alter reproduction in *Daphnia* at low concentrations. Additionally (and not

testing my hypotheses), demonstrating if and when the effects of NOM become negative to

Daphnia could be most useful.

References (Chapter 1)

Adema, D. M. M. 1978. "*Daphnia magna* as a Test Animal in Acute and Chronic Toxicity Tests." *Hydrobiologia* 59 (2): 125-134.

Al-Reasi, Hassan A., D. Scott Smith, and Chris M. Wood. 2012. "Evaluating the Ameliorative Effect of Natural Dissolved Organic Matter (DOM) Quality on Copper Toxicity to *Daphnia magna:* Improving the BLM." *Ecotoxicology* 21 (2): 524-537.

Al-Reasi, Hassan A., Chris M. Wood, and D. Scott Smith. 2011. "Physicochemical and Spectroscopic Properties of Natural Organic Matter (NOM) from various Sources and Implications for Ameliorative Effects on Metal Toxicity to Aquatic Biota." *Aquatic Toxicology (Amsterdam, Netherlands)* 103 (3-4): 179-190.

Andersen, Henrik Rasmus, Bent Halling-Sorensen, and Kresten Ole Kusk. 1999. "A Parameter for Detecting Estrogenic Exposure in the Copepod *Acartia Tonsa*." *Ecotoxicology and Environmental Safety* 44 (1): 56-61.

Ashforth, Dawn and Norman D. Yan. 2008. "The Interactive Effects of Calcium Concentration and Temperature on the Survival and Reproduction of *Daphnia pulex* at High and Low Food Concentrations." *Limnology and Oceanography* 53 (2): 420-432.

ASTM, 2012. Standard Guide for Conducting Daphnia magna Life-Cycle Toxicity test. E1193-97 (reapproved 2012). American Society for Testing Materials, West Conshohocken.

Bahrs, H., R. Menzel, G. Kubsch, R. Stosser, A. Putschew, T. Heinze, and C. E. Steinberg. 2012. "Does Quinone Or Phenol Enrichment of Humic Substances Alter the Primary Compound from a Non-Algicidal to an Algicidal Preparation?" *Chemosphere* 87 (11): 1193-1200.

Benzie, John A. H. 2005. *The Genus Daphnia (Including Daphniopsis) : (Anomopoda, Daphniidae)*. Guides to the Identification of the Microinvertebrates of the Continental Waters of the World,. Ghent: Kenobi Productions ;.

Bertilsson, Stefan and Lars J. Tranvik. 1998. "Photochemically Produced Carboxylic Acids as Substrates for Freshwater Bacterioplankton." *Limnology and Oceanography* 43 (5): 885-895.

Bouchnak, Rihab and Christian E. W. Steinberg. 2010. "Modulation of Longevity in *Daphnia magna* by Food Quality and Simultaneous Exposure to Dissolved Humic Substances." *Limnologica* 40 (2, Sp. Iss. SI): 86-91.

Carey, James R. 1993. *Applied Demography for Biologists with Special Emphasis to Insects*. New York: Oxford University Press.

Celis-Salgado, Martha Patricia, Allegra Cairns, Natalie Kim, and Norman D. Yan. 2008. "The FLAMES Medium: A New, Soft-water Culture and Bioassay Medium for Cladocera." *Verhandlungen des Internationalen Verein Limnologie* 30 (2): 265-271.

Chen, Lei, Chaofeng Shen, Xianjin Tang, Chen Chen, and Yingxu Chen. 2012. "Estrogenic Effects of Dissolved Organic Matter and its Impact on the Activity of 17[Beta]-Estradiol." *Environmental Science and Pollution Research International* 19 (2): 522-8.

Cole, Jonathan J., Stephen R. Carpenter, Michael L. Pace, Matthew C. Van de Bogert, James L. Kitchell, and James R. Hodgson. 2006. "Differential Support of Lake Food Webs by Three Types of Terrestrial Organic Carbon." *Ecology Letters* 9 (5): 558-568.

Curtis, P. Jefferson and David W. Schindler. 1997. "Hydrologic Control of Dissolved Organic Matter in Low-Order Precambrian Shield Lakes." *Biogeochemistry (Dordrecht)* 36 (1): 125-138.

Daan, N. and J. Ringelberg. 1969. "Further Studies on the Positive and Negative Phototactic Reaction of *Daphnia magna*." *Netherlands Journal of Zoology* 19 (4): 525-540.

Dahlen, J., S. Bertilsson, and C. Pettersson. 1996. "Effects of UV-A Irradiation on Dissolved Organic Matter in Humic Surface Waters." *Environment International* 22 (5): 501-506.

De Lange, H. J. and M. T. Arts. 1999. "Seston Composition and the Potential for *Daphnia* Growth." *Aquatic Ecology* 33 (4): 387-398.

DeSellas, Anna M., Andrew M. Paterson, Jon N. Sweetman, and John P. Smol. 2008. "Cladocera Assemblages from the Surface Sediments of South-Central Ontario (Canada) Lakes and their Relationships to Measured Environmental Variables." *Hydrobiologia* 600: 105-119.

Dodson, Stanley I. and Takayuki Hanazato. 1995. "Commentary on Effects of Anthropogenic and Natural Organic Chemicals on Development, Swimming Behavior, and Reproduction of *Daphnia*, a Key Member of Aquatic Ecosystems." *Environmental Health Perspectives* 103 (SUPPL. 4): 7-11.

Efron, Bradley. 1981. "Nonparametric Estimates of Standard Error: The Jackknife, the Bootstrap and Other Methods." *Biometrika* 68 (3): 589-599.

Engert, A., S. Chakrabarti, N. Saul, M. Bittner, R. Menzel, and C. E. Steinberg. 2013. "Interaction of Temperature and an Environmental Stressor: *Moina Macrocopa* Responds with Increased Body Size, Increased Lifespan, and Increased Offspring Numbers Slightly Above its Temperature Optimum." *Chemosphere* 90 (7): 2136-2141.

Environment Canada. 2007. Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*. Report EPS 1/RM/21 Feb

Environment Canada. 1996. Biological Test Method: Acute Lethality Test Using *Daphnia* spp. Report EPS 1/RM/11 May

Ertel, J. R., J. I. Hedges, and E. M. Perdue. 1984. "Lignin Signature of Aquatic Humic Substances." *Science* 223 (4635): 485-487.

Euent, Sandra, Ralph Menzel, and Christian E. W. Steinberg. 2008. "Gender-Specific Lifespan Modulation in *Daphnia Magna* by a Dissolved Humic Substances Preparation." *Annals of Environmental Science*: 7-10.

Evans, Christopher D., Pippa J. Chapman, Joanna M. Clark, Don T. Monteith, and Malcolm S. Cresser. 2006. "Alternative Explanations for Rising Dissolved Organic Carbon Export from Organic Soils." *Global Change Biology* 12 (11): 2044-2053.

Fee, E. J., R. E. Hecky, S. E. M. Kasian, and D. R. Cruikshank. 1996. "Effects of Lake Size, Water Clarity, and Climatic Variability on Mixing Depths in Canadian Shield Lakes." *Limnology and Oceanography* 41 (5): 912-920.

Fellman, Jason B., Eran Hood, and Robert G. M. Spencer. 2010. "Fluorescence Spectroscopy Opens New Windows into Dissolved Organic Matter Dynamics in Freshwater Ecosystems: A Review." *Limnology and Oceanography* 55 (6): 2452-2462.

Findlay, Stuart E. G. 2005. "Increased Carbon Transport in the Hudson River: Unexpected Consequence of Nitrogen Deposition?" *Frontiers in Ecology and the Environment* 3 (3): 133-137.

Freeman, C., C. D. Evans, D. T. Monteith, B. Reynolds, and N. Fenner. 2001. "Export of Organic Carbon from Peat Soils." *Nature* 412 (6849): 785.

Freeman, C., N. Fenner, N. J. Ostle, H. Kang, D. J. Dowrick, B. Reynolds, M. A. Lock, D. Sleep, S. Hughes, and J. Hudson. 2004. "Export of Dissolved Organic Carbon from Peatlands Under Elevated Carbon Dioxide Levels." *Nature* 430 (6996): 195-198.

Freese, H., M. and D. Martin-Creuzburg. 2013. "Food Quality of Mixed Bacteria-Algae Diets for *Daphnia magna*." *Hydrobiologia* 715: 63-76.

Gellis, S. S., and G. L. Clarke. 1935. "Organic Matter in Dissolved and in Collodial Form as Food for *Daphnia magna*." *Physiological Zoology* 8 (2):127-137.

Gennings, Chad, Lewis A. Molot, and Peter J. Dillon. 2001. "Enhanced Photochemical Loss of Organic Carbon in Acidic Waters." *Biogeochemistry (Dordrecht)* 52 (3): 339-354.

Goad, L. J. 1981. "Sterol biosynthesis and metabolism in marine invertebrates." *Pure and Applied Chemistry* 53 (4):837-852.

Government of Canada. 2001. Determination of the Aerobic Colony Count in Foods. Microbiology Evaluation Division, Ottawa. MGHPB-18

Glover, Chris N. and Chris M. Wood. 2005. "The Disruption of *Daphnia Magna* Sodium Metabolism by Humic Substances: Mechanism of Action and Effect of Humic Substance Source." *Physiological and Biochemical Zoology* 78 (6): 1005-1016.

Gunn, M. John. 1995. *Restoration and Recovery of an Industrial Region: Progress in Restoring the Smelter-Damaged Landscape Near Sudbury, Canada*. Springer Series on Environmental Management. New York: Springer-Verlag.

Guthrie, J. W., N. M. Hassan, M. S. A. Salam, I. I. Fasfous, C. A. Murimboh, J. Murimboh, C. L. Chakrabarti, and D. C. Gregoire. 2005. "Complexation of Ni, Cu, Zn, and Cd by DOC in some Metal-Impacted Freshwater Lakes: A Comparison of Approaches using Electrochemical Determination of Free-Metal-Ion and Labile Complexes and a Computer Speciation Model, WHAM V and VI." *Analytica Chimica Acta* 528 (2): 205-218.

Haitzer, Markus, Gudrun Abbt-Braun, Walter Traunspurger, and Christian E. W. Steinberg. 1999. "Effects of Humic Substances on the Bioconcentration of Polycyclic Aromatic Hydrocarbons: Correlations with Spectroscopic and Chemical Properties of Humic Substances." *Environmental Toxicology and Chemistry* 18 (12): 2782-2788.

Hanski, I. and E. Ranta. 1983. "Coexistence in a Patchy Environment: Three Species of *Daphnia* in Rock Pools." *Journal of Animal Ecology* 52 (1): 263.

Helms, John R., Aron Stubbins, Jason D. Ritchie, Elizabeth C. Minor, David J. Kieber, and Kenneth Mopper. 2008. "Absorption Spectral Slopes and Slope Ratios as Indicators of Molecular Weight, Source, and Photobleaching of Chromophoric Dissolved Organic Matter." *Limnology and Oceanography* 53 (3): 955-969.

Hessen, D. O., T. Andersen, and A. Lyche. 1990. "Carbon Metabolism in a Humic Lake Pool Sizes and Cycling through Zooplankton." *Limnology and Oceanography* 35 (1): 84-99.

Höss, Sebastian, Matthias Bergtold, Markus Haitzer, Walter Traunspurger, and Christian E. W. Steinberg. 2001. "Refractory Dissolved Organic Matter can Influence the Reproduction of *Caenorhabditis Elegans* (Nematoda)." *Freshwater Biology* 46 (1): 1-10.

Höss, Sebastian, Ingrid Juettner, Walter Traunspurger, Gerd Pfister, Karl-Werner Schramm, and Christian E. W. Steinberg. 2002. "Enhanced Growth and Reproduction of *Caenorhabditis Elegans* (Nematoda) in the Presence of 4-Nonylphenol." *Environmental Pollution* 120 (2): 169-172.

Hofmann, Sylva, Maxim A. Timofeyev, Anke Putschew, Nadine Saul, Ralph Menzel, and Christian E. W. Steinberg. 2012. "Leaf Litter Leachates have the Potential to Increase Lifespan, Body Size, and Offspring Numbers in a Clone of *Moina Macrocopa*." *Chemosphere* 86 (9): 883-890.

Hudson, Naomi, Andy Baker, and Darren Reynolds. 2007. "Fluorescence Analysis of Dissolved Organic Matter in Natural, Waste and Polluted Waters - A Review." *River Research and Applications* 23 (6): 631-649.

Jaffé, R., D. McKnight, N. Maie, R. Cory, W.H. McDowell, and J.L. Campbell. 2008. "Spatial and Temporal Variations in DOM composition in Ecosystems: The Importance of Long-term Monitoring of Optical Properties." *Journal of Geophysical Research* 113: 1-15.

Janosek, J., M. Bittner, K. Hilscherova, L. Blaha, J. P. Giesy, and I. Holoubek. 2007. "AhR-Mediated and Antiestrogenic Activity of Humic Substances." *Chemosphere* 67 (6): 1096-1101.

Jaser, Wolfgang, Gabriele F. Severin, Uta Juetting, Ingrid Juettner, Karl-Werner Schramm, and Antonius Kettrup. 2003. "Effects of 17alpha-Ethinylestradiol on the Reproduction of the Cladoceran Species Ceriodaphnia Reticulata and Sida Crystallina." *Environment International* 28 (7): 633-638.

Jones, Roger I. 1992. "The influence of humic substances on lacustrine planktonic food chains." *Hydrobiologia* 229: 73-91.

Kankaala, P. 1988. "The Relative Importance of Algae and Bacteria as Food for *Daphnia longispina* Cladocera in a Polyhumic Lake." *Freshwater Biology* 19 (3): 285-296.

Keller, W., Andrew M. Paterson, Keith M. Somers, Peter J. Dillon, Jocelyne Heneberry, and Andrea Ford. 2008. "Relationships between Dissolved Organic Carbon Concentrations, Weather, and Acidification in Small Boreal Shield Lakes." *Canadian Journal of Fisheries and Aquatic Sciences* 65 (5): 786-795.

Keller, W. and N. D. Yan.1990. "Inferred effects of lake acidification on *Daphnia galeata mendotae*." *Environmental Science and Technology* 24: 1259-1261.

Kerr, Jason Grainger and M. Catherine Eimers. 2012. "Decreasing Soil Water Ca2+ Reduces DOC Adsorption in Mineral Soils: Implications for Long-Term DOC Trends in an Upland Forested Catchment in Southern Ontario, Canada." *Science of the Total Environment* 427: 298-307.

Klöcking, R., and R. Helbig. 2001. Medical Aspects and Applications of Humic substances In: Hofrichter M., and A. Steinbüchel (eds) Biopolymers. Vol. 1: Lignin, Humic Substances, and Coa. Wiley-VCH, Weinheim, pp379-392.

Lampert W. and U. Schober. 1990. "The Importance of "Threshold" Food Concentrations." In: Evolution and Ecology of Zooplankton Communities. Kerfoot W.C. Ed. University Press of New England. 264-267.

Leenheer, Jerry A. and Jean-Philippe Croue. 2003. "Characterizing Aquatic Dissolved Organic Matter." *Environmental Science and Technology* 37 (1): 18A-26A.

Luider, Chad D., John Crusius, Richard C. Playle, and P. Jeff Curtis. 2004. "Influence of Natural Organic Matter Source on Copper Speciation as Demonstrated by Cu Binding to Fish Gills, by Ion Selective Electrode, and by DGT Gel Sampler." *Environmental Science and Technology* 38 (10): 2865-2872.

Lutz, Ilka, Zhang Jie, Robert Opitz, Werner Kloas, Xu Ying, Ralph Menzel, and Christian E. W. Steinberg. 2005. "Environmental Signals: Synthetic Humic Substances Act as Xeno-Estrogen and Affect the Thyroid System of *Xenopus Laevis*." *Chemosphere* 61 (8): 1183-1188.

Marcial, Helen S. and Atsushi Hagiwara. 2007. "Multigenerational Effects of 17 Beta-Estradiol and Nonylphenol on Euryhaline Cladoceran Diaphanosoma Celebensis." *Fisheries Science* 73 (2): 324-330

Martin-Creuzburg, D. and E. Von Elert. 2004. "Impact of 10 Dietary Sterols on Growth and Reproduction of *Daphnia galeata*." *Journal of Chemical Ecology* 30 (3): 483-500.

Martin-Creuzburg, D., B. Beck., and H.M. Freese. 2011. "Food Quality of Heterotrophic Bacteria for *Daphnia magna*: Evidence for a Limitation by Sterols. *Federation of European Microbiological Societies* 76: 592-601.

McKnight, Diane M., Elizabeth W. Boyer, Paul K. Westerhoff, Peter T. Doran, Thomas Kulbe, and Dale T. Andersen. 2001. "Spectrofluorometric Characterization of Dissolved Organic Matter for Indication of Precursor Organic Material and Aromaticity." *Limnology and Oceanography* 46 (1): 38-48.

Meinelt, Thomas, Andrea Paul, Thuy My Phan, Elke Zwirnmann, Angela Krueger, Andreas Wienke, and Christian E. W. Steinberg. 2007. "Reduction in Vegetative Growth of the Water Mold *Saprolegnia Parasitica* (Coker) by Humic Substance of Different Qualities." *Aquatic Toxicology* 83 (2): 93-103.

Meinelt, Thomas, Kurt Schreckenbach, Klaus Knopf, Andreas Wienke, Angelika Stueber, and Christian E. W. Steinberg. 2004. "Humic Substances Affect Physiological Condition and Sex Ratio of Swordtail (*Xiphophorus Helleri Heckel*)." *Aquatic Sciences* 66 (2): 239-245.

Meyer, J. S., C. G. Ingersoll, L. L. Mcdonald, and M. S. Boyce. 1986. "Estimating Uncertainty in Population Growth Rates Jackknife Vs. Bootstrap Techniques." *Ecology* 67 (5): 1156-1166.

Miller, R. G. 1974. "The Jackknife a Review." *Biometrika* 61 (1): 1-15.

Molot, L. A., W. Keller, P. R. Leavitt, R. D. Robarts, M. J. Waiser, M. T. Arts, T. A. Clair, et al. 2004. "Risk Analysis of Dissolved Organic Matter-Mediated Ultraviolet B Exposure in Canadian Inland Waters." *Canadian Journal of Fisheries and Aquatic Sciences* 61 (12): 2511-2521.

Monteith, Donald T., John L. Stoddard, Christopher D. Evans, Heleen A. de Wit, Martin Forsius, Tore Hogasen, Anders Wilander, et al. 2007. "Dissolved Organic Carbon Trends Resulting from Changes in Atmospheric Deposition Chemistry." *Nature* 450 (7169): 537.

Murray, R. E. and R. E. Hodson. 1984. "Microbial Biomass and Utilization of Dissolved Organic Matter in the Okefenokee Swamp USA Ecosystem." *Applied and Environmental Microbiology* 47 (4): 685-692.

Mushak, Paul. 2007. "Hormesis and its Place in Nonmonotonic Dose-Response Relationships: Some Scientific Reality Checks." *Environmental Health Perspectives* 115 (4): 500-506.

Nadella, Sunita R., John L. Fitzpatrick, Natasha Franklin, Carol Bucking, Scott Smith, and Chris M. Wood. 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." *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* 149 (3): 340-348.

OECD 2012. *Daphnia magna* reproduction test. OECD Guidelines for Testing of Chemicals, 211. OECD, Paris.

OECD 2004. *Daphnia* sp., Acute Immobilisation Test. OECD Guidline for Testing of Chemicals.202. OECD, Paris.

Pace, Michael L., Jonathan J. Cole, Stephen R. Carpenter, James F. Kitchell, James R. Hodgson, Matthew C. Van do Bogert, Darren L. Bade, Emma S. Kritzberg, and David Bastviken. 2004. "Whole-Lake Carbon-13 Additions Reveal Terrestrial Support of Aquatic Food Webs." *Nature* 427 (6971): 240-243.

Palmer, Michelle E., Norman D. Yan, Andrew M. Paterson, and Robert E. Girard. 2011. "Water Quality Changes in South-Central Ontario Lakes and the Role of Local Factors in Regulating Lake Response to Regional Stressors." *Canadian Journal of Fisheries and Aquatic Sciences* 68 (6): 1038-1050.

Perdue, E.M. 2009. "Natural Organic Matter" in G.E. Likens, Encyclopedia of Inland Waters 2: 806-819. Oxford:Elsevier

Prairie, Yves T. 2008. "Carbocentric Limnology: Looking Back, Looking Forward." *Canadian Journal of Fisheries and Aquatic Sciences* 65 (3): 543-548.

Riessen, H. P. 1999. "Chaoborus Predation and Delayed Reproduction in *Daphnia*: A Demographic Modeling Approach." *Evolutionary Ecology* 13 (4): 339-363.

Ringelberg, J. 1964. "The positively phototactic reaction of *Daphnia magna* Straus: A contribution to the understanding of diurnal vertical migration." *Netherlands Journal of Sea Research* 2: 841-847.

Ringelberg, J. and B. J. G. Flik. 1994. "Increased Phototaxis in the Field Leads to Enhanced Diel Vertical Migration." *Limnology and Oceanography* 39 (8): 1855-1864.

Ristow, Michael and Kim Zarse. 2010. "How Increased Oxidative Stress Promotes Longevity and Metabolic Health: The Concept of Mitochondrial Hormesis (Mitohormesis)." *Experimental Gerontology* 45 (6): 410-418.

Rostad, Colleen E. and Jerry A. Leenheer. 2004. "Factors that Affect Molecular Weight Distribution of Suwannee River Fulvic Acid as Determined by Electrospray ionization/mass Spectrometry." *Analytica Chimica Acta* 523 (2): 269-278.

Sabbah, Shai, Raico Lamela Laria, Suzanne M Gray, and Craig W Hawryshyn. 2010. "Functional Diversity in the Color Vision of Cichlid Fishes." *BMC Biology* 8: 133-149.

Salonen, Kalevi, and Taina Hammar. 1986. "On the Importance of Dissolved Organic Matter in Nutrition of Zooplankton in Some Lake Waters." *Oecologia* 68 (2): 246-253.

Schindler, D.W., S.E. Bayley, P.J. Curtis, B.R. Parker, M.P. Stainton, C.A. Kelly. 1992. Natural and man-caused factors affecting the abundance and cycling of dissolved organic substances in Precambrian shield lakes. *Developments in Hydrobiology* 73:1-21

Schmitt-Kopplin, Philippe, Norbert Hertkorn, Hans-Rolf Schulten, and Antonius Kettrup. 1998. "Structural Changes in a Dissolved Soil Humic Acid during Photochemical Degradation Processes Under O2 and N2 Atmosphere." *Environmental Science and Technology* 32 (17): 2531-2541.

Schulten, H. -R and G. Gleixner. 1999. "Analytical Pyrolysis of Humic Substances and Dissolved Organic Matter in Aquatic Systems: Structure and Origin." *Water Research* 33 (11): 2489-2498.

Schwartz, Melissa L., P. Jefferson Curtis, and Richard C. Playle. 2004. "Influence of Natural Organic Matter Source on Acute Copper, Lead, and Cadmium Toxicity to Rainbow Trout (*Oncorhynchus Mykiss*)." *Environmental Toxicology and Chemistry* 23 (12): 2889-2899.

Senesi, Nicole. 1990. "Molecular and Quantitative Aspects of the Chemistry of Fulvic Acid and its Interactions with Metal Ions and Organic Chemicals. Part I The Electron Spin Resonance Approach. *Analytica Chimica Acta* 232: 51-75

Senesi, N., T. M. Miano, M. R. Provenzano, and G. Brunetti. 1991. "Characterization Differentiation and Classification of Humic Substances by Fluorescence Spectroscopy." *Soil Science* 152 (4): 259-271.

Spitze, K. 1991. "*Chaoborus* Predation and Life-History Evolution in *Daphnia pulex* - Temporal Pattern of Population Diversity, Fitness, and Mean-Life History." *Evolution* 45 (1): 82-92.

Steinberg, Christian E.W., Sebastian Höss, and Rainer Brüggemann. 2002. "Further Evidence that Humic Substances Have the Potential to Modulate the Reproduction of the Nematode *Caenorhabditis elegans*." *International Review of Hydrobiology* 87 (1): 121-133.

Steinberg, Christian E.W., Andrea Paul, Stephan Pflugmacher, Thomas Meinelt, Renate Klöcking, and Claudia Wiegand. 2003. "Pure Humic Substances Have the Potential to Act as Xenobiotic Chemicals – A Review." *Fresenius Environmental Bulletin* 12 (5): 391-401.

Steinberg, Christian E. W., Sebastian Hoess, Werner Kloas, Ilka Lutz, Thomas Meinelt, Stephan Pflugmacher, and Claudia Wiegand. 2004. "Hormonelike Effects of Humic Substances on Fish, Amphibians, and Invertebrates." *Environmental Toxicology* 19 (4): 409-411.

Steinberg, Christian E. W., Nadia Ouerghemmi, Steffen Herrmann, Rihab Bouchnak, Maxim A. Timofeyev, and Ralph Menzel. 2010. "Stress by Poor Food Quality and Exposure to Humic Substances: *Daphnia Magna* Responds with Oxidative Stress, Lifespan Extension, but Reduced Offspring Numbers." *Hydrobiologia* 652 (1): 223-236.

Steinberg, Christian E. W., Laura Vicentic, Ramona Rauch, Rihab Bouchnak, Albert L. Suhett, and Ralph Menzel. 2010. "Exposure to Humic Material Modulates Life History Traits of the Cladocerans *Moina Macrocopa* and *Moina Micrura*." *Journal of Chemistry and Ecology* 26 (Suppl. 2): 135-143.

Suhett, Albert L., Christian E. W. Steinberg, Jayme M. Santangelo, Reinaldo L. Bozelli, and Vinicius F. Farjalla. 2011. "Natural Dissolved Humic Substances Increase the Lifespan and Promote Transgenerational Resistance to Salt Stress in the *Cladoceran Moina Macrocopa*." *Environmental Science and Pollution Research International* 18 (6): 1004-1014.

Taipale, Ssami, Michael T. Brett, Katja Pulkkinen, and Martin J. Kainz. 2012. "The Influence of Bacteria-Dominated Diets on Daphnia magna somatic growth, reproduction, and Lipid Composition." *Federation of European Microbiological Societies* 82: 50-32.

Taipale, Sami, Paula Kankaala, Heikki Hamalainen, and Roger I. Jones. 2009. "Seasonal Shifts in the Diet of Lake Zooplankton Revealed by Phospholipid Fatty Acid Analysis." *Freshwater Biology* 54 (1): 90-104.

Tatarazako, Norihisa and Shigeto Oda. 2007. "The Water Flea *Daphnia Magna* (Crustacea, Cladocera) as a Test Species for Screening and Evaluation of Chemicals with Endocrine Disrupting Effects on Crustaceans." *Ecotoxicology* 16 (1): 197-203.

Thurman, E. M. 1985. *Organic Geochemistry of Natural Waters /*. Developments in Biogeochemistry. Dordrecht ;: M. Nijhoff ;.

Timofeyev, Maxim A., Zhanna M. Shatilina, Aleksey V. Kolesnichenko, Darya S. Bedulina, Viktoria V. Kolesnichenko, Stephan Pflugmacher, and Christian E. W. Steinberg. 2006. "Natural Organic Matter (NOM) Induces Oxidative Stress in Freshwater Amphipods

Gammarus Lacustris Sars and *Gammarus Tigrinus* (Sexton)." *Science of the Total Environment* 366 (2-3): 673-681.

Timofeyev, Maxim A., Zhanna M. Shatilina, Aleksey V. Kolesnichenko, Viktoria V. Kolesnichenko, and Christian E. W. Steinberg. 2006. "Specific Antioxidant Reactions to Oxidative Stress Promoted by Natural Organic Matter in Two Amphipod Species from Lake Baikal." *Environmental Toxicology* 21 (2): 104-110.

Timofeyev, Maxim A., Claudia Wiegand, B. Kent Burnison, Zhanna M. Shatilina, Stephan Pflugmacher, and Christian E. W. Steinberg. 2004. "Impact of Natural Organic Matter (NOM) on Freshwater Amphipods." *Science of the Total Environment* 319 (1-3): 115.

USEPA 2002. Short-Term Methods for Estimating the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th ed. U.S. Environmental Protection Agency Office of Water, Washington. EPA-821-R-02-012

USEPA 2002. Short-Term Methods for Estimating Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, 4th Ed. Us. Environmental Protection Agency Office of Water, Washington. EPA-821-R-02-013

Wacker, A. and D. Martin-Creuzburg. 2007. "Allocation of Essential Lipids in *Daphnia Magna* during Exposure to Poor Food Quality." *Functional Ecology* 21 (4): 738-747.

Watts, Matthew M., David Pascoe, and Kathleen Carroll. 2002. "Population Responses of the Freshwater Amphipod Gammarus Pulex (L.) to an Environmental Estrogen, 17alpha-Ethinylestradiol." *Environmental Toxicology and Chemistry* 21 (2): 445-450.

Wissel, B., W. J. Boeing, and C. W. Ramcharan. 2003a. "Effects of Water Color on Predation Regimes and Zooplankton Assemblages in Freshwater Lakes." *Limnology and Oceanography* 48 (5): 1965-1976.

Wissel, B., N.D. Yan, and C.W. Ramcharan. 2003b. "Predation and Refugia: Implications for *Chaoborus* abundance and species composition." *Freshwater Biology* 48: 1421-1431.

Yan, Norman D., Keith M. Somers, Robert E. Girard, Andrew M. Paterson, W. Keller, Charles W. Ramcharan, James A. Rusak, Ron Ingram, George E. Morgan, and John M. Gunn. 2008. "Long-Term Trends in Zooplankton of Dorset, Ontario, Lakes: The Probable Interactive Effects of Changes in pH, Total Phosphorus, Dissolved Organic Carbon, and Predators." *Canadian Journal of Fisheries and Aquatic Sciences* 65 (5): 862-877.

Yan, Norman D., Wendel Keller, Norman M. Scully, David R. S. Lean, and Peter J. Dillon. 1996. "Increased UV-B Penetration in a Lake Owing to Drought-Induced Acidification." *Nature* 381 (6578): 141-143.

Young, S., V. A. Taylor, and E. Watts. 1984. "Visual Factors in *Daphnia* Feeding." *Limnology and Oceanography* 29 (6): 1300-1308.

Acknowledgements

Table 1. NOM collection sites, including several chemical characteristic site descriptions. No samples of the outflow of Clearwater Lake were taken. Chemical data for Lake Laurentian is from July 2005, all other site data are from June and July of 2011.

NOM	Type	Coordinates	DOC (mg·L^{-1})	pH	Conductivity (µS·cm^{-1})	Ni (µg·L^{-1})	Cu (µg·L^{-1})
Clearwater Lake (CW)	Lake	46224'N, 81245'W	3	6.72	58.8	42.8	8.5
Daisy Lake (DL)	Lake	46450'N, 80888'W	2.5	6.71	33.3	55.3	8.9
Lake Laurentian (LL)	Lake	46447'N, 80961'W	7.1	6.46	146	56.0	15.0
Clearwater Lake Outflow (SSW)	Stream	462244'N, 81246'W					
Laurentian Wetland (LW)	Wetland	46450'N, 80942'W	65.75	4.62	30.2	303.6	236.2

Table 2. Major ion and nickel concentrations of NOM isolates at a nominal concentration of 6 mg C·L^{-1} combined with the synthetic soft water medium, FLAMES-without-EDTA.

	Ca (mg L^{-1})	Mg (mg L^{-1})	Na (mg L^{-1})	K (mg L^{-1})	Cl (mg L^{-1})	SO$_4$ (mg L^{-1})	Ni (µg L^{-1})
Control	2.64	0.735	0.89	0.345	0.48	9.45	0
CW	8.28	2.28	14.7	0.98	19.1	26.4	40.27
DL	2.76	0.83	4.87	0.37	2.1	14.3	15.23
LL	9.6	2.74	22.4	1.12	40.2	17.1	46.91
SSW	6.92	1.8	21.2	1.1	31.4	15.3	31.55
LW	2.46	0.73	4.11	0.365	3.54	8.4	4.94

Table 3. Results from stepwise regression were used to determine the ion of greatest influence on each health metric. These figures were used to measure *Daphnia* health and for the regression equation obtaining residuals.

Metric	Ion	Regression Equation	R^2	P
Mean generation time, T_bar	Ca	-.190x + 11.759	.250	0.000
Intrinsic rate of natural increase, r	Ca	.009x + .285	.189	0.000
Specific growth rate, g	Ni	.013x + 2.595	.365	0.000
Time to primiparity	Ca	-.178x + 10.438	.138	0.004
Mean clutch	Ca	.507x + 10.270	.097	0.016

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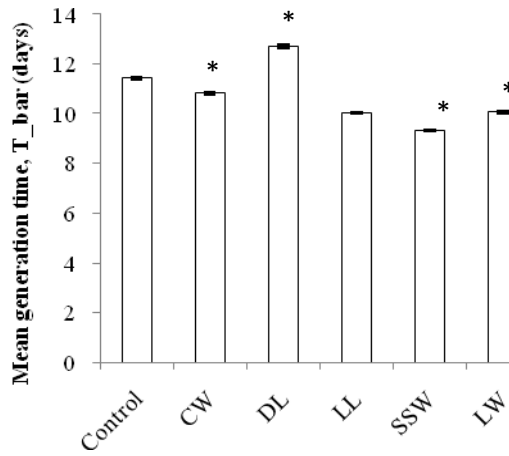
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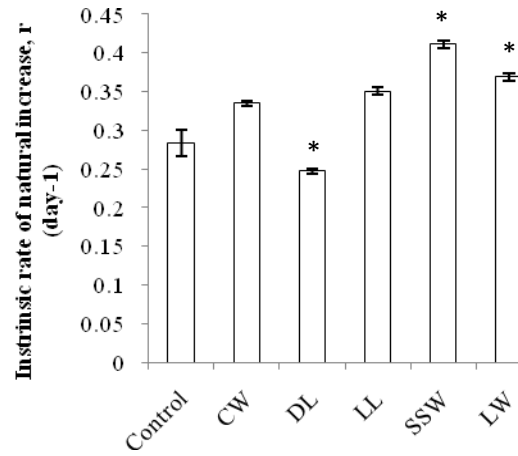
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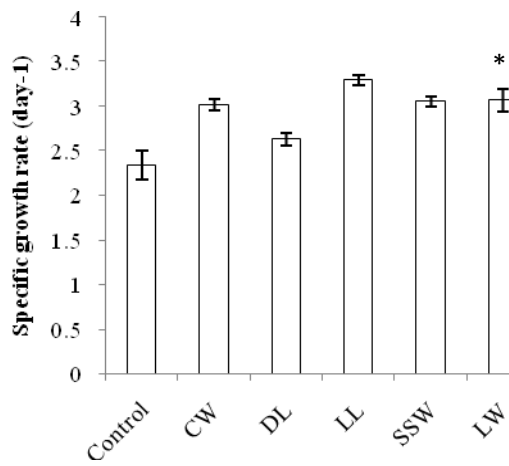
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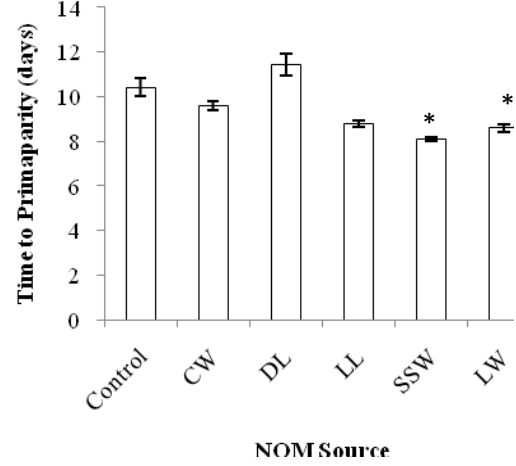
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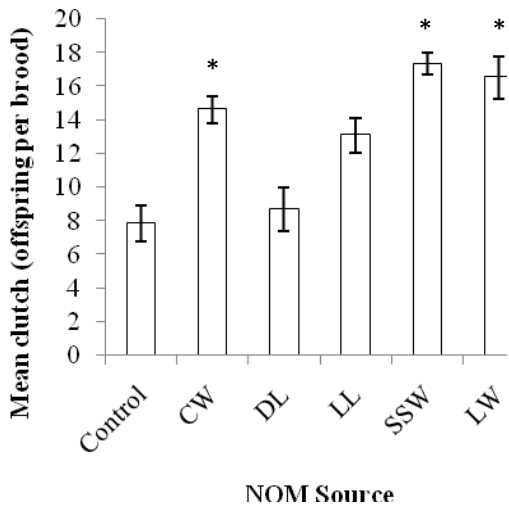
Figure 1. Five health matrices ((A) cohort generation time (T_bar); (B) intrinsic rate of natural increase (r); (C) specific growth rate (g); (D) time to primiparity; (E) and mean clutch) for a *Daphnia pulex/pulicaria* hybrid exposed to five different NOM isolates. * indicates significant difference (P = 0.05) between NOM isolate and control. Error bars are standard error. Clearwater Lake (CW), Daisy Lake (DL), Lake Laurentian (LL), Outflow of Clearwater Lake (SSW), and Laurentian Wetland (LW)
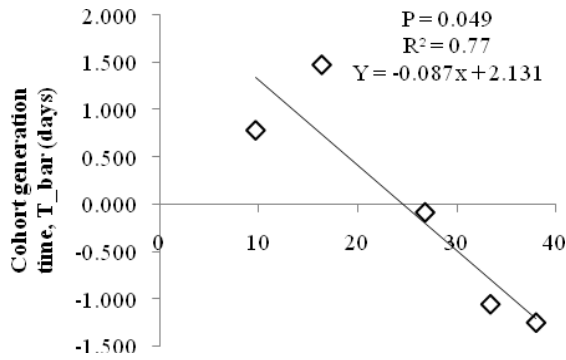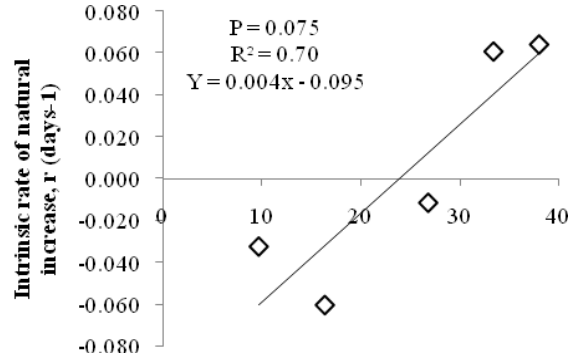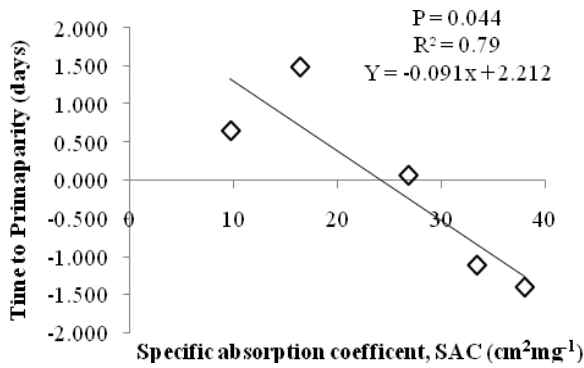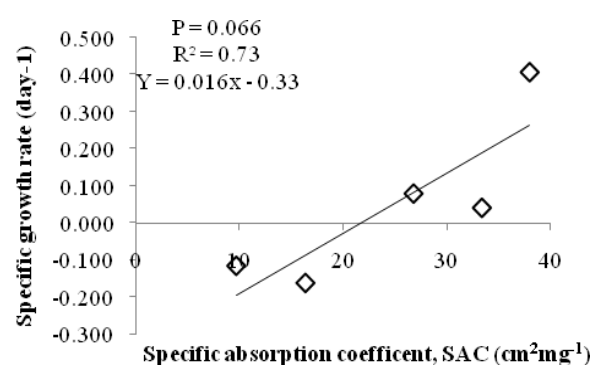
Figure 2. The regression between four of the *Daphnia* health matrices ((A) cohort generation time (T_bar); (B) intrinsic rate of natural increase (r); (C) time to primiparity; (D) and specific growth rate (g)) and specific absorption coefficient (SAC cm^2mg^{-1} an index of aromaticity and colour). Regression analysis was run on the residuals of a linear regression with calcium for r, T_bar, time to primiparity, and with nickel, for g.

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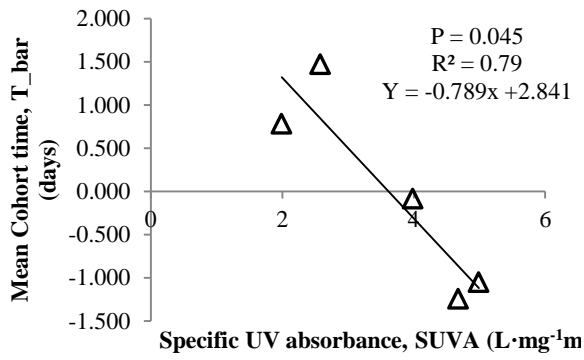
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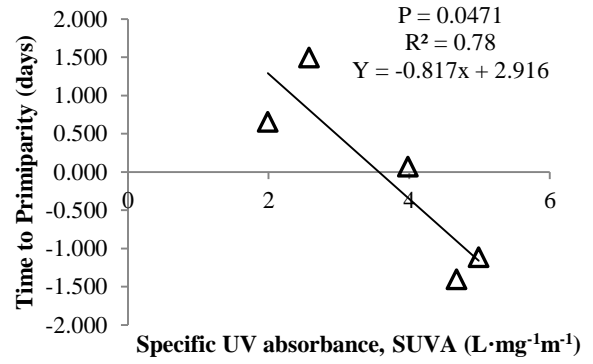
Figure 3. The regression between (A) mean generation time (T_bar, days), and (B) time to primiparity and specific UV absorbance (SUVA L·mg^{-1}M^{-1} an index of aromaticity). Regression analysis was based on the residuals of linear regression between calcium and T_bar.

A.

B.
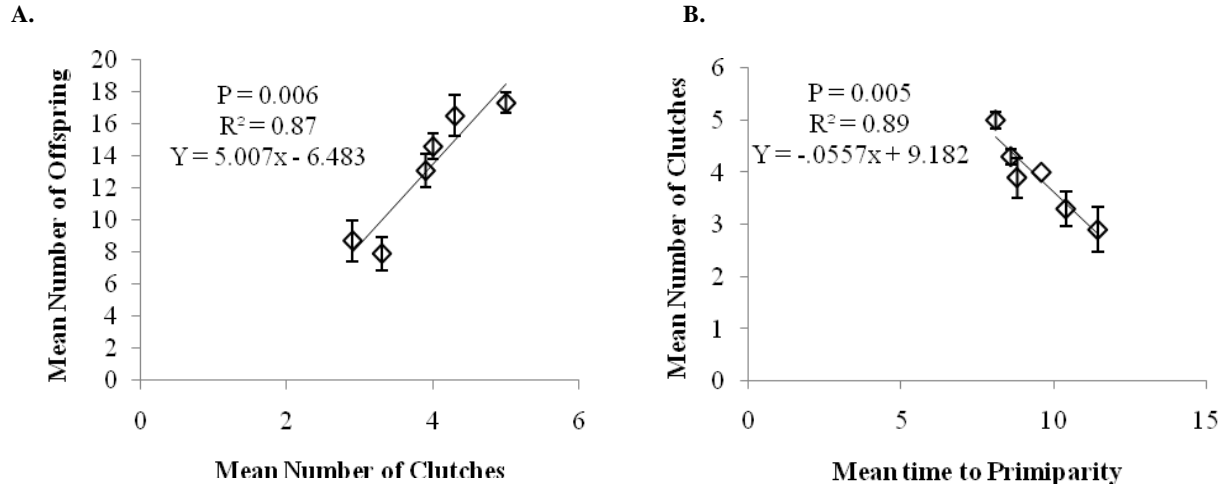
Fig 4. The relationship between the (A) mean number of offspring produced and the mean number of clutches for each of the NOM isolates during the 18 day exposure; and the relationship between (B) the mean number of clutches produced and the mean time to primiparity for each of the NOM isolates during the 18 day exposure.

Table 4. Bacterial counts (± relative uncertainty) for the control, the solution used to dilute the NOM isolates (FLAMES without EDTA), and the solutions containing 6 mg C·L^{-1} of four out of the five NOM isolates used in the study.

Solution	Colony forming unit (CFU)
Control	66 ± 5.94
FLAMES without EDTA	550 ± 53.9
Clearwater Lake	420 ± 46.62
Daisy Lake	100 ± 7.4
Outflow of Clearwater Lake	290 ± 38.57
Laurentian Wetlands	550 ± 53.9

Table 5. The decrease in absorbance at 340 nm after 48 hours of exposure to a 16-hr light and 8-hr dark cycle at 20 °C of solutions containing 6 mg C·L^{-1} of the five NOM isolates.

Solution	% Decrease in Absorbance
Clearwater Lake	0.0
Daisy Lake	8.9
Lake Laurentian	16.6
Outflow of Clearwater Lake	15.1
Laurentian Wetlands	6

Chapter 2

Effects of the Structure of Natural Organic Matter (NOM) on the Amelioration of Acute

Nickel Toxicity to a Wild *Daphnia*

Abstract

Natural organic matter (NOM) complexes metals and decreases their toxicity to aquatic biota. While increasing NOM concentration increases mitigation of metal toxicity, NOM structure may also play a role, but this structural effect of NOM on metal toxicity has received little study. Hence, I investigated how the structural heterogeneity of NOM affects nickel toxicity to a native Canadian *Daphnia pulex/pulicaria* hybrid that had been isolated from a lake with a history of metal contamination. To quantify structure, NOM isolates from thirteen different natural sources were characterized with six different spectroscopic techniques. The addition of 7 mg $C \cdot L^{-1}$ of NOM decreased toxicity, i.e. increased Ni LC_{50}s; seven of the isolates did so to a significant degree. Both specific absorbance coefficient (SAC) and specific UV absorbance (SUVA) techniques, used as aromatic indices, were positively related to toxicity amelioration. The protective effect of the NOM was best described by segmented regression rather than linear regression with ameliorative effect of NOM increasing to a plateau at 19.3 $cm^2 mg^{-1}$ for SAC and 3.03 $L \cdot mg^{-1} m^{-1}$ for SUVA. Phenolic functional groups within NOM structure bind with metals and seem to be the main constituents alleviating metal toxicity. There is growing use of the Biotic Ligand Model (BLM) in setting standards for metal toxicity and a quality factor (F) has been used to quantify amelioration differences in NOM structure. However, the addition of the quality factor F, improved the R^2 between measured and predicted Ni LC_{50}s by 5%.

Introduction:

Natural organic matter (NOM) is a ubiquitous (Ertel et al. 1984; Guthrie et al. 2005) heterogeneous (Hudson et al., 2007) substance made up of decomposed woody plant debris, decayed animals, and microbes (Al-Reasi et al. 2011, Timofeyev et al. 2004, Chem et al. 2012) found in ground and surface waters (Ertel et al., 1984). Its composition depends on the chemistry and physics of the aquatic environment and the available parent molecules in play (Senesi 1990). NOM is largely comprised of humic substances, the main constituents of which are humic acid (HA) and fulvic acid (FA) (Thurman 1985; Al-Reasi et al. 2011; Schindler et al. 1992; Sekaly et al., 1999; Guthrie et al., 2005). These acids are polydisperse macromolecules (Baker and Khalili 2003) with a wide range of functional moieties (Celo et al., 2001), including carboxylic, phenolic (Sekaly et al., 1999), nitrogenous, and sulfitic sites (Takacs et al. 1999) which are known to complex with cationic divalent metals (Doig and Liber 2007). When NOM complexes and chelates with metals (Hutchinson and Sprague, 1987), the resulting metal-organic matter complexes are too large and polar to cross biological membranes (Richards et al. 2001); thus, the NOM has a protective effect as the formerly dissolved, potentially toxic metals become unavailable to aquatic organisms (Al-Reasi et al. 2011; Baken et al., 2011). As functional group make-up varies with NOM source, it follows that the protective abilities of NOM against metal toxicity would vary among sources (Al-Reasi et al., 2011).

Recent advances in absorbance and fluorescence techniques (Fellman et al. 2010) have allowed for easy, non-invasive (Senesi et al. 1990), cost effective and reliable (Jaffe et al. 2008) means of characterizing NOM. Absorbance and fluorescence spectroscopy are able to distinguish molecular variability between natural sources of NOM as well as between humic and fulvic acid portions of an NOM source (Senesi et al., 1991). Absorbance metrics such as the

specific absorbance coefficient (SAC) (a measure of relative NOM colour (Curtis and Schindler) and aromaticity (Schwartz et al., 2004)), the specific UV absorbance (another measure of aromaticity (Weishaar et al., 2003), and the ratio of 254 nm and 365 nm (a proxy inversely related to molecular weight (Helms et al., 2008) have all been useful in characterizing NOM samples in metal toxicity studies. Considered to be more sophisticated than absorbance measures (Luider et al., 2004), fluorescence spectroscopy is a sensitive (Borisover et al. 2009), rapid, and precise method for characterizing NOM (Fellman et al. 2010). It provides information on the molecular size, chemical composition and aromatic properties of humic substances (Hudson et al. 2007). It has also provided insight into the relationship between toxicity and metal bioavailability. Such indices include the fluorescence Index (FI) (McKnight et al. 2001) which distinguishes allochthonous (terrestrial in origin) from autochtonous (produced within the water column from macrophytes, algae and bacteria, or microbrial and photodegradation of terrestrially derived NOM (Leenheer and Coure, 2003; Curtis and Schindler, 1997; McKnight et al., 2001; Al Reasi et al., 2011) origin of the FA portion of the NOM. There are also Emissions and Excitation Matrices (EEMs) which are considered more sensitive than other techniques, providing a unique finger print for NOM (Luider et al. 2004). Although the fluorescent structures make up only a minor portion of humic substance composition, their variety permits useful chemical characterization of the NOM, i.e. differentiating and classifying various humic substances based on their origin and nature (Senesi et al. 1991). EEMs in combination with parallel factor analysis (PARAFAC) designate the main components of NOM and establish their relative concentrations, thereby allowing data within the same study to be compared and trends in quality identified (Nadella et al. 2009). These spectroscopic characteristic techniques described above have been used to identify specific characteristics of NOM which correspond to

greater protection against metals in toxicity bioassays. These studies have found that more allochthonous (low FI values) (Playle, 1998; Luider et al., 2004; Al Reasi et al., 2011, 2012) based NOMs are darker in colour, have higher SAC values (MacDonald et al., 2002; Schwartz et al., 2004; Glover et al., 2005a; Gheorghiu et al., 2010), are more aromatic in nature (high ABS_{254}) (Pempkowiak et al., 1999; De Schamphelaere et al., 2004; Luider et al., 2004), have higher molecular weight (Kozuch and Pempkowiak 1996; Al Reasi et al., 2011, 2012) and greater HA concentration (based on EEMs) (Ryan et al., 2004; Nadella et al., 2009; Gheorghiu et al., 2010; Al Reasi et al., 2011, 2012). These allochthonous NOMs are associated with greater protection against metal toxicity to aquatic biota. Acute toxicity studies have indicated a positive relationship between SAC and NOM protection for Cu (Schwartz et al., 2004; Al-Reasi et al., 2012) and Pb (Schwartz et al., 2004) , while Al-Reasi et al., (2012) also demonstrated a negative correlation with acute Cu toxicity and the molecular weight of NOM. Decreases in acute Cu and Cd toxicity have also been associated with lower FA content and higher HA content, indeed, NOM with higher FI values has been correlated with decreases in Pb and Cd toxicity (Al-Reasi et al., 2011). There are no current studies focusing on effects of the composition of NOM on Ni toxicity to freshwater biota.

The Canadian Council of Ministers of the Environment (CCME), the United States Environmental Protection Agency (EPA), and European Union are currently using the Biotic Ligand Model (BLM) (Deleebeeck et al., 2008, and McGeer in press) to quantify the toxicity of metals in natural waters. In BLMs, only one structural parameter of NOM is taken into account: the quantity of HA. When HA% has not been measured or included in toxicity prediction with the BLM it is often generalized to 10% BLMs (The Biotic Ligand Model Windows Interface, 2005). NOM-quality studies which have attempted to incorporate the actual HA% parameter

into the model have had mixed results, from substantial to only marginal improvements. Ryan et al. (2004) found moderate improvements with the inclusion of HA%, however they described the model as insufficient in explaining differences between NOM source with the inclusion of NOM concentration and HA% for Cu toxicity. Al-Reasi et al. (2012) found a much stronger relationship between predicted and measured Cu LC_{50}s when relative HA% representing specific NOM sources was included in the BLM. Other methods used to take NOM structure into consideration include altering dissolved organic carbon (DOC) with a so-called "quality" factor so concentration represents aromatic content (Richards et al., 2001). This alteration improved the predictive capabilities of a Pb BLM for rainbow trout (Oncorhynchus mykiss) (MacDonal et al. 2002).

Nickel is considered to be a magnesium ionoregulator to *Daphnia* (Pane et al., 2003b) and a respiratory toxicant to fish (Pane et al., 2003) at acute concentrations. It was first considered to be appropriate for adoption into a BLM model by Wu et al. (2003). At that time, little toxicity data existed for Ni and even less dealt with Ni-organic matter complexation. Due to Ni's low binding affinity to NOM and the high concentrations required for acute toxicity, NOM concentration was not thought to play a major role in Ni mitigation (Wu et al., 2003). More recently, this opinion has been shifting, as, for example, Ashworth and Alloway (2008) were surprised to find the high affinity Ni had for sewage sludge. Moreover, a study by Kozlova et al., (2009) discovered that not only did NOM concentration significantly alter LC_{50} concentration of acute Ni to *Daphnia pulex*, but the protection offered by two different NOM sources varied as well. A study by Baken et al. (2011) observed a 10-fold difference in Ni affinity for 23 distinct NOM sources. Though Baken et al. (2011) studied the largest number of NOM sources, their study did not involve toxicity to organisms. The above results suggest that

NOM structural differences will indeed influence Ni toxicity to biota, but this has yet to be tested for several NOM sources. Additionally, there have been no attempts to relate NOM structural variability to Ni mitigation.

My objectives were: 1) to determine if the protective effects of NOM on acute Ni toxicity to a wild *Daphnia pulex/pulicaria* clone differed among various NOM isolates; 2) to establish which NOM characterization method best described the relationship between NOM structure and the mitigation of Ni toxicity; and 3) to verify if a BLM could be modified, and its predictions of Ni toxicity to daphniids improved, by including the best NOM characterization method.

Material and Methods

NOM collection:

NOM collections were made using a portable reverse osmosis (RO) apparatus. NOM isolates obtained from RO extraction have demonstrated similar protective qualities to those of natural waters (De Schamphelaere et al., 2004; De Schamphelaere et al., 2005). It is also the most common collection method used with metal toxicity studies (Al-Reasi et al. 2011) and is one of the most chemically mild methods used in obtaining NOM (Perdue 2009). The NOM collection sites spanned almost 1000 km in Ontario, and were selected to vary in recent logging, industrial and fire history, factors we assumed would all influence NOM composition. In particular, NOM was collected at three sites from White River, five sites from Sudbury, and five sites from the Muskoka region. The streams, wetlands and lakes which made up the site locations (Table 1) were chosen to cover water bodies which had been affected by various stressors including; fire, logging, metal pollution, acid rain and as well as pristine sites. Sites were also chosen to ensure a range of NOM colour as streams and wetlands are known to have

greater terrigenous content (Wong 2009) due to inputs from soil material (Leichtfried, 2007) as well as groundwater and leaf litter (Allan, 1995).   In an effort to remove all minor and major cations from the NOM, a cation exchange resin charged with 4 N HCl was utilised (Schwartz et al., 2004); in this process, the pH of NOM concentrates was decreased to approximately 2 (Resin USF C-211 (H) Cation, Siemens). Following this resination step, NOM isolates were kept cold at 4°C and dark prior to use in the bioassays.

Culture Toxicity Testing:

    All bioassays were performed with a *Daphnia pulex/pulicaria* hybrid obtained from McFarlane Lake in Sudbury, Ontario, a region with a long, well-studied history of metal contamination (Gunn, 1995). . This pond (*pulex*) / lake (*pulicaria*) hybrid is quite common in Sudbury area lakes and is among the initial *Daphnia* to re-settle lakes recovering from high metal concentrations and acidification (Yan unpub. Data). The daphniid cultures were maintained in the FLAMES laboratory at the Dorset Environmental Science Centre, at 20° C in Conviron E7/2 growth chambers programmed to provide a 24-h deil cycle of 16 daylight hours (at 100 µmol m^{-2} s^{-1}) and 8 night hours of darkness, with 20 minute crepuscular transitions . At the time of this study, this hybrid line had been maintained for three years in this fashion. *D. pulex/pulicaria* were cultured in 1 L glass beakers in approximately 800 ml of the soft water FLAMES medium (Celis-Salgado et al. 2008). The medium was prepared and aerated at least 24 hours prior to use. Animals were fed 4 mg C·L^{-1} with a non-axenic algal combination of *Pseudokirchneriella subcapitata, Scenedesmus obliquus, Chlorella kessleri, Cyclotella* sp., and *Ankistrodesmus falcatus.* All algae were procured in pure stocks from the Canadian Phycological Culture Centre (previously the University of Toronto Culture Collection); they were raised in batch cultures in the FLAMES laboratory. For the stock daphniid cultures, each

beaker was initiated with thirty *Daphnia*; beakers were inoculated with algae three times a week and the medium was changed twice a week.

Static acute 48-h toxicity assays were conducted using neonates of less than 24-h, without the use of the first brood (Environment Canada, 1996). The end point was immobility at the completion of 48-h, which was assumed to indicate mortality. The animals were not fed (OECD 2004) for the duration of the test. The soft water medium, FLAMES, was modified for the 48-h tests by removal of the main chelator, ethylenediaminetetraacetic acid (EDTA), resulting in FLAMES-without-EDTA. A preliminary test demonstrated 100% survival with *Daphnia* in FLAMES and EDTA-free-FLAMES for up to four days. NOM solutions were brought up to a pH of 7.0 with 0.1 M NaOH and FLAMES-without-EDTA was spiked with the NOM solution to obtain a final NOM concentration of 7 mg $C \cdot L^{-1}$ (Table 2). For each NOM source, a series of solutions were prepared: one control of FLAMES-without-EDTA, one with only NOM and the remaining four solutions contained NOM and Ni. The Ni concentrations were 500, 1000, 2000, and 4000 $\mu g \cdot L^{-1}$ to ensure the acute LC50 was within range as well as incorporate a Ni concentration which killed 100% of exposed *Daphnia*. Each Ni treatment of each NOM source had six replicates, each containing eight animals held in 130 mL of test solution. Before use, all containers were base and acid washed to completely remove organic materials and metals. Animals were rinsed twice in FLAMES-without-EDTA to minimize transfer of EDTA and algae from the culture medium. Test solutions were prepared 24 hours in advance and not aerated. Nickel was added as $NiCl_2$ due to preliminary test findings that only minor differences between the LC_{50}s obtained with $NiCl_2$ and $NiSO_4$ existed.

 Chemical analysis:

The pH, dissolved oxygen, and temperature of each test solution were measured at the initiation of the experiment and at test completion. At the initiation of the test, solutions were also analysed for alkalinity, colour, conductivity, ammonia, phosphorous, dissolved inorganic carbon, sulphide, chloride, calcium, magnesium, potassium, and sodium. At time 0 and test completion, two 10 mL samples were collected for total nickel and dissolved nickel analysis. Each sample was acidified to 1% with 70% reagent grade nitric acid. Dissolved nickel samples were filtered with a 0.45 µm Acrodisc® HT Tuffryn Membrane, PALL, NY filter.

The pH of solutions was measured with an Accumet® Basic AB15 pH Meter, Fisher Scientific and pHC2001-8 electrode (Radiometer analytical, France) calibrated with buffer solutions of pH 4, 7 and 10. Dissolved oxygen and temperature were measured with a ProODO digital professional series YSI. Alkalinity, colour, conductivity, ammonia, phosphorous, dissolved inorganic carbon, sulfate and chloride, calcium, magnesium, potassium, sodium and DOC were measured by the Ministry of the Environment at the Dorset Environmental Science Centre following their standard methods (MOE 1983).   Ni was measured *via inductively* coupled plasma atomic emission spectroscopy (ICPAES, Varian Inc.) at Wilfred Laurier University, Waterloo, Ontario. Measured Ni concentrations were within 10% of nominal values.

NOM Characterization:

NOM quality was characterized by absorbance and fluorescence measurements. For absorbance measures, NOM solutions (10 mg $C \cdot L^{-1}$) were prepared by diluting the NOM stock solutions with the appropriate quantity of water which had been run through a double cycle of reverse osmosis and measured a present conductivity of 18.2 MEG Ω. Solutions were adjusted to a pH of 7.0 ± 0.1 with a 0.1 M NaOH solution and filtered with a 3-ml syringe (Luer-LOK Tip Franklin Lakes, NJ, USA) fitted with a 0.45-µm Acrodisc® syringe filter (Pall Corporation, Ann

Arbor, MI, USA). Absorbance was measured at 254, 340 and 365 nm in a 1 cm quartz spectrophotometer cuvet (VWR, West Chester, PA, USA) with a UV-Vis spectrophotometer (Thermo Scientific Genesys, G 10s UV-Vis, Madison, WI, USA)(Al-Reasi et al. 2012). A specific absorbance coefficient (SAC), used as an aromatic index, was obtained using the equation from Curtis and Schindler (1997) SAC = ((2.303* absorbance at 340 nm)/(pathlength))/ ([DOC]/1000). A second measure of aromaticity, known as the specific UV absorbance (SUVA), was obtained with the following equation:

$$SUVA = Abs_{254} \, (m^{-1})/[DOC \, (mg \cdot L^{-1})]$$ (Weishaar et al., 2003)

To obtain the approximate molecular weight of the NOM molecules, absorbance at 254 nm was divided by the absorbance at 365 nm (Dahlen et al. 1996). SAC, SUVA and molecular weight measurements were obtained in triplicate for each NOM source.

Fluorescence values were obtained from Livingstone et al. (Wilfred Laurier University, unpublished). Emission intensity was measured at 450 nm and 500 nm; the excitation wavelength of 370 nm was used to determine the fluorescence index (FI) (McKnight et al. 2001). This was designated to indicate the source of NOM as either allochthonous or autochtonous in origin. EEMs, in combination with parallel factor analysis (PARAFAC), were used to designate the main components of NOM and establish their relative concentrations (Nadella et al., 2009). The use of PARAFAC allowed for the quantification of the relative concentrations of each of these components.

Statistics:

LC_{50}s were obtained using nominal Ni concentrations. The LC_{50} values were calculated using the trimmed Spearman-Karber method (Hamilton et al. 1977) programmed into Microsoft Excel. An LC_{50} was determined for each replicate; LC_{50} values were averaged for

determinations in presence of each specific NOM. All values have been reported as mean ±

standard error (Figure 1A). Any replicates which had less than 90% survival in the controls were

removed from analysis. This occurred for only one control. For WR1 and WR3 only three

replicates were used as the other three replicates had been somehow contaminated.

While we did follow established procedures, treatment with cation exchange resin did not

remove all cations from the NOM (Table 2). To correct for the effects of cations on our

endpoints, a linear regression between LC_{50}s and Ca (which is known to influence *Daphnia*

health (Ashforth and Yan 2008) and acute Ni toxicity (Kozlova et al., 2009)) was performed to

obtain the residuals (Figure 2A). Because the NOM isolate, SSW, was identified as an

influential outlier (Figure 2A), a regression analysis between calcium and acute nickel LC_{50}s was

conducted a second time with the removal of SSW (Figure 2B). Residuals from the second

regression analysis were used in further statistical analyses. Data were analysed using SPSS

Statistics 19.0 for Windows (SPSS Inc., Chicago, IL, USA). While the Shapiro_Wilk test

identified the data as normally distributed, the Levene's test indicated the data did not abide by

the assumption of homogeneity. Therefore, a Welch one-way analysis of variance (ANOVA)

was employed to detect variation in LC_{50}s with various NOMs. The ANOVA was followed by

the post hoc Games-Howell test ($P < 0.05$) (Figure 1B), also used when the assumption of

homogeneity is not achieved. The relationship between LC_{50} residuals and each NOM structural

characteristic were initially analysed by linear regression. Visual observation suggested that this

analysis did not completely capture the relationship. Segmented linear regression with SegReg

(**http://www.waterlog.info/segreg.htm**) was employed as SegReg tests data with seven different

functions and determines the best function and breakpoint based on maximizing the statistical

coefficient of explanation. As data must be positive to be analysed by Segreg, the lowest LC_{50} residual value was subtracted from each NOM isolate prior to analysis.

LC_{50} Prediction using the BLM for Acute Ni toxicity

The BLM (Windows version 2.2.3, HydroQual Inc.) developed for *Daphnia pulex* in moderately soft water by Kozlova et al. (2009) was used to predict LC_{50}s for the *Daphnia* hybrid in soft water in the current study. The BLM was optimized for the current data set using five different control series by gradually manually altering the critical value in the Ni BLM to improve the relationship between the actual measured LC50s of the five controls and the predicted LC50s for these series. In an attempt to include NOM structure and improve BLM predictions, two different methods were employed. These were based on the relationships obtained between the residuals of LC_{50}s and the characteristics of the NOM which were found to have a relationship. The first incorporated the significant relationship between SAC and the relative HA% obtained from PARAFAC to estimate HA content (Al Reasi et al., 2012) (Figure 4). The second used the quality factor, F, described by Schwartz et al. (2004):

$$F = (0.31 \cdot \ln(SAC)) \cdot [DOC]$$

The quality factor decreases the concentration of DOC in order to decrease the concentration of binding sites, giving NOMs with greater aromatic content higher DOC concentrations and those with less aromaticity a lower concentration of DOC. These values were compared with the original measured values (not residuals) by linear regression analysis to determine if improvements were made to BLM predictions.

Results:

For all the series run, only one control had less than 90% survival; this was for a replicate without any NOM added. One replicate of the Hp3 NOM-only series had a survival rate of 87.5%, this was also true for one replicate of the PL-NOM-only series. Two replicates of the SSW-NOM-only series had 87.5% survival. For all other NOM isolates there was 100% survival in all replicates.

The mean 48-h Ni LC_{50} for the *Daphnia pulex/pulicaria* hybrid in FLAMES medium without EDTA was 702.76 $\mu g \cdot L^{-1}$ (Fig. 1), at its low measured hardness of 9.22 $mg \cdot L^{-1}$ ($CaCO_3$).

The addition of 7 mg $C \cdot L^{-1}$ of NOM increased acute nickel LC_{50} concentrations in every NOM treatment. Increases in LC_{50} values were significant for seven of the 12 NOM isolates used. While the mean nickel LC_{50} for the control was 702.76 ($\mu g \cdot L^{-1}$), the addition of NOM raised the mean LC_{50} values from 931.08 (WR2) to 1898.0 (LL) ($\mu g \cdot L^{-1}$); an almost two-fold range in values (Figure 1A and B).

The protection provided by NOM varied with source. There was a two-fold range in mean nickel LC_{50} values with the addition of twelve NOM sources. The mean LC_{50} produced by WR2 was significantly less than six of the other NOM isolates (DL, HL, WR3, HP3, PC1, LW). The NOM isolate, WR2, had the lowest Ni LC_{50} of all at 931.08 $\mu g \cdot L^{-1}$, while LC_{50}s for isolates of significantly greater value ranged from 1405.0 $\mu g \cdot L^{-1}$ for HL to 1511.5 $\mu g \cdot L^{-1}$ for LW.

PL had a significantly lower mean LC_{50} (1393.2 $\mu g \cdot L^{-1}$) than DL (1407.83 $\mu g \cdot L^{-1}$). LW was significantly greater than four of the other NOM isolates (WR2, PL, HP3 and PC1) (Figure 1B).

The variation in Nickel LC_{50} residuals were related to two of the six NOM characteristic metrics (Table 3). Specific absorption coefficient (SAC), which is considered an index of colour (Curtis and Schindler 1997) and aromaticity (Haitzer et al., 1999; Richards et al., 2001), varied between

9.67 and 38 cm^2mg^{-1}. Specific UV absorbance (SUVA), the second parameter used to measure aromaticity (Baker et al., 2011), ranged between 1.99 and 4.67 L $mg^{-1}m^{-1}$. SegReg determined that a segment regression out performed linear regression. Between the SAC values of 9.67 and 19.35 cm^2mg^{-1}, there was a positive relationship with Ni LC_{50} residuals where SAC explained 60% of the variation seen in LC_{50}s. This relationship ceased after this value, thus, any additional increases in SAC/aromaticity did not result in a greater LC_{50}. For SUVA, 1.99 to 3.03 L·$mg^{-1}m^{-1}$, explained 67.7% of the variance in LC_{50}s up to that maximum, while stopping above this point. Therefore, the colour and aromatic content of NOM did correlate to the ability of natural NOM to protect *Daphnia* against acute Ni toxicity to a point. While none of the absorbance matrices were related to FI (data not shown), both SAC $_{340}$ (Figure 4A) and SUVA (Figure 4B) were positively related to HA%. Though the relationship between HA% was not significantly related to changes in nickel LC_{50}s, the NOMs with higher relative humic acid content do appear to have greater aromatic content.

The LC_{50} toxicity predictions made with the BLM were all within the accepted two-fold range (Santore et al., 2002), but all samples (except those from WR2) were under predicted by as much as 41% of the measured value. Changing the critical value from 5.1 (used by Kozlova et al. (2009)) to 6.37 made minor improvements to BLM predictions, expanding the R^2 value from 0.5922 to 0.6025 and increasing the predicted values while decreasing the differences between measured and predicted to 25% (with the exception of WR2 which increased from 37% to 69%). BLM predictions were not improved by the inclusion of relative HA% based on the relationship between SAC and HA% (results not shown). Using the F factor for determining DOC concentration improved BLM predictions (increasing the R^2 value to 0.6548)(Figure 5); improvements in this relationship were primarily due to those NOM isolates with SAC values

less than 19.35. The NOM isolate WR2 was only over predicted by 44% while 95% were predicted within 1.24 fold and 50% of those samples were within 1.1 fold. The slope of the relationship also changed from 0.5922 with the original BLM predictions to 0.7405 with the inclusion of the F factor.

Discussion:

The measured Ni LC_{50} for the *D. pulex/pulcaria* hybrid in FLAMES medium was higher than what Kozlova et al. (2009) observed for *D. pulex* in harder water. Deleebeck et al. (2008) proposed that differences in clone sensitivity explained the disparity in Ni toxicity between two clones of *Daphnia magna*. The differences which arose in this study may have been due to the use of a hybrid originating from a metal-contaminated lake. Disturbance is credited as a main cause of hybridization (reviewed by Allendorf et al., 2001); furthermore, the hybrid-superiority hypothesis suggests that in certain environments, hybrids are superior (Moore 1977). Anthropogenic disturbances have been related to the dominance of hybrid species in *Daphnia* populations. An example of this is given by Keller et al. (2008) who found that *Daphnia galeata-hyalina-cucullata* hybrids dominated over parent lines in lakes which had a history of eutrophication in Europe north and south of the Alps. The *D. pulex/pulicaria* hybrid used for this present study was obtained from an environment which had been disturbed with high concentrations of metals (Valois et al., 2011). I hypothesize that its higher tolerance to Ni than a parent line—such as *D. pulex*—is another example of the hybrid-superiority hypothesis.

The NOM isolate, SSW, was identified as an influential outlier as it had a lower than expected LC_{50}; this may be due to its high calcium concentration of over 7 mg·L^{-1}. While Ca is known to protect against acute Ni toxicity (Kozlova et al. 2009), at high Ca to Ni ratios, Ca can so thoroughly out-compete Ni for available ionic-bonding sites while simultaneously reducing

Ni's electrostatic binding potential at other sites, that the resulting weak and unstable Ni-NOM complexes can ultimately lead to higher concentrations of the free Ni ion (Mandal et al., 2000). This would result in a lower than expected LC_{50}. However, this is normally the case when Ca is several orders of magnitude greater than Ni (unlike the Ni : Ca ratio for this NOM isolate, which was 4 : 7) It is also possible that other metals which were not measured remained in the NOM isolate and contributed to the lower than expected LC_{50}. This would, though, be expected to cause high mortality in the NOM-only series.

The addition of 7 mg $C \cdot L^{-1}$ of NOM from seven out of 12 sites significantly increased acute Ni LC_{50}s, i.e. had a protective effect. These results are comparable to those in the literature. While Deleebeeck et al. (2008a) found a relationship between acute Ni EC_{50} values and DOC for *D. magna* in eight European lakes, the relationship was weak ($R^2 = 0.33$). Pane et al. (2003b) suggested that the discrepancies found in acute Ni LC_{50}s for *D. magna* in their study compared to the results of a study by Biesinger and Christensen (1992) were attributed to differences in DOC concentration from 1 to 3.6 mg $C \cdot L^{-1}$. Cloran et al. (2010) also found that additions of one to 18 mg $C \cdot L^{-1}$ provided protection against 2150 $\mu g \cdot L^{-1}$ of Ni for *D. magna*. NOM is known to bind Ni (Livens 1991; Mandal et al., 1999), and so it would be expected that the addition of NOM to the synthetic medium used in this study would increase acute Ni LC_{50}s.

The relationship between SAC and the ability of NOM and its fundamental components (dissolved organic matter (DOM) and humic substances (HS)) to ameliorate metal toxicity has been well established for Cu (Richards et al., 2001; De Schamphelaere et al., 2004; Ryan et al., 2004; Schwartz et al., 2004; Al-Reasi et al., 2011; 2012) and to a lesser extend Cd (Schwartz et al., 2004), inorganic Hg (Klink et al., 2005) and Pb (Schwartz et al., 2004) (Al-Reasi et al., 2001). This relationship has yet to be well established for Ni. Kozlova et al. (2009) observed

that additions of NOM from two different sources both significantly increased Ni EC50 values

for *D. pulex*; above 10 mg C·L^{-1} the two NOMs ameliorated Ni toxicity to varying degrees. The

darker of the two isolates provided a greater amount of protection than the lighter. Doig and

Liber (2007) identified that neither source nor fraction (HA or FA) provided differences in

protection against acute Ni toxicity to *Hyalella azteca*. Chan (2013) found that 10 out of 12

NOM isolates provided a significant amount of protection against acute Ni toxicity to *H. azteca*

and that the protection varied amongst NOM isolates. The differences in protection were not,

however, related to aromaticity or colour. This may be due to the fact that Ca was not accounted

for as it was in the present study.

NOM is a composite system wherein a continuum of binding sites complex with metals,

each with different stability constants (Hertkorn et al., 2004). Donor atoms from O-, N-, and S-

binding sites on NOM (Mandal et al., 2003) are most likely to bind with metal cations (Hertkorn

et al., 2004; Mandal et al., 2003). Metals which tightly retain their valence electrons have more

substantial interactions with oxygen (Al Reasi et al., 2012; Mandal et al., 2003; Smith et al.,

2002).   Oxygen containing functional groups such as carboxylic acids and phenolics are

considered to provide the main sites of metal binding (Carbonaro et al., 2011; Perdue 2009),

which is also considered true for Ni (Nachtegaal and Sparks 2003). Carboxyl functional groups

are more abundant than phenolic, with an estimated ratio of 2.7 ± 0.4 for NOM (Perdue 2009)

and are considered weak (Richards et al., 2004; Swartz et al., 2004). The stronger phenolic-type

binding sites have an average pKa between 8 -10 (Christensen and Christensen 2000). While

higher values of SAC are associated with greater aromaticity, it is these phenol groups which

have been suggested as the source of this protection (Schwartz et al., 2004; Al-Reasi et al.,

2012). The concept that phenolic functional groups provide the main source of protection is

substantiated by Leenheer et al., (1998) who observed it was not the direct association of metals with aromatic structures, but the phenolic functional groups themselves which participated in metal binding.

To establish a correlation between SAC and LC_{50}s, the quantity of metal bound to NOM must have enough of an impact on the free metal ion to decrease the amount which can bind to the biotic ligand. Al Reasi et al. (2011) refer to this as an overlap of the 'toxicological' and 'analytical window'. With large amounts of metal, the strong binding sites can become saturated and changes to aromatic content will not exert enough influence on the free metal ion to alter LC_{50}s. The opposite is also true; a large number of strong binding sites with low levels of metal may not prove effective at alleviating metal burdens either. It appears that for acute Ni toxicity to the *Daphnia* hybrid I used, only a small overlap exists between the two windows, and this occurs solely below a SAC of 19.35 (cm^2mg^{-1}) and SUVA 3.03 ($L \cdot mg^{-1}M^{-1}$). The lack of overlap is not entirely unexpected, because high concentrations of Ni are required for acute toxicity (unlike other more potent metals such as Cu and Pb). Furthermore, binding constants for Ni are much lower between free metal ions and NOM ligands; these equilibrium constants, when juxtaposed, score Cu with phenolic functional groups from $12 - 15$, over Ni's weaker 8 - 10 (Carbonaro et al., 2011). This is because Ni has a lower rate coefficient for water exchange (Mandal et al., 1999b) which means it is less likely to shed water molecules in order to interact with another ligand, which is especially important in aquatic environments. Ni also has a lower ionization potential which leads to lower electrostatic attractions (Baker and Khalili 2003). Therefore, high Ni concentrations and weaker binding constants make it less likely for the 'toxicological' and 'analytical' windows to overlap and show a relevant relationship between LC_{50}s and aromaticity.

The benefits of adding NOM isolates from various sources may also be due to direct effects of NOM on *Daphnia*. Geiger (Chapter 1) demonstrated that the addition of five unfiltered NOM isolates decreased time to primiparity and mean generation time; increased specific growth rate in the same hybrid used in this study; and indicated that these changes in life history were related to SAC. In multiple-stress situations, the addition of NOM and its components (HSs) have also been shown to decrease stress and promote health matrices. Bouchnak and Steinber (2010) noted that when *D. magna* was fed a poor quality diet of pure yeast, the addition of HSs increased fecundity and longevity. Suhett et al. (2011) maintained that in the presence of salt stress, the addition of dissolved HSs extended mean lifespan of the cladoceran, *Moina macrocopa*; it also diminished the salt-induced reduction of somatic growth. Direct effects of NOM on biota have also been directly related to abatements in metal toxicity. NOM either adsorbs to cell surfaces (observed with filtered NOM samples) (Campbell et al., 1997) or integrates into lipid membranes, increasing membrane permeability (observed with unfiltered NOM samples) (Vigneault et al., 2000), stimulating an increase in Na uptake rates (Glover et al., 2005), preventing ion-regulatory metals from interrupting the influx of Na+ (Matsuo et al., 2004), and limiting access to certain metals passing through the membrane (Glover et al., 2005). Unfiltered NOM has also led to changes in epithelium membrane voltage of trout gills, with the magnitude of this change relating to SAC (Galvez et al., 2008).

The relationship between measured and BLM-predicted Ni LC_{50}s was improved by using the quality factor F to take NOM aromaticity into consideration. Haitzer et al., (1999) found that a specific absorption measurement was an effective way to qualify NOM concentration in terms of toxicity amelioration. Based on these results (and in combination with their own), Richards et al., (2001) came up with the F quality factor to incorporate NOM quality into a biotic ligand

model. By multiplying the concentration of NOM by the quality factor F, the number of binding sites on the NOM decreases (Richards et al., 2001; Macdonald et al., 2002). Macdonald et al. (2002) found that inclusion of the F quality factor made minor improvements to the predictive capabilities of a Pb-gill binding model. Inclusion of the quality factor seems to make the BLM more comprehensive.

As an example, if we look at one of Plastic Lake's measured LC_{50s} of 1369.5 µg L^{-1}, the BLM predicted 1584.95 µg L^{-1} when species sensitivity is taken into account. When its measured DOC concentration of 8.14 mg L^{-1} is multiplied by the F factor using its SAC value of 10.36 cm^2mg^{-1}, the concentration changes to 5.9 mg L^{-1} and decreases the predicted LC_{50} to 1396.9 µg L^{-1}, ultimately improving the predictions of the BLM.

In conclusion, NOM at environmentally relevant concentrations decreases Ni toxicity to varying degrees depending on NOM source. Increasing the aromaticity of NOM mitigates Ni toxicity up until a value where the protection provided by NOM plateaus. The inclusion in biotic ligand models of Ni toxicity of the quality factor, F, which accounts for the increasing aromaticity and thus protective effects of NOM aromatic structure can improve the predictions of acute Ni toxicity to daphniids.

Acknowledgements

References Chapter 2

Allan, J. David. 1995. *Stream Ecology : Structure and Function of Running Waters*. 1st ed. ed. London: Chapman & Hall.

Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenburg. 2001. "The Problems with Hybrids: Setting Conservation Guidelines." *Trends in Ecology & Evolution* 16 (11): 613-622.

Al-Reasi, Hassan A., D. Scott Smith, and Chris M. Wood. 2012. "Evaluating the Ameliorative Effect of Natural Dissolved Organic Matter (DOM) Quality on Copper Toxicity to *Daphnia magna*: Improving the BLM." *Ecotoxicology* 21 (2): 524-537.

Al-Reasi, Hassan A., Chris M. Wood, and D. Scott Smith. 2011. "Physicochemical and Spectroscopic Properties of Natural Organic Matter (NOM) from various Sources and Implications for Ameliorative Effects on Metal Toxicity to Aquatic Biota." *Aquatic Toxicology (Amsterdam)* 103 (3-4): 179-190.

Ashforth, Dawn and Norman D. Yan. 2008. "The Interactive Effects of Calcium Concentration and Temperature on the Survival and Reproduction of *Daphnia pulex* at High and Low Food Concentrations." *Limnology and Oceanography* 53 (2): 420-432.

Ashworth, D. J. and B. J. Alloway. 2008. "Influence of Dissolved Organic Matter on the Solubility of Heavy Metals in Sewage-Sludge-Amended Soils." *Communications in Soil Science and Plant Analysis* 39 (3-4): 538-550.

Baken, Stijn, Fien Degryse, Liesbeth Verheyen, Roel Merckx, and Erik Smolders. 2011. "Metal Complexation Properties of Freshwater Dissolved Organic Matter are Explained by its Aromaticity and by Anthropogenic Ligands." *Environmental Science & Technology* 45 (7): 2584-2590.

Baker, H. and F. Khahli. 2003. "Comparative Study of Binding Strengths and Thermodynamic Aspects of Cu(II) and Ni(II) with Humic Acid by Schubert's Ion-Exchange Method." *Analytica Chimica Acta* 497 (1-2): 235-248.

Borisover, Mikhail, Yael Laor, Arkady Parparov, Nadezhda Bukhanovsky, and Marcos Lado. 2009. "Spatial and Seasonal Patterns of Fluorescent Organic Matter in Lake Kinneret (Sea of Galilee) and its Catchment Basin." *Water Research* 43 (12): 3104-3116.

Bouchnak, Rihab and Christian E. W. Steinberg. 2010. "Modulation of Longevity in *Daphnia magna* by Food Quality and Simultaneous Exposure to Dissolved Humic Substances." *Limnologica* 40 (2, Sp. Iss. SI): 86-91.

Campbell, P. G. C., M. R. Twiss, and K. J. Wilkinson. 1997. "Accumulation of Natural Organic Matter on the Surfaces of Living Cells: Implications for the Interaction of Toxic Solutes with Aquatic Biota." *Canadian Journal of Fisheries and Aquatic Sciences* 54 (11): 2543-2554.

Carbonaro, Richard F., Yasemin B. Atalay, and Dominic M. Di Toro. 2011. "Linear Free Energy Relationships for Metal-Ligand Complexation: Bidentate Binding to Negatively-Charged Oxygen Donor Atoms." *Geochimica Et Cosmochimica Acta* 75 (9): 2499-2511.

Celis-Salgado, Martha Patricia, Allegra Cairns, Natalie Kim, and Norman D. Yan. 2008. "The FLAMES Medium: A New, Soft-Water Culture and Bioassay Medium for Cladocera." *International Association of Theoretical and Applied Limnology, Vol 30, Pt 2, Proceedings* 30: 265-271.

Celo, V., J. Murimboh, M. S. A. Salam, and C. L. Chakrabarti. 2001. "A Kinetic Study of Nickel Complexation in Model Systems by Adsorptive Cathodic Stripping Voltammetry." *Environmental Science & Technology* 35 (6): 1084-1089.

Chen, Lei, Chaofeng Shen, Xianjin Tang, Chen Chen, and Yingxu Chen. 2012. "Estrogenic Effects of Dissolved Organic Matter and its Impact on the Activity of 17[Beta]-Estradiol." *Environmental Science and Pollution Research International* 19 (2): 522-8.

Christensen, J. B. and T. H. Christensen. 2000. "The Effect of pH on the Complexation of Cd, Ni and Zn by Dissolved Organic Carbon from Leachate-Polluted Groundwater." *Water Research* 34 (15): 3743-3754.

Curtis, P. Jefferson and David W. Schindler. 1997. "Hydrologic Control of Dissolved Organic Matter in Low-Order Precambrian Shield Lakes." *Biogeochemistry (Dordrecht)* 36 (1): 125-138.

Dahlen, J., S. Bertilsson, and C. Pettersson. 1996. "Effects of UV-A Irradiation on Dissolved Organic Matter in Humic Surface Waters." *Environment International* 22 (5): 501-506.

De Schamphelaere, K. A. C., J. L. Stauber, K. L. Wilde, S. J. Markich, P. L. Brown, N. M. Franklin, N. M. Creighton, and C. R. Janssen. 2005. "Toward a Biotic Ligand Model for Freshwater Green Algae: Surface-Bound and Internal Copper are Better Predictors of Toxicity than Free Cu2+-Ion Activity when pH is Varied." *Environmental Science & Technology* 39 (7): 2067-2072.

De Schamphelaere, K. A. C., F. M. Vasconcelos, F. M. G. Tack, H. E. Allen, and C. R. Janssen. 2004. "Effect of Dissolved Organic Matter Source on Acute Copper Toxicity to *Daphnia magna*." *Environmental Toxicology and Chemistry* 23 (5): 1248-1255.

Deleebeeck, Nele M. E., Karel A. C. De Schamphelaere, and Colin R. Janssen. 2008. "A Novel Method for Predicting Chronic Nickel Bioavailability and Toxicity to *Daphnia magna* in Artificial and Natural Waters." *Environmental Toxicology and Chemistry* 27 (10): 2097-2107.

Doig, Lorne E. and Karsten Liber. 2007. "Nickel Speciation in the Presence of Different Sources and Fractions of Dissolved Organic Matter." *Ecotoxicology and Environmental Safety* 66 (2): 169-177.

Environment Canada. 1996. Biological Test Method: Acute Lethality Test Using *Daphnia* spp. Report EPS 1/RM/11 May

Ertel, J. R., J. I. Hedges, and E. M. Perdue. 1984. "Lignin Signature of Aquatic Humic Substances." *Science (Washington D C)* 223 (4635): 485-487.

Fellman, Jason B., Eran Hood, and Robert G. M. Spencer. 2010. "Fluorescence Spectroscopy Opens New Windows into Dissolved Organic Matter Dynamics in Freshwater Ecosystems: A Review." *Limnology and Oceanography* 55 (6): 2452-2462.

Galvez, Fernando, Andrew Donini, Richard C. Playle, D. Scott Smith, Michael J. O'Donnell, and Chris M. Wood. 2008. "A Matter of Potential Concern: Natural Organic Matter Alters the Electrical Properties of Fish Gills." *Environmental Science & Technology* 42 (24): 9385-9390.

Gheorghiu, C., D. S. Smith, H. A. Al-Reasi, J. C. McGeer, and M. P. Wilkie. 2010. "Influence of Natural Organic Matter (NOM) Quality on Cu-Gill Binding in the Rainbow Trout (*Oncorhynchus mykiss*)." *Aquatic Toxicology* 97 (4): 343-352.

Glover, C. N., R. C. Playle, and C. M. Wood. 2005. "Heterogeneity of Natural Organic Matter Amelioration of Silver Toxicity to *Daphnia magna*: Effect of Source and Equilibration Time." *Environmental Toxicology and Chemistry* 24 (11): 2934-2940.

Glover, C. N., S. K. Sharma, and C. M. Wood. 2005. "Heterogeneity in Physicochemical Properties Explains Differences in Silver Toxicity Amelioration by Natural Organic Matter to *Daphnia magna*." *Environmental Toxicology and Chemistry* 24 (11): 2941-2947.

Gunn, M. John. 1995. *Restoration and Recovery of an Industrial Region: Progress in Restoring the Smelter-Damaged Landscape Near Sudbury, Canada*. Springer Series on Environmental Management. New York: Springer-Verlag.

Guthrie, J. W., N. M. Hassan, M. S. A. Salam, I. I. Fasfous, C. A. Murimboh, J. Murimboh, C. L. Chakrabarti, and D. C. Gregoire. 2005. "Complexation of Ni, Cu, Zn, and Cd by DOC in some Metal-Impacted Freshwater Lakes: A Comparison of Approaches using Electrochemical Determination of Free-Metal-Ion and Labile Complexes and a Computer Speciation Model, WHAM V and VI." *Analytica Chimica Acta* 528 (2): 205-218.

Haitzer, Markus, Gudrun Abbt-Braun, Walter Traunspurger, and Christian E. W. Steinberg. 1999. "Effects of Humic Substances on the Bioconcentration of Polycyclic Aromatic Hydrocarbons: Correlations with Spectroscopic and Chemical Properties of Humic Substances." *Environmental Toxicology and Chemistry* 18 (12): 2782-2788.

Hamilton, M. A., R. C. Russo, and R. V. Thurston. 1977. "Trimmed Spearman-Karber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays." *Environmental Science & Technology* 11 (7): 714-719.

Helms, John R., Aron Stubbins, Jason D. Ritchie, Elizabeth C. Minor, David J. Kieber, and Kenneth Mopper. 2008. "Absorption Spectral Slopes and Slope Ratios as Indicators of Molecular Weight, Source, and Photobleaching of Chromophoric Dissolved Organic Matter." *Limnology and Oceanography* 53 (3): 955-969.

Hertkorn, N., E. M. Perdue, and A. Kettrup. 2004. "A Potentiometric and Cd-113 NMR Study of Cadmium Complexation by Natural Organic Matter at Two Different Magnetic Field Strengths." *Analytical Chemistry* 76 (21): 6327-6341.

Hudson, Naomi, Andy Baker, and Darren Reynolds. 2007. "Fluorescence Analysis of Dissolved Organic Matter in Natural, Waste and Polluted Waters - A Review." *River Research and Applications* 23 (6): 631-649.

Hutchinson, N. J. and J. B. Sprague. 1987. "Reduced Lethality of Al, Zn and Cu Mixtures to American Flagfish by Complexation with Humic Substances in Acidified Soft Waters." *Environmental Toxicology and Chemistry* 6 (10): 755-765.

Jaffe, R., D. McKnight, N. Maie, R. Cory, W. H. McDowell, and J. L. Campbell. 2008. "Spatial and Temporal Variations in DOM Composition in Ecosystems: The Importance of Long-Term Monitoring of Optical Properties." *Journal of Geophysical Research-Biogeosciences* 113 (G4): G04032.

Keller, W., Andrew M. Paterson, Keith M. Somers, Peter J. Dillon, Jocelyne Heneberry, and Andrea Ford. 2008. "Relationships between Dissolved Organic Carbon Concentrations, Weather, and Acidification in Small Boreal Shield Lakes." *Canadian Journal of Fisheries and Aquatic Sciences* 65 (5): 786-795.

Klinck, J., M. Dunbar, S. Brown, J. Nichols, A. Winter, C. Hughes, and R. C. Playle. 2005. "Influence of Water Chemistry and Natural Organic Matter on Active and Passive Uptake of Inorganic Mercury by Gills of Rainbow Trout (*Oncorhynchus mykiss*)." *Aquatic Toxicology* 72 (1-2): 161-175.

Kozlova, Tatiaria, Chris M. Wood, and James C. McGeer. 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." *Aquatic Toxicology* 91 (3): 221-228.

Kozuch, J. and J. Pempkowiak. 1996. "Molecular Weight of Humic Acids as a Major Property of the Substances Influencing the Accumulation Rate of Cadmium by a Blue Mussel (*Mytilus edulis*)." *Environment International* 22 (5): 585-589.

Leenheer, J. A., G. K. Brown, P. MacCarthy, and S. E. Cabaniss. 1998. "Models of Metal Binding Structures in Fulvic Acid from the Suwannee River, Georgia." *Environmental Science & Technology* 32 (16): 2410-2416.

Leenheer, Jerry A. and Jean-Philippe Croue. 2003. "Characterizing Aquatic Dissolved Organic Matter." *Environmental Science and Technology* 37 (1): 18A-26A.

Leichtfried, Maria. 2007. "The Energy Basis of the Consumer Community in Streams Yesterday, Today and Tomorrow..." *International Review of Hydrobiology* 92 (4-5): 363-377.

Luider, Chad D., John Crusius, Richard C. Playle, and P. Jeff Curtis. 2004. "Influence of Natural Organic Matter Source on Copper Speciation as Demonstrated by Cu Binding to Fish Gills, by Ion Selective Electrode, and by DGT Gel Sampler." *Environmental Science & Technology* 38 (10): 2865-2872.

Macdonald, A., L. Silk, M. Schwartz, and R. C. Playle. 2002. "A Lead-Gill Binding Model to Predict Acute Lead Toxicity to Rainbow Trout (*Oncorhynchus mykiss*)." *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology* 133 (1-2): 227-242.

Mandal, R., M. S. A. Salam, C. L. Chakrabarti, and M. H. Back. 2003. "An Electrochemical Investigation of Complexation of Pb(II) by a Well-Characterized Fulvic Acid in Model Systems - Effect of Competition with Major Cations and Trace Metals." *Electroanalysis* 15 (10): 903-906.

Mandal, R., M. S. A. Salam, J. Murimboh, N. M. Hassan, C. L. Chakrabarti, M. H. Back, and D. C. Gregoire. 2000. "Competition of Ca(II) and mg(II) with Ni(II) for Binding by a Well-Characterized Fulvic Acid in Model Solutions." *Environmental Science & Technology* 34 (11): 2201-2208.

Mandal, R., A. L. R. Sekaly, J. Murimboh, N. M. Hassan, C. L. Chakrabarti, M. H. Back, D. C. Gregoire, and W. H. Schroeder. 1999. "Effect of the Competition of Copper and Cobalt on the Lability of Ni(II)-Organic Ligand Complexes, Part II: In Freshwaters (Rideau River Surface Waters)." *Analytica Chimica Acta* 395 (3): 323-334.

Matsuo, A. Y. O., R. C. Playle, A. L. Val, and C. M. Wood. 2004. "Physiological Action of Dissolved Organic Matter in Rainbow Trout in the Presence and Absence of Copper: Sodium Uptake Kinetics and Unidirectional Flux Rates in Hard and Softwater." *Aquatic Toxicology* 70 (1): 63-81.

McKnight, Diane M., Elizabeth W. Boyer, Paul K. Westerhoff, Peter T. Doran, Thomas Kulbe, and Dale T. Andersen. 2001. "Spectrofluorometric Characterization of Dissolved Organic Matter for Indication of Precursor Organic Material and Aromaticity." *Limnology and Oceanography* 46 (1): 38-48.

Nachtegaal, M. and D. L. Sparks. 2003. "Nickel Sequestration in a Kaolinite-Humic Acid Complex." *Environmental Science & Technology* 37 (3): 529-534.

Nadella, Sunita R., John L. Fitzpatrick, Natasha Franklin, Carol Bucking, Scott Smith, and Chris M. Wood. 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." *Comparative Biochemistry and Physiology Part C Toxicology & Pharmacology* 149 (3): 340-348.

OECD 2004. *Daphnia* sp., Acute Immobilisation Test. OECD Guidline for Testing of Chemicals.202. OECD, Paris.

Pane, E. F., J. C. McGeer, and C. M. Wood. 2004. "Effects of Chronic Waterborne Nickel Exposure on Two Successive Generations of *Daphnia magna*." *Environmental Toxicology and Chemistry* 23 (4): 1051-1056.

Pane, E. F., J. G. Richards, and C. M. Wood. 2003. "Acute Waterborne Nickel Toxicity in the Rainbow Trout (Oncorhynchus Mykiss) Occurs by a Respiratory rather than Ionoregulatory Mechanism." *Aquatic Toxicology* 63 (1): 65-82.

Pane, E. F., C. Smith, J. C. McGeer, and C. M. Wood. 2003. "Mechanisms of Acute and Chronic Waterborne Nickel Toxicity in the Freshwater Cladoceran, *Daphnia magna*." *Environmental Science & Technology* 37 (19): 4382-4389.

Pempkowiak, J., J. Kozuch, H. Grzegowska, and E. T. Gjessing. 1999. "Biological Vs. Chemical Properties of Natural Organic Matter Isolated from Selected Norwegian Lakes." *Environment International* 25 (2-3): 357-366.

Perdue, E.M. 2009. "Natural Organic Matter" in G.E. Likens, Encyclopedia of Inland Waters 2: 806-819. Oxford: Elsevier

Playle, R. C. 1998. "Modelling Metal Interactions at Fish Gills." *Science of the Total Environment* 219 (2-3): 147-163.

Richards, J. G., P. J. Curtis, B. K. Burnison, and R. C. Playle. 2001. "Effects of Natural Organic Matter Source on Reducing Metal Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) and on Metal Binding to their Gills." *Environmental Toxicology and Chemistry* 20 (6): 1159-1166.

Santore, R. C., R. Mathew, P. R. Paquin, and D. DiToro. 2002. "Application of the Biotic Ligand Model to Predicting Zinc Toxicity to Rainbow Trout, Fathead Minnow, and *Daphnia magna*." *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology* 133 (1-2): 271-285.

Schindler, D. W., S. E. Bayley, P. J. Curtis, B. R. Parker, M. P. Stainton, and C. A. Kelly. 1992. "Natural and Man-Caused Factors Affecting the Abundance and Cycling of Dissolved Organic-Substances in Precambrian Shield Lakes." *Hydrobiologia* 229: 1-21.

Schwartz, Melissa L., P. Jefferson Curtis, and Richard C. Playle. 2004. "Influence of Natural Organic Matter Source on Acute Copper, Lead, and Cadmium Toxicity to Rainbow Trout (*Oncorhynchus mykiss*)." *Environmental Toxicology and Chemistry* 23 (12): 2889-2899.

Sekaly, A. L. R., R. Mandal, N. M. Hassan, J. Murimboh, C. L. Chakrabarti, M. H. Back, D. C. Gregoire, and W. H. Schroeder. 1999. "Effect of metal/fulvic Acid Mole Ratios on the

Binding of Ni(II), Pb(II), Cu(II), Cd(II), and Al(III) by Two Well-Characterized Fulvic Acids in Aqueous Model Solutions." *Analytica Chimica Acta* 402 (1-2): 211-221.

Senesi, N. 1990. "Molecular and Quantitative Aspects of the Chemistry of Fulvic-Acid and its Interactions with Metal-Ions and Organic-Chemicals .2. the Fluorescence Spectroscopy Approach." *Analytica Chimica Acta* 232 (1): 77-106.

Senesi, N., T. M. Miano, M. R. Provenzano, and G. Brunetti. 1991. "Characterization Differentiation and Classification of Humic Substances by Fluorescence Spectroscopy." *Soil Science* 152 (4): 259-271.

Smith, D. S., R. A. Bell, and J. R. Kramer. 2002. "Metal Speciation in Natural Waters with Emphasis on Reduced Sulfur Groups as Strong Metal Binding Sites." *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology* 133 (1-2): 65-74.

Suhett, Albert L., Christian E. W. Steinberg, Jayme M. Santangelo, Reinaldo L. Bozelli, and Vinicius F. Farjalla. 2011. "Natural Dissolved Humic Substances Increase the Lifespan and Promote Transgenerational Resistance to Salt Stress in the Cladoceran *Moina macrocopa*." *Environmental Science and Pollution Research* 18 (6): 1004-1014.

Takacs, M., J. J. Alberts, and P. K. Egeberg. 1999. "Characterization of Natural Organic Matter from Eight Norwegian Surface Waters: Proton and Copper Binding." *Environment International* 25 (2-3): 315-323.

The Biotic Ligand Model Windows Interface, Version 2.2.3: User's Guide and Reference Manual, HydroQual, Inc, Mahwah, NJ, April 2005.

Thurman, E. M. 1985. *Organic Geochemistry of Natural Waters /*. Developments in Biogeochemistry. Dordrecht ;: M. Nijhoff ;.

Timofeyev, Maxim A., Claudia Wiegand, B. Kent Burnison, Zhanna M. Shatilina, Stephan Pflugmacher, and Christian E. W. Steinberg. 2004. "Impact of Natural Organic Matter (NOM) on Freshwater Amphipods." *Science of the Total Environment* 319 (1-3): 115.

Valois, Amanda E., W. Bill Keller, and Charles W. Ramcharan. 2011. "Recovery in a Multiple Stressor Environment: Using the Reference Condition Approach to Examine Zooplankton Community Change Along Opposing Gradients." *Journal of Plankton Research* 33 (9): 1417-1429.

Vigneault, B., A. Percot, M. Lafleur, and P. G. C. Campbell. 2000. "Permeability Changes in Model and Phytoplankton Membranes in the Presence of Aquatic Humic Substances." *Environmental Science & Technology* 34 (18): 3907-3913.

Weishaar, J. L., G. R. Aiken, B. A. Bergamaschi, M. S. Fram, R. Fujii, and K. Mopper. 2003. "Evaluation of Specific Ultraviolet Absorbance as an Indicator of the Chemical

Composition and Reactivity of Dissolved Organic Carbon." *Environmental Science & Technology* 37 (20): 4702-4708.

Wong, C.Y. Jessica. 2009. "Source Tracing of Dissolved Organic Matter (DOM) in Watersheds Using UV and Fluorescence Spectroscopy." *Unpublished Master's Thesis, University of Toronto, Ontario, Canada*

Table 1. The description of sites where NOM isolates were obtained. For measurements below detection, BD is used, detection limit for Ni < 0.18 µg L^{-1} and for Cu <0.11 µg L^{-1}. Clearwater outflow analysis remains incomplete, however it is expected to be similar to those of Clearwater Lake. Metal levels of the White River sites are expected to be very low.

NOM Source	Type	Condition	Coordinates	DOC (mg·L^{-1})	pH	Conductivity (µS·cm^{-1})	Nickel (µg L^{-1})	Copper (µg L^{-1})
Lake Laurentian (LL)	Lake	Metal Contaminated	46447'N, 80.961'W	7.1	6.45	146	56	15
Laurentian Wetland (LW)	Wetlands	Metal Contaminated	46450'N, 80942'W	65.75	4.62	30.2	303.6	236.2
Daisy Lake (DL)	Lake	Metal Contaminated	46450'N, 80888'W	2.5	6.71	33.3	55.3	8.9
White River 1 (WR1)	Stream	Pristine	48751'N, 85173'W	17.03	7.4	102.1	BD	3.7
White River 2 (WR2)	Stream	Logged	48653'N, 85364'W	4.26	7.6	133.5	BD	2.6
White River 3 (WR3)	Stream	Fire	48431'N, 85350'W	9.9	7.6	197.6	BD	BD
Clearwater Lake (CW)	Lake	Metal Contaminated	46224'N, 81245'W	3	6.72	58.8	42.8	8.5
Clearwater outflow (SSW)	Stream	Metal Contaminated	462244'N, 81246'W					
Harp Lake (HL)	Lake	Pristine	45228'N, 79085'W	4.5	6.5	32.2	0.4	0.8
Harp Inflow 3 (Hp3)	Stream	Pristine	45224'N, 79084'W	18.4	5.84	71.4	1.1	0.8
Plastic Lake (PL)	Lake	Acidified	45107'N, 78496'W	3.2	5.5	11.4	0.4	0.3
Plastic inflow 1 (PC1)	Stream below wetland	Acidified	45107'N, 78497'W	22.2	4.64	24.4	0.5	1.1
Plastic inflow 108 (PC108)	Stream	Acidified	45109'N, 78497'W	3.3	5.03	17.4	1.2	0.7

Table 2. The number of replicates used to determine mean LC_{50}s; mean dissolved organic carbon (DOC); mean Ca concentration; and mean pH of control and FLAMES with the addition of NOM isolate. Measured as the mean ± standard deviation.

Treatments	N	DOC (mg L^{-1})	pH	Ca (mg L^{-1})
FLAMES no added NOM	5	1.75 ± 1.04	6.5 ± 0.14	2.55 ± .09
Lake Laurentian	6	7.8 ± 1.49	6.65 ± 0.15	4.52 ± 0.28
Laurentian Wetland	6	7.3 ± 2.06	6.53 ± 0.13	2.49 ± 0.04
Daisy Lake	6	6.81 ± 2.14	6.58 ± 0.11	2.86 ± 0.04
White River 1	3	7.56 ± 0.11	6.58 ± 0.09	2.86 ± 0.03
White River 2	6	7.13 ± 0.17	6.94 ± 0.04	3.28 ± 0.06
White River 3	3	7.89 ± 1.52	6.54 ± 0.06	2.76 ± 0.04
Clearwater Lake	6	6.86 ± 0.07	6.71 ± 0.02	4.24 ± 0.09
Clearwater outflow	6	9.04 ± 0.19	6.81 ± 0.03	7.37 ± 0.09
Harp Lake	6	6.69 ± 0.03	6.77 ± 0.04	3.81 ± 0.03
Harp Inflow 3	6	6.99 ± 0.06	6.72 ± 0.06	4.16 ± 0.02
Plastic Lake	6	8.0 ± 0.63	6.67 ± 0.08	4.51 ± 0.13
Plastic Lake Inflow 1	6	8.0 ± 0.27	6.54 ± 0.08	2.46 ± 0.03
Plastic Lake Inflow 108	6	7.31 ± 0.27	6.76 ± 0.08	4.96 ± 0.05

A.

B.
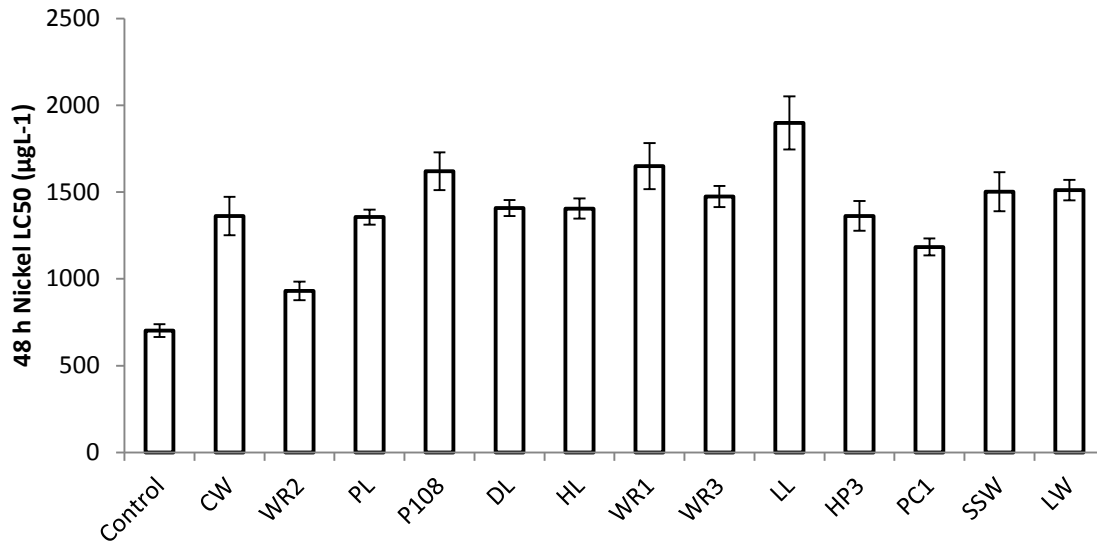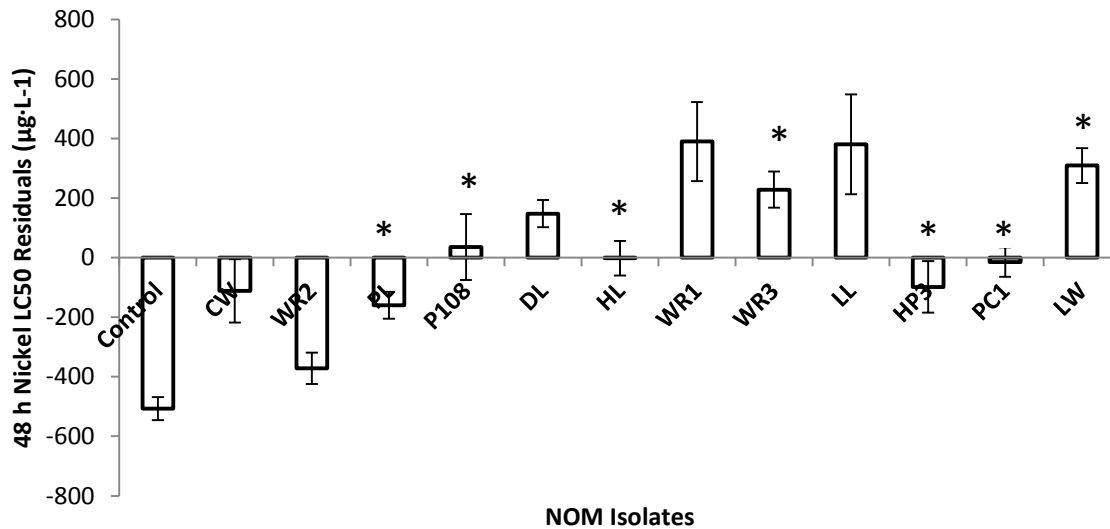
Figure 1. (A) Meansured 48-h 50% lethal concentration (LC$_{50}$) based on nominal nickel concentrations to *Daphnia pulex/pulicaria* with the addition of NOM from thirteen different sites. (B) LC50s residuals obtained from the relationship between measured LC50s and calcium concentration, SSW was not included. All residuals are more positive than the control. NOM treatments are arranged in order of its SAC value. All NOM was added as a nominal value of 7 mg C·L^{-1}. LC$_{50}$s which were significantly different from the control are denoted by *.
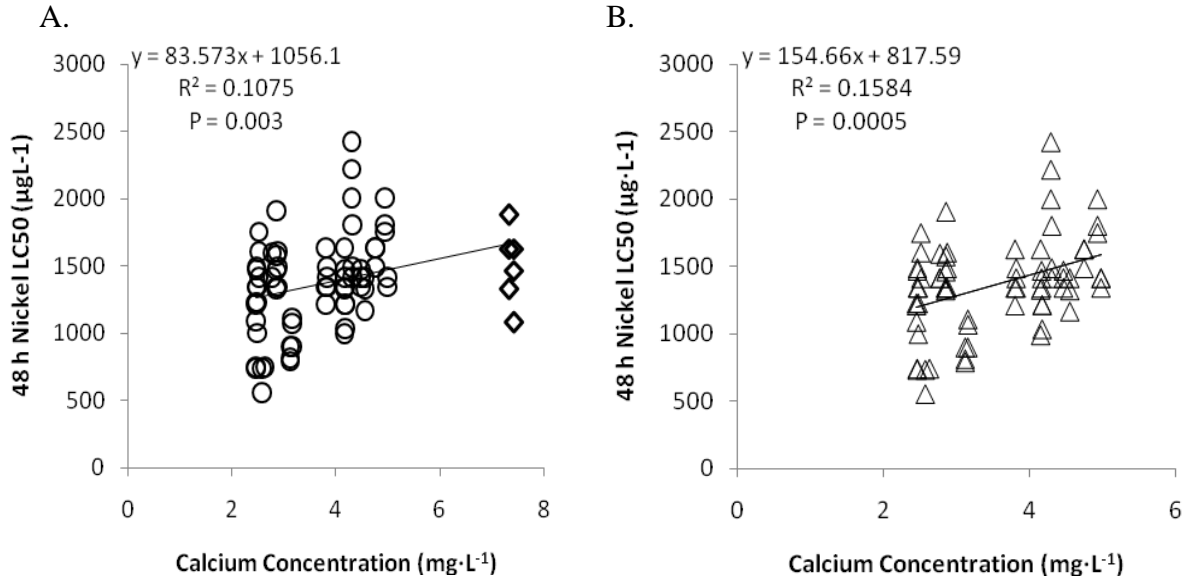
Figure 2. Relationship between 48-h nickel LC$_{50}$s and calciuming concentration (A) including the influential outlier SSW ($\lozenge$) (B) without the influential outlier.

Table 3. Correlation coefficient for linear regression between each spectroscopic technique and 48-h Ni LC_{50}s.

Spectroscopic Technique	Correlation Coefficient
Specific absorption coefficient (SAC)	0.556
Specific UV absorbance (SUVA)	0.578
Molecular Weight	-0.512
Fluorescent index	0.138
Relative percent humic acid (HA%)	0.172
Relative percent fulvic acid (FA%)	-0.104

A.
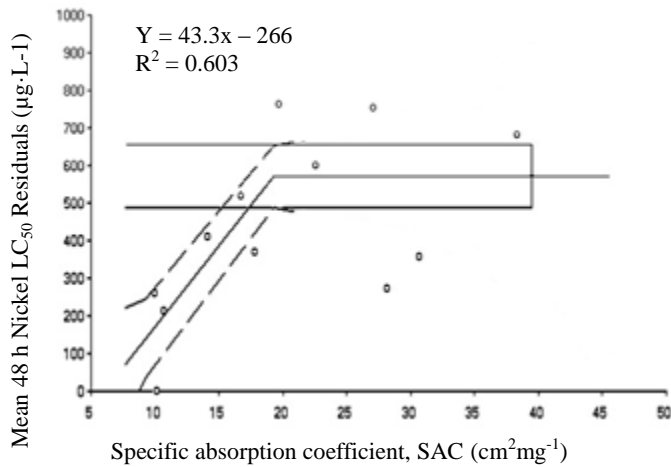
B.
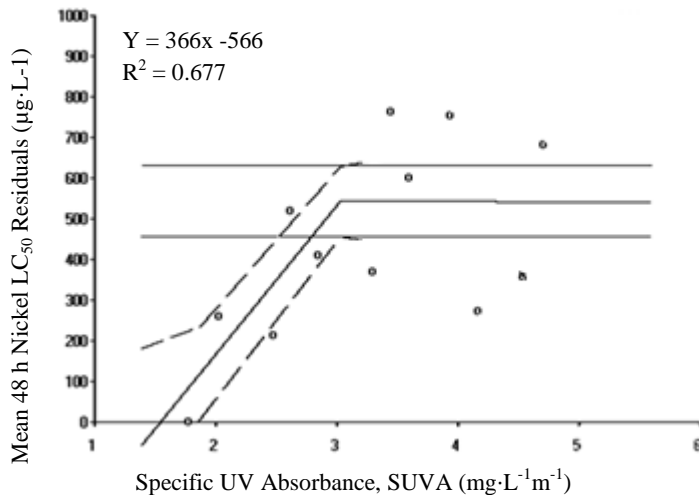
Figure 3. Relationship between mean 48-h Ni LC_{50}, using residuals from linear regression between Ni LC_{50} and Ca concentrations. and (A) SAC (a proxy for aromaticity and colour) and (B) SUVA (another proxy for aromaticity). The residuals used for A and B were from the relationship in Figure 2B without the inclusion of SSW. LC_{50} break-point and its 90% confidence block of break-point is shown as well as the 90% confidence belt. Linear regression equations are for the relationship to the left of the breakpoint.
NOM sources were added at a nominal concentration of 7 mg C•L^{-1}.

A.
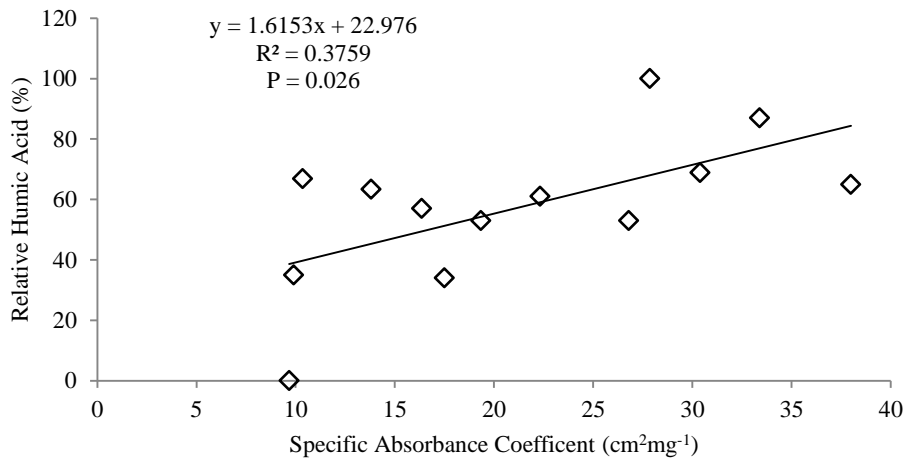
B.
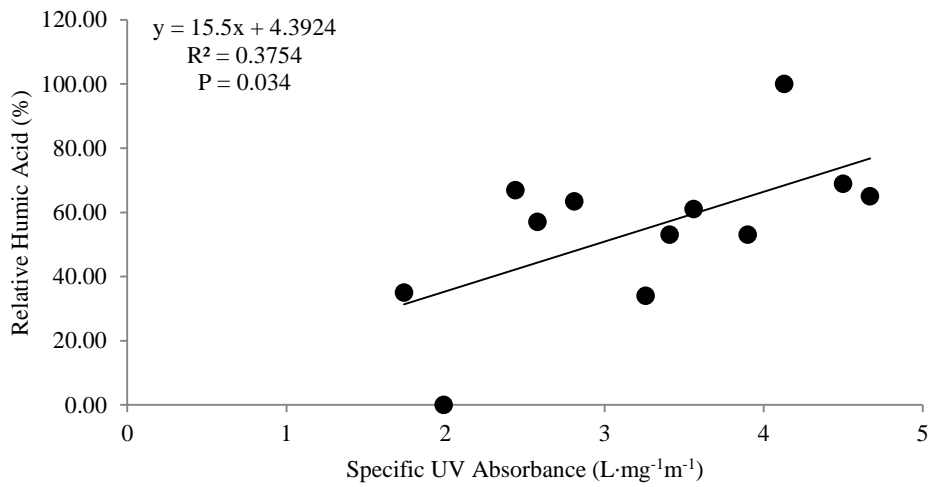
Figure 4. The relationship between relative humic acid percent obtained from PARAFAC and (A) specific absorbance coefficient, SAC, and (B) specific UV absorbance, SUVA.
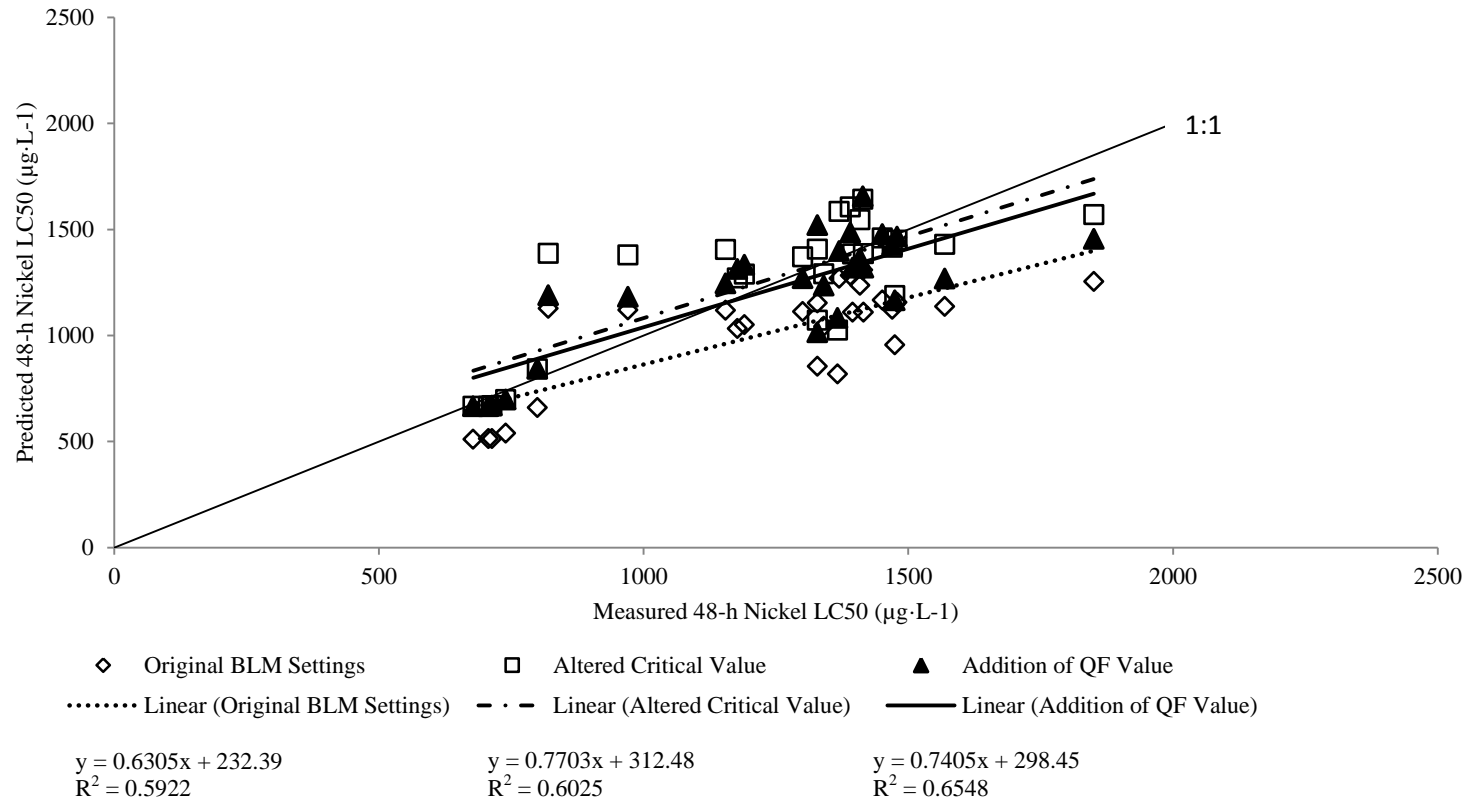
Figure 5. The relationship between BLM-predicted Ni LC_{50} ($\mu g \cdot L^{-1}$) values and measured LC_{50}s. Predicted values from the BLM include those with the critical value obtained from (◊) Kozlova et al., (2009): 5.1; and when the critical value was corrected for (□) this study: 6.37; as well as predicted values obtained when NOM structure was incorporated using the (▲) F quality factor.

Concluding Remarks

It was formerly held that natural organic matter (NOM) was a large and inert substance having little to no effect on aquatic biota (Steinberg et al., 2002). While this paradigm has shifted over the last decade, this thesis adds to the growing literature exploring the direct effects NOM indeed has on aquatic biota. Not only does it concern how NOM aids in ameliorating toxic environments, it also attempts to explain which structural features of NOM cause these observed changes and demonstrates how this knowledge can improve predictive models.

The first chapter of this thesis discusses how NOM from four out of five different sources decreased time to primiparity and mean generation time; it also increased specific growth rate and intrinsic rate of natural increase of a wild *Daphnia* species. Three of these changes in life history traits were related to the colour and aromatic content of NOM isolates as measured by absorbance characteristic techniques, i.e. specific absorbance coefficient measured at 340 nm (SAC) and specific UV absorbance (SUVA). In the second chapter of this thesis, these same NOM characteristics were related to acute Ni toxicity mitigation when 12 different NOM isolates were added to Ni-spiked water. Seven out of the 12 isolates provided a significant amount of protection. NOM with a measured SAC of 19.35 cm^2mg^{-1} and below had a positive relationship with measured LC_{50}s, however, isolates with higher SAC values had no relationship with measured toxicity. The decrease in Ni mitigation may not be due directly to aromatic structures, but also to phenolic functional groups of NOM. Altering DOC concentrations in the Biotic Ligand Model with the quality factor (a method used to incorporate SAC) improved the relationship between measured and predicted acute Ni LC_{50}s far better than any other prescribed method of NOM character inclusion. While NOM remains a complex substance, delineating its general characteristics has proven useful in interpreting the results obtained in this study. Colour

and aromatic content specifically appear to be major factors influencing both direct effects as well as toxicity mitigation.

With NOM on the rise in northeastern North America, and northern and central Europe (Monteith et al., 2007), future research may do well to focus on how these life history traits are affected by this increase in NOM concentration, paying heed to its varying aromatic contents. I suggest: 1) taking on such investigations by performing similar experiments as Menzel et al. (2012) to determine if phenolic/quinonoid moieties are the functional groups responsible for changes in *Daphnia* life history; 2) examining entire genome DNA microarray experiments over a range of NOM sources to determine if changes in time to maturation and growth are due to hormesis in nature from NOM-induced stress (Steinberg et al., in press); and 3) exploring indirect effects by performing chronic toxicity studies to determine if NOM structurally-induced differences persist at lower Ni concentrations. This research is a stepping stone in gaining a greater understanding of how NOM characteristics influence its wide role in aquatic ecology and the above are but a few suggestions in its development.

NOM cannot be thought of as a single entity. As observed in this thesis, the differences between NOM characteristics influence its direct effects on aquatic biota as well as its indirect effects by altering the protection it provides against acute Ni toxicity. Understanding the effects of NOM on aquatic biota and determining which aspects of NOM have the greatest influence can provide insight to agencies in setting water quality standards as well as understanding how changes in the environment (such as increasing NOM) will affect aquatic biota in the long term.

References (Concluding Remarks)

Menzel, Ralph, Stefanie Menzel, Sophie Tiedt, Georg Kubsch, Reinhardt Stoesser, Hanno Baehrs, Anke Putschew, Nadine Saul, and Christian E. W. Steinberg. 2011. "Enrichment of Humic Material with Hydroxybenzene Moieties Intensifies its Physiological Effects on the Nematode *Caenorhabditis Elegans*." *Environmental Science & Technology* 45 (20): 8707-8715.

Meinelt, Thomas, Andrea Paul, Thuy My Phan, Elke Zwirnmann, Angela Krueger, Andreas Wienke, and Christian E. W. Steinberg. 2007. "Reduction in Vegetative Growth of the Water Mold *Saprolegnia Parasitica* (Coker) by Humic Substance of Different Qualities." *Aquatic Toxicology* 83 (2): 93-103.

Steinberg, C. E. W., S. Hoss, and R. Bruggemann. 2002. "Further Evidence that Humic Substances have the Potential to Modulate the Reproduction of the Nematode *Caenorhabditis Elegans*." *International Review of Hydrobiology* 87 (1): 121-133.

Steinberg, C.E.W., K. Pietsch, N. Saul, S. Mezel, S. C. Swain, S. R. Stürzenbaum and R. Menzel. In press. "Transcript Expression Patterns Illuminate the Mechanistic Background of Hormesis in *Caenorhabditis Elegans* Maupas." *Dose-Response*

Appendix A:

Photos of portable reverse osmosis apparatus used to collect natural organic matter (NOM) samples and of five of the NOM isolates collected.

A.

B.

Appendix B:

Raw survival and reproductive data for chronic study looking at the effects of natural organic matter (NOM) on *Daphnia*.

The raw data collected for the experiment described in Chapter 1 is presented in the following table. Ten *D. pulex/pulicaria* individuals were exposed to five different NOM isolates at a nominal concentration of 7 mg C·L^{-1} for 18 days. Day 1 is the start of the experiment with *Daphnia* less than 24 hours in age. Experimental animals are labelled #1-10 in the following table. Each table lists the number of neonates produced by each individual daphniid. Mortality is marked by an underscore.

			Adult Survival and number of neonates born									
Treatment	Date	Day	1	2	3	4	5	6	7	8	9	10
FLAMES	22-Nov-2011	1	0	0	0	0	0	0	0	0	0	0
	23-Nov-2011	2	0	0	0	0	0	0	0	0	0	0
	24-Nov-2011	3	0	0	0	0	0	0	0	0	0	0
	25-Nov-2011	4	0	0	0	0	0	0	0	0	0	0
	26-Nov-2011	5	0	0	0	0	0	0	0	0	0	0
	27-Nov-2011	6	0	0	0	0	0	0	0	0	0	0
	28-Nov-2011	7	0	0	0	0	0	0	0	0	0	0
	29-Nov-2011	8	0	0	0	0	0	0	0	0	0	0
	30-Nov-2011	9	0	0	2	0	0	9	0	0	0	0
	01-Dec-2011	10	5	6	0	2	7	0	0	0	1	0
	02-Dec-2011	11	0	0	0	0	0	0	0	0	0	7
	03-Dec-2011	12	8	0	5	5	10	0	0	11	10	0
	04-Dec-2011	13	0	0	0	0	0	0	1	0	0	0
	05-Dec-2011	14	0	10	6	0	0	0	1	0	0	0
	06-Dec-2011	15	8	12	0	8	11	15	0	13	12	0
	07-Dec-2011	16	0	0	0	0	0	0	0	0	0	0
	08-Dec-2011	17	13	0	9	6	0	0	0	11	0	10
	09-Dec-2011	18	0	9	0	0	1	13	0	0	16	0
Clearwater Lake	22-Nov-2011	1	0	0	0	0	0	0	0	0	0	0
	23-Nov-2011	2	0	0	0	0	0	0	0	0	0	0
	24-Nov-2011	3	0	0	0	0	0	0	0	0	0	0
	25-Nov-2011	4	0	0	0	0	0	0	0	0	0	0
	26-Nov-2011	5	0	0	0	0	0	0	0	0	0	0
	27-Nov-2011	6	0	0	0	0	0	0	0	0	0	0

Location	Date	Day										
	28-Nov-2011	7	0	0	0	0	0	0	0	0	0	0
	29-Nov-2011	8	0	0	0	0	0	0	0	0	0	0
	30-Nov-2011	9	9	9	0	0	0	8	9	0	0	0
	01-Dec-2011	10	0	0	6	8	0	0	0	5	7	6
	02-Dec-2011	11	0	0	0	0	7	0	0	0	0	0
	03-Dec-2011	12	19	16	9	0	0	22	17	0	18	19
	04-Dec-2011	13	0	0	0	13	13	0	0	15	0	0
	05-Dec-2011	14	0	0	0	0	0	0	0	0	14	0
	06-Dec-2011	15	15	18	4	16	0	15	19	13	0	18
	07-Dec-2011	16	0	18	0	0	15	0	0	0	0	0
	08-Dec-2011	17	20	0	16	0	0	23	20	0	21	25
	09-Dec-2011	18	0	21	0	19	21	0	0	17	0	0
Daisy Lake	22-Nov-2011	1	0	0	0	0	0	0	0	0	0	0
	23-Nov-2011	2	0	0	0	0	0	0	0	0	0	0
	24-Nov-2011	3	0	0	0	0	0	0	0	0	0	0
	25-Nov-2011	4	0	0	0	0	0	0	0	0	0	0
	26-Nov-2011	5	0	0	0	0	0	0	0	0	0	0
	27-Nov-2011	6	0	0	0	0	0	0	0	0	0	0
	28-Nov-2011	7	0	0	0	0	0	0	0	0	0	0
	29-Nov-2011	8	0	0	0	0	0	0	0	0	0	0
	30-Nov-2011	9	0	0	0	0	0	0	0	0	0	0
	01-Dec-2011	10	6	0	0	0	0	0	_	0	8	0
	02-Dec-2011	11	0	0	0	5	0	0		0	0	4
	03-Dec-2011	12	11	0	4	0	0	7		0	0	0
	04-Dec-2011	13	0	4	0	4	5	0		5	14	8
	05-Dec-2011	14	0	0	8	0	0	0		0	0	0

	Date											
	06-Dec-2011	15	16	0	0	0	9	0		7	18	0
	07-Dec-2011	16	0	7	0	12	0	0		0	18	13
	08-Dec-2011	17	0	0	0	0	0	0		0	0	0
	09-Dec-2011	18	19	8	0	17	14	0		17	19	20
Lake Laurentian	22-Nov-2011	1	0	0	0	0	0	0	0	0	0	0
	23-Nov-2011	2	0	0	0	0	0	0	0	0	0	0
	24-Nov-2011	3	0	0	0	0	0	0	0	0	0	0
	25-Nov-2011	4	0	0	0	0	0	0	0	0	0	0
	26-Nov-2011	5	0	0	0	0	0	0	0	0	0	0
	27-Nov-2011	6	0	0	0	0	0	0	0	0	0	0
	28-Nov-2011	7	0	0	0	0	0	0	0	0	0	0
	29-Nov-2011	8	0	0	0	0	0	0	0	0	6	5
	30-Nov-2011	9	<u>5</u>	11	9	6	7	10	7	5	0	0
	01-Dec-2011	10		0	0	0	0	0	0	0	0	16
	02-Dec-2011	11		0	0	0	0	0	0	0	14	0
	03-Dec-2011	12		19	18	17	14	18	12	16	0	0
	04-Dec-2011	13		0	0	0	0	0	0	0	20	20
	05-Dec-2011	14		16	10	14	2	10	0	18	0	0
	06-Dec-2011	15		0	0	0	16	0	15	0	0	0
	07-Dec-2011	16		24	0	0	0	0	0	0	16	16
	08-Dec-2011	17		0	20	20	17	22	0	23	0	0
	09-Dec-2011	18		0	0	0	0	0	17	0	17	15
Clearwater Lake Outflow	22-Nov-2011	1	0	0	0	0	0	0	0	0	0	0
	23-Nov-2011	2	0	0	0	0	0	0	0	0	0	0
	24-Nov-2011	3	0	0	0	0	0	0	0	0	0	0
	25-Nov-2011	4	0	0	0	0	0	0	0	0	0	0

	Date											
	26-Nov-2011	5	0	0	0	0	0	0	0	0	0	0
	27-Nov-2011	6	0	0	0	0	0	0	0	0	0	0
	28-Nov-2011	7	0	0	0	0	0	0	0	0	0	0
	29-Nov-2011	8	5	8	5	0	6	6	7	4	6	8
	30-Nov-2011	9	0	0	0	14	0	0	0	0	0	0
	01-Dec-2011	10	18	16	18	0	14	20	17	16	16	20
	02-Dec-2011	11	0	0	0	0	0	0	0	0	0	0
	03-Dec-2011	12	26	0	26	20	0	24	0	4	0	22
	04-Dec-2011	13	0	22	0	0	23	0	22	21	20	3
	05-Dec-2011	14	0	0	0	0	0	0	0	0	0	0
	06-Dec-2011	15	22	19	21	15	16	21	18	21	9	24
	07-Dec-2011	16	0	0	0	0	0	0	0	0	0	0
	08-Dec-2011	17	0	0	31	18	0	26	0	0	0	28
	09-Dec-2011	18	26	21	0	0	21	0	22	23	20	0
Laurentian Wetland	22-Nov-2011	1	0	0	0	0	0	0	0	0	0	0
	23-Nov-2011	2	0	0	0	0	0	0	0	0	0	0
	24-Nov-2011	3	0	0	0	0	0	0	0	0	0	0
	25-Nov-2011	4	0	0	0	0	0	0	0	0	0	0
	26-Nov-2011	5	0	0	0	0	0	0	0	0	0	0
	27-Nov-2011	6	0	0	0	0	0	0	0	0	0	0
	28-Nov-2011	7	0	0	0	0	0	0	0	0	0	0
	29-Nov-2011	8	5	6	6	0	5	0	0	0	0	0
	30-Nov-2011	9	0	0	0	10	0	7	6	6	9	5
	01-Dec-2011	10	13	12	17	0	0	0	0	0	0	0
	02-Dec-2011	11	0	0	0	0	12	0	0	0	0	0
	03-Dec-2011	12	0	0	27	23	0	21	16	19	20	19

Date											
04-Dec-2011	13	25	15	0	0	0	0	0	0	0	0
05-Dec-2011	14	0	0	0	0	0	0	21	20	20	0
06-Dec-2011	15	22	9	23	22	0	24	0	0	0	22
07-Dec-2011	16	0	0	0	0	4	0	0	0	0	0
08-Dec-2011	17	0	0	29	27	0	21	21	13	29	30
09-Dec-2011	18	21	16	0	0	12	0	0	0	0	0

Appendix C:

Raw survival data for preliminary study investigating *Daphnia* survival without food in both FLAMES and FLAMES without EDTA

The raw survival and reproductive data collected for a preliminary experiment used in Chapter 2 is presented in the following table. Ten *D. pulex/pulicaria* individuals were placed in FLAMES and FLAMES-without-EDTA. Animals were not fed for the seven days of the experiment. Day 1 is the start of the experiment with *Daphnia* >24 hours in age. Experimental animals are labelled #1-10. Each table lists the survival of individual *Daphnia*. Mortality is marked by an underscore.

Treatment	Date	Day	**Adult Survival and number of neonates born**									
			1	2	3	4	5	6	7	8	9	10
FLAMES	16-Jul-2011	1	0	0	0	0	0	0	0	0	0	0
	17-Jul-2011	2	0	0	0	0	0	0	0	0	0	0
	18-Jul-2011	3	0	0	0	0	0	0	0	0	0	0
	19-Jul-2011	4	0	0	0	0	0	0	0	0	0	0
	20-Jul-2011	5	0	0	0	0	0	0	0	0	0	0
	21-Jul-2011	6	0	0	0	0	0	0	0	0	0	0
	22-Jul-2011	7	0	0	0	0	0	0	0	0	0	0
FLAMES without EDTA	16-Jul-2011	1	0	0	0	0	0	0	0	0	0	0
	17-Jul-2011	2	0	0	0	0	0	0	0	0	0	0
	18-Jul-2011	3	0	0	0	0	0	0	0	0	0	0
	19-Jul-2011	4	0	0	0	0	0	0	0	0	0	0
	20-Jul-2011	5	0	0	0	0	0	0	_	0	0	0
	21-Jul-2011	6	0	0	0	0	_	0		0	0	0
	22-Jul-2011	7	0	0	0	0		0		0	0	0

Appendix D:

Raw mortality data for 48-hour acute Ni toxicity tests.

The raw mortality data collected for acute toxicity tests used in Chapter 2 is presented in the following table. Thirteen different natural organic matter samples were tested to determine their protective effects on *Daphnia*. NOM concentrations were at a nominal 6 mg C·L^{-1}. For each NOM isolate, eight *Daphnia* were placed in containers at four Ni concentrations of 500 µg L^{-1}, 1000 µg L^{-1}, 2000 µg L^{-1}, and 4000 µg L^{-1} plus a container with only NOM and a container with FLAMES-without-EDTA. This was repeated six times for each NOM isolate. An "X" denotes occasion when animals were not subjected to that treatment.

Treatment	Replicate	Number of animals dead at Ni Concentrations (µg L^{-1})					
		0 FLAMES WITHOUT EDTA	0 NOM only	500	1000	2000	4000
No NOM added	1	0	X	3	8	8	8
No NOM added	2	0	X	1	7	8	8
No NOM added	3	0	X	0	0	7	8
No NOM added	4	0	X	1	7	8	8
No NOM added	5	0	X	0	0	7	8
No NOM added	6	1	X	1	6	8	8
Clearwater Lake	1	0	0	2	0	8	8
Clearwater Lake	2	0	0	3	1	8	8
Clearwater Lake	3	0	0	1	1	8	8
Clearwater lake	4	0	0	0	0	7	8
Clearwater Lake	5	0	0	0	0	8	8
Clearwater Lake	6	0	0	0	0	5	8
White River 2	1	0	0	0	5	8	8
White River 2	2	0	0	0	3	8	8
White River 2	3	0	0	0	5	6	8
White River 2	4	0	0	0	5	8	8
White River 2	5	0	0	0	6	8	8
White River 2	6	0	0	0	7	7	8
Plastic Lake	1	0	0	0	1	8	8
Plastic Lake	2	0	0	0	0	8	8
Plastic Lake	3	0	0	0	1	7	8
Plastic Lake	4	0	1	0	1	8	8

Plastic Lake	5	0	0	1	1	7	8
Plastic Lake	6	0	0	0	0	8	8
Plastic Lake Inflow 108	1	0	0	0	1	8	8
Plastic Lake Inflow 108	2	0	0	0	4	8	8
Plastic Lake Inflow 108	3	0	0	0	2	8	8
Plastic Lake Inflow 108	4	0	0	0	2	8	8
Plastic Lake Inflow 108	5	0	0	0	3	7	8
Plastic Lake Inflow 108	6	0	0	0	4	7	8
Daisy Lake	1	0	0	1	1	7	8
Daisy Lake	2	0	0	0	1	8	8
Daisy Lake	3	0	0	0	1	8	8
Daisy Lake	4	0	0	0	1	8	8
Daisy Lake	5	0	0	0	2	6	8
Daisy Lake	6	0	0	0	0	7	8
Harp Lake	1	0	0	0	0	6	8
Harp Lake	2	0	0	0	2	7	8
Harp Lake	3	0	0	0	3	7	8
Harp Lake	4	0	0	0	0	7	8
Harp Lake	5	0	0	0	0	8	8
Harp Lake	6	0	0	0	1	8	8
White River 1	1	0	0	1	1	5	8
White River 1	2	0	0	0	1	4	8
White River 1	3	0	0	0	2	6	8
White River 3	1	0	0	0	2	8	8
White River 3	2	0	0	0	3	6	8
White River 3	3	0	0	0	3	8	8
Lake Laurentian	1	0	0	0	0	6	8

Lake Laurentian	2	0	0	0	0	7	8
Lake Laurentian	3	0	0	0	0	6	8
Lake Laurentian	4	0	0	0	0	4	8
Lake Laurentian	5	0	0	0	0	3	8
Lake Laurentian	6	0	0	0	0	3	7
Harp Inflow 3	1	0	1	0	0	8	8
Harp Inflow 3	2	0	0	1	0	7	8
Harp Inflow 3	3	0	0	0	0	6	8
Harp Inflow 3	4	0	0	0	0	8	8
Harp Inflow 3	5	0	0	0	1	7	8
Harp Inflow 3	6	0	0	0	1	7	8
Plastic Lake Inflow 1	1	0	0	0	1	8	8
Plastic Lake Inflow 1	2	0	0	0	4	8	8
Plastic Lake Inflow 1	3	0	0	0	2	8	8
Plastic Lake Inflow 1	4	0	0	0	2	8	8
Plastic Lake Inflow 1	5	0	0	0	3	7	8
Plastic Lake Inflow 1	6	0	0	0	4	7	8
Clearwater outflow	1	0	0	1	0	3	8
Clearwater outflow	2	0	0	1	0	7	8
Clearwater outflow	3	0	0	0	0	6	8
Clearwater outflow	4	0	1	0	1	7	8
Clearwater outflow	5	0	1	0	0	7	8
Clearwater outflow	6	0	0	0	0	6	8
Laurentian Wetland	1	0	0	0	1	7	8
Laurentian Wetland	2	0	0	0	2	7	8
Laurentian Wetland	3	0	0	0	0	7	8
Laurentian Wetland	4	0	0	0	0	8	8

Laurentian Wetland	5	0	0	0	1	5	8
Laurentian Wetland	6	0	0	0	1	6	8

Appendix E:

Table of abbreviations used in the thesis.

Abbreviation	Name
NOM	Natural Organic Matter
HA	Humic Acid
FA	Fulvic Acid
DOM	Dissolved Organic Matter
POM	Particulate Organic Matter
EPA	Eicosapentaenoic Acid
ALA	α-linolenic Acid
MSR	Multixenobiotic Resistance System
HS	Humic Substance
HSs	Humic Substances
SRNOM	Suwanne River NOM
SAC	Specific Absorbance Coefficient
SUVA	Specific UV Absorbance
FI	Fluorescence Index
EEMs	Emission and Excitation Matrices
PARAFAC	Parallel Factor Analysis
BLM	Biotic Ligand Model
DOC	Dissolved Organic Carbon
WHAM	Windermere Humic Aqueous Model
F	Quality Factor
LC_{50}	Lethal Concentration of 50% of the population

ASTM	American Society for Testing Materials
OECD	
US-EPA	United States Environmental Potential Agency
TALER	Terrestrial Aquatic Linkages for Ecosystem Recovery
RO	Reverse Osmosis
FLAMES	Field Laboratory for the Assessment of Multiple Ecological Stressors
EDTA	Ethylenediaminetetraacetic Acid
r	Intrinsic Rate of Natural Increase
g	Specific Growth Rate
T_bar	Mean Generation Time
ANOVA	
CFU	Colony Forming Units
E2	17β-estradiol
EE	17α-ethinylestradiol
CCME	The Canadian Council of Ministers of the Environment
MOE	Ministry of the Environment
LL	Lake Laurentian
LW	Laurentian Wetland
DL	Daisy Lake
CW	Clearwater Lake
SSW	Clearwater Lake Outflow
WR1	White River 1
WR2	White River 2
WR3	White River 3

HL	Harp Lake
Hp3	Harp Inflow 3
PL	Plastic Lake
PC1	Plastic Lake Inflow 1
PC108	Plastic Lake Inflow 108