Proceedings of the 36th Annual Aquatic Toxicity Workshop: September 27 to 30, 2009, La Malbaie, Québec

Comptes rendus du 36ième atelier annuel sur la toxicité aquatique: du 27 au 30 septembre 2009, La Malbaie, Québec

Editors/Éditeurs

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Preface/Préface

The 36th Annual Aquatic Toxicity Workshop was held at the Fairmont Le Manoir Richelieu in La Malbaie, Québec, from September 27-30, 2009. The Workshop included three plenary presentations, 157 platform and 89 poster presentations. Total attendance was 350.
This workshop was one of a continuing series of annual workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industries, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These workshops are administered by a Board of Directors and organized by local organizing committees. The Proceedings are published with the support of the Department of Fisheries and Oceans.

Le 36e édition annuelle de l'atelier de toxicologie aquatique a eu lieu au Fairmont Le Manoir Richelieu à La Malbaie au Québec, du 27 au 30 septembre 2009. Le programme scientifique de l'atelier comportait 3 conférences plénières par des orateurs invités, 157 conférences régulières et 89 communications par affichage. 350 personnes ont assisté à l'atelier.
L'atelier a permis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces ateliers annuels, administrés par un conseil d'administration national et organisés par un comité organisateur local, réunissent des représentants des secteurs industriels, gouvernementaux et universitaires que le domaine intéresse. Ceux-ci y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements et la définition de critères pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés avec la participation du ministère des Pêches et des Océans.

Editors' comments/Remarques des éditeurs

These proceedings contain papers, abstracts or extended abstracts of all presentations at the workshop. An author index is also included. The papers and abstracts were subject to limited review by the editors but were not subjected to full formal or external review. In most cases, the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned nor rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The editors would like to thank Dr. Jill Graham and Ms. Monica Lyons for their assistance in the preparation of these proceedings.

Ces comptes rendus présentent les articles ou les résumés, courts ou longs, de toutes les communications présentées lors de la 36^{e} édition annuelle de l'atelier de toxicologie aquatique. Un index des auteurs est aussi inclus. Les articles et les résumés ont été révisés sommairement par les éditeurs, mais n'ont pas fait l'objet d'une révision exhaustive ou d'une révision externe. La longueur et la forme des articles varient parce qu'ils sont pour la plupart publiés tel que soumis. Veuillez communiquer directement avec les auteurs pour toutes remarques concernant leurs travaux. Toutes les déclarations et opinions paraissant dans les présents compte rendus appartiennent aux conférenciers et ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation de leur emploi.

Les éditeurs désirent remercier le Dr. Jill Graham et Mme Monica Lyons pour leur aide dans la préparation de ces comptes rendus.

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Le castor, un ingénieur de l'écosystème qui augmente les concentrations en méthylmercure et en nutriments dans les ruisseaux des Laurentides.

ROY, V. [1], AMYOT, M. [1] ET CARIGNAN, R. [1]

[1]Université de Montréal.

L′accroissement actuel des populations de castors dans plusieurs régions du Québec, et plus particulièrement dans les Laurentides, entraîne une accumulation de zones forestières inondées résultant de la création de barrages de castors. Des échantillons d′eau ont été récoltés au cours de l′été 2006 dans 17 barrages de castors et trois de ces barrages ont été suivis de mars à octobre 2007. Les étangs de castors contribuent à augmenter en moyenne les concentrations de méthylmercure (MeHg) d′un facteur de 6, entre l'amont et l'aval du ruisseau, et à des concentrations moyennes de 1,2 $ng \cdot l^{-1}$. De plus, les retenues de castors accroissent en moyenne les concentrations en nutriments (PT, PTD, NT, COD) d′un facteur de 2 à 3 entre l'amont et l'aval. Les étangs de castors créés dans les 10 dernières années et ceux situés sur des sites à prédominance de conifères montrent les plus fortes concentrations en MeHg (jusqu'à 4,5 $ng \cdot l^{-1}$). La période hivernale sous couvert de glace et l'été sont deux moments où l'efficacité de la méthylation du mercure inorganique est la plus élevée (jusqu' à 80% MeHg/HgT). Suivant ces résultats, nous démontrons que les étangs de castors modifient la chimie de l'eau des ruisseaux laurentiens à divers degrés selon leurs âges, la végétation environnante et les saisons.

Contaminants anciens I - Organiques
Legacy contaminants I - Organics

Tracking legacy and emerging contaminants in the Oslofjord, Norway.

BREEDVELD, GD [1,2], ARP, H-P [1] and CORNELISSEN, G [1,3]

[1]Dep. Env. Eng. Norwegian Geotechnical Institute (NGI); [2]Dep. of Geosciences, University of Oslo, Norway; [3]Dep. of Applied Env. Science, University of Stockholm, Sweden.

Extensive studies during recent years have focused on the chemical record in sediment cores from the inner Oslofjord. This is a fjord with limited circulation, receiving contaminants from industrial and urban runoff as well as diffuse sources. Sediment cores show a clear transition hydrocarbons (PAH) are emerging during this period. As a result of the development of hydropower dams, the PAH signal diminished after WW II, while PCB-related to electrification emerged. As a result of stringent regulations, polychlorinated biphenyl (PCB) levels have been decreasing the last 20 years. However, despite the ban on the use of Tributyltinn (TBT) in anti-fouling paint, levels are still rising as a result of the enormous contaminant load present in the marine environment. Detailed analysis of sediment cores covering the last 20 years revealed increased

sedimentation rates of numerous emerging contaminants that have not been part of the regular monitoring program of the Norwegian Environmental Authorities. Brominated flame retardants and chlorinated paraffins (CP) are showing increasing depositional rates. This indicates that releases to the environment have been increasing and there has been little source control.

Based on these findings, the question arises whether the sedimentary deposit might be a secondary source of contaminants for the marine environment at present or in the future. Passive sampling methods using polyoxymethylene (POM) have been used to determine activities of PAH and PCB in air, water and sediments of the inner Oslofjord. Results indicate that atmospheric PAH might be a significant source of concentrations found in the surface water. In bottom water, contaminated sediments contribute to the observed PAH concentrations. For PCB, sediment is the major source even though some atmospheric input still can be observed.

The developed methodology for source identification will generate important input data for the development of regional remediation strategies for contaminated fjords in Norway.

Temporal and spatial variations in PCB contamination of sediments and source apportionment in a section of the Rhône River, France.

ROUX, G. [1], MIÈGE, C. [2], ROY, A. [2], MAHLER, B. [3], LEFÈVRE, I. [4], BONTE, P. [4], DESMET, M. [1], PERSAT, H. [4], VAN METRE, P. [3] and BABUT, M. [2]

[1]ENTPE, [2]Cemagref, [3]US Geological Survey, [4]CNRS.

In fall 2005, fish contamination by dioxin-like PCBs above the threshold for fish consumption was observed in the Rhône River, in the vicinity of Lyon (France). This observation triggered a series of investigations on sediment contamination along the mainstem of the Rhône. In addition to surface sampling in depositional areas along a ~120 km reach, short cores were collected from 3 locations and analyzed for PCBs and age-dating radionuclides. These data could help regional authorities identify the principal PCB sources among the potential ones, provided that distinct congeners' source patterns can be identified. PCB congener pattern analysis in sediments is challenging because weathering processes can modify the original patterns. Further, in a large river basin like the Rhône, at mid- or downstream sites the various upstream sources are mixed. We applied two different factor analysis techniques in order to characterize source profiles and their relative contribution to total PCB content in each sediment sample. These results will be discussed in relation to the hydrology, geography, and water management of the area.

Environmentally relevant concentrations of PCB126 affect survival skills in marine fish larvae.

COUILLARD, C. [1], LÉGARÉ, B. [1], DIONNE, Z. [1] and BERNIER, A. [2]
[1]Fisheries And Oceans Canada, [2]Institut des Sciences de la Mer.

Early life stages from a model fish species, *Fundulus heteroclitus*, were exposed to environmentally realistic doses of 3, 3', 4, 4', 5-pentachlorobiphenyl (PCB126) to evaluate its effects on ecologically relevant responses: growth and behaviour. A few hours after fertilisation, eggs were treated topically with PCB126 (3.1 to 50 pg/egg). Four days post-hatching (dph), morphological changes (body length and malformations), spontaneous locomotor activity, the rate of capture of artemia and whole body EROD activity were evaluated. To assess the contribution of retarded development to the responses observed in PCB126-treated larvae, untreated larvae collected at 0.5, 1, 2, 3, 4 dph were examined. PCB126 did not increase the mortality or malformation rates. Body length and spontaneous locomotor activity were altered only in larvae treated with the highest dose. The active swimming speed was increased and the rate of travel unchanged in PCB126-treated larvae (50 pg/egg). In contrast, both active swimming speed and rate of travel were reduced in 1 dph compared to 4 dph untreated larvae. Treatment with PCB126 caused a dose-responsive increase in EROD activity and reduction in prey capture ability. The lowest observed effective dose for both of these responses was 6.3 pg PCB126/egg or 6.3 TCDD-toxic equivalents $pg \cdot g^{-1}$ egg. The ability to capture prey increased as post-hatching development progressed. EROD activity was low and showed little variation from 0.5 to 4 dph. Thus, behavioural dysfunction is a more sensitive response to PCB126 than morphological alterations, and it occurs at environmentally-relevant concentrations. Other mechanisms than retarded development appear to be involved in PCB126-induced neurobehavioural dysfunctions.

American eel may be more sensitive to TCDD than rainbow trout.

CUTLER, G. [1] and HODSON, P. [2]
[1]University of Guelph, [2]Queen's University.

American eel is a Species of Concern because of a catastrophic decline in recruitment of juveniles to L. Ontario. Eels have virtually disappeared from L. Ontario, and there are fewer eels in many rivers throughout its range. As a consequence, the fisheries of Lake Ontario and the St. Lawrence estuary have declined sharply. One potential cause is maternal transfer of embryo-toxic dioxin-like compounds that bioaccumulate during the growth phase of eels in contaminated ecosystems. Eels accumulate these compounds to the same extent as salmonids, and lake trout reproductive failure in L. Ontario has been linked to dioxin contamination. Dioxin toxicity to eel embryos is difficult to assess in the laboratory because low survival rates obscure chemical effects. However, the toxicity of dioxin-like compounds to fish embryos can be predicted from their potency for inducing cytochrome P4501A (CYP1A) enzyme activity in juvenile fish. We compared the potency of IP-injected 2,3,7,8-tetrachlordibenzo-p-dioxin (TCDD) to juvenile eels (21-137 g) with potency to juvenile rainbow trout (12-49 g), a standard test species. Preliminary data suggest that eels may be 10 times more

sensitive to TCDD than rainbow trout. Assays of CYP1A activity in livers of adult silver eels caught at various locations in Eastern Canada, including L. Ontario, showed strong correlations among location, dioxin equivalents in fish tissue, and enzyme activity. Overall, these data support the idea that eels are sensitive to dioxin-like compounds, and that the extent of contamination typical of L. Ontario could be an important factor in reproductive success.

Nanotoxicologie
Nanotoxicology

Investigation of nano-gold glycodendrimer's interactions with algal cells and its effect on photochemical processes.

PERREAULT, F. [1], SAISON, C. [1], BOGDAN, N. [1], MORIN, M. [1], GOGOT, J. [1], TARDY-LAPORTE, C. [1], CLAVERIE, J. [1] and POPOVIC, R. [1]

[1]University of Québec in Montréal.

Combination of gold nanoparticles with glycodendrimer is particularly interesting for molecular recognition of biomolecule in medical and pharmaceutical applications. Because of this growing interest in nano-gold glycodendrimer, their possible environmental impacts demand urgent evaluation. In this study, we used glycodendrimer-coated gold nanoparticles, designed for specific lectin binding process, to determine their binding affinity for algae cell membranes. Here, we investigated the effects of nano-gold glycodendrimer on photosynthetic activity of green alga *Chlamydomonas reinhardtii*. Since algal cell wall contains glycoproteins, permitting interactions with carbohydrate-coated nanoparticles, we also investigated a cell wall deficient mutant to further understand the role of the cell wall in such interaction. We found that nanoparticles' interactions with algal cells may reduce algal growth and affect photosystem II activity, indicating that gold nanoparticles may interfere with the whole physiological state of algae. By using a cell wall deficient mutant, less affected than wild type *C. reinhardtii*, we indicated that nanoparticles' interactions with algal cells may be via cell wall components. Flux cytometry and confocal laser scanning microscopy of algal cells treated with fluorescent nano-gold glycodendrimer indicated distribution of nanoparticles in different cellular compartments. The results of this study are further discussed concerning possible environmental impacts of nano-gold glycodendrimer.

The radio-labeling approach to study the biodynamic of nanoparticles in aquatic organisms.

PELLETIER, É. [1], AL-SID-CHEIKH, M. [1], ZUYKOV, M. [1] and ROULEAU, C. [2]

[1]Université du Québec à Rimouski, [2]Institut Maurice Lamontagne.

Environmental concerns about engineered nanoparticles (nps) and various nanomaterials increased in the last few years as domestic and industrial applications of these newly developed nanostructures increased exponentially. Carbon nanotubes as well as metal and mineral nanoparticles are exceedingly difficult to trace and quantify in all environmental compartments due to their nanometric size and their tendency to coagulate

in presence of natural organic matter such as humic and fulvic acids. The Laboratory of MARINE Radioecology of Rimouski is developing a radio-labeling approach by preparing 14C tagged nanotubes and 110mAg nanoparticles. The dynamic of freshly labeled nanoparticles in seawater was determined, and whole-body autoradiography (WBA) was used to visualize and quantify both the accumulation and depuration of 110mAg in tissues of scallop *Chamys islandica* and fish *Salvelinus alpinus*. The WBA technique provided the first quantitative results on the location of labeled Ag nanoparticles in tissues of each species, and thus contributes to the discussion on the toxicity of nanoparticles in organs targeted during bioaccumulation and those retaining nps for weeks during depuration. The radio-labeling approach is also developed to study the adsorption and incorporation of nanomaterials in shells of bivalves (*Mytilus edulis*) by exposing these animals for a few hours to a solution containing labeled nps and following their distribution in shells for months by alpha tracks autoradiography and laser ablation ICP-MS. The advantages and pitfalls of this approach are discussed.

In vitro evaluation of the toxicity of Quantum dots (CdS/CdTe) on different species.

BRUNEAU, A. [1], FORTIER, M. [1], GAGNÉ, F. [2], AUFFRET, M. [3] and FOURNIER, M. [1]
[1]INRS-Institut Armand Frappier, [2]Environment Canada, [3]Brest University.

Nanoparticles are used in various industries such as medical, pharmaceutical and cosmetic. However, there is little toxicological data on these new elements. The purpose of this study was to examine the toxic effects of quantum dots (CdS/CdTe, Vivenano ®) on the immune systems of three species. *In vitro* tests were performed on mice and fish lymphocytes and macrophages. *In vitro* tests were also performed on the bivalves' hemocytes. To estimate the toxicity of these nanoparticles, we performed the following tests: the cellular viability, the lymphocyte proliferation and the phagocytosis. We then compared the sensitivity of the three species within a range of 0 to 952 $\mu g \cdot ml^{-1}$of cadmium present in the Qdots. The tests that were performed resulted in lethal doses of 50% (LD50). The first results allowed us to assert that the immune cells (lymphocytes and macrophages) of the "terrestrial species" are less sensitive (LD50 mouse: lymphocytes = 88.5 $\mu g \cdot ml^{-1}$ and macrophages = 210 $\mu g \cdot ml^{-1}$) than those of the fish cells (LD50 fish: lymphocytes = 42.6 $\mu g \cdot ml^{-1}$ and macrophages = 195 $\mu g \cdot ml^{-1}$) but more sensitive than those of the bivalves hemocytes (LD50 > 952 $\mu g \cdot ml^{-1}$). Future investigations are planned to study more species and perform a broader range of tests.

Effect of core-shell copper oxide nanoparticles on cell culture morphology and photosynthesis in *Chlamydomonas reinhardtii*.

SAISON, C. [1], PERRAULT, F. [1], DAIGLE, J-C. [1], GOGOT, J. [1], TARDY-LAPORTE, C. [1], FORTIN, C. [2], CLAVERIE, J. [1], MORIN, M. [1] and POPOVIC, R. [1]

[1]University of Québec in Montréal, [2]Institut National de la Recherche Scientifique, Centre Eau, Terre et Environnement.

The effect of core-shell copper oxide nanoparticles on algal cellular systems is still not well understood. Documenting these effects is urgent since core-shell copper oxide nanoparticles are currently a component of boats' antifouling paint. This paint may induce alteration effects in aquatic ecosystems. Here, the toxic effect of core-shell copper oxide nanoparticles on green alga *Chlamydomonas reinhardtii* was investigated with regards to the change of algal cellular population structure, primary photochemistry of photosystem II, and formation of reactive oxygen species when algal cultures have been exposed 6 h to 0.004, 0.01 and 0.02 $g \cdot L^{-1}$of core-shell copper oxide nanoparticles. We found that core-shell copper oxide nanoparticles induce cellular aggregation processes and deterioration of photosynthesis. The inhibition effect of photosynthetic electron transport induced a strong energy dissipation process via a non-photochemical pathway. The deterioration effect was found to be induced by reactive oxygen species formed by core-shell copper oxide nanoparticles.

Études de suivi des effets environnementaux
Environmental effects monitoring

Metal mining effluent effects on fish and invertebrates: national patterns of effects over the first two phases of the Environmental Effects Monitoring Program.

LOWELL, R. [1], WALKER, S. [1], TESSIER, C. [1], BUCKLAND, B. [1], RING, B. [1] and BEST, A. [1]

[1]Environment Canada.

The National Environmental Effects Monitoring (EEM) Program has recently completed the second three-year phase of metal mining monitoring, which tracks the effects of mining effluents across the country. The first phase of the program showed overall inhibitory effects on fish and invertebrates on a national scale, although this varied depending on ore type, habitat, fish species and gender, and other site-specific factors. For some mines and receiving environments, stimulatory effects were also measured. The national patterns and site-specific heterogeneity in effects are being further investigated in Phase 2 of the program. Meta-analyses, multivariate analyses, and effect size summaries are being used to examine effects on a broad geographic scale. The key response variables are the magnitudes of measured effects on core fish (condition, relative gonad and liver size, age, size-at-age) and invertebrate (density, taxon richness, evenness, Bray-Curtis Index) endpoints. Statistical comparisons among different mine groupings allow questions to be addressed that can not be evaluated at the level of the individual mine. As ongoing EEM data-collection progresses, these national-scale

analyses are helping to provide a more comprehensive picture of metal mining response patterns in Canada.

The effect of decades of acid and metal inputs to the fish and aquatic invertebrate communities of the Lynn River and two connected lakes.

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For over 30 years in Lynn Lake, Manitoba, two adjacent abandoned mine sites have been releasing highly acidic water (pH < 3) with metal concentrations often in the $mg \cdot Kg^{-1}$ mg· range into local water bodies. This has resulted in significant reductions in water quality within localized reaches of the Lynn River. The purpose of this study was to determine the effect of this contaminated runoff and impaired water quality on exposed aquatic invertebrate and fish communities. The study area comprised approximately 20 km of the Lynn River, and included the connected nearfield Eldon Lake and farfield Cockeram Lake. Parameters used in the assessment included total abundance, species richness, sculpin abundance, and young-of-year abundance for riverine fish communities, and species richness, diversity, evenness and EPT Index for the riverine invertebrate communities. Within the nearfield riverine sites, the invertebrate and fish communities indicated slight to severe impact, respectively, but a farfield site only 4 km downstream of the last input showed no impact on either the invertebrate or fish community. In contrast, both nearfield Eldon Lake and farfield Cockeram Lake showed no impacts, and contained a diverse fish community comparable to what was expected in the Study Area. White Sucker, Northern Pike, and Walleye were present, robust, and of normal weight for their size. Despite decades of contaminated runoff and poor-to-marginal local water quality, the Lynn River and connected lakes still supported surprisingly diverse fish and aquatic invertebrate communities. The data indicate that application of "standard" water quality guidelines is de facto "overprotection".

Using a field-based multi-trophic artificial stream bioassay to assess the effects of complex mining mixtures.

RAMILO, L. [1], DUBE, M. [1], NIYOGI, S. [1] and RICKWOOD, C. [2]

[1]University of Saskatchewan, [2]Natural Resources Canada.

A field-based, multi-trophic artificial stream study was conducted in August 2008 to assess the effects of three independently treated metal mining effluents (MME) to fathead minnow (*Pimephales promelas*) in a 21 day reproduction bioassay. Junction Creek in Sudbury, ON, Canada receives inputs from three mining-related wastewater treatment plants (minewater effluent [GME], stormwater effluent [NME] and processing effluent [CCME]). All three MME's were tested as well as reference/dilution water collected from the Vermilion River. Metals were analyzed in several media (water, sediments) and tissues (algae, *Chironomus dilutus*, female fathead minnow body, ovary, liver, gills). Significant increases in metal concentrations were observed in the water and algae tissues in all treatments compared to reference with no appreciable concentrations

in the sediments. Tissue analysis of *C. dilutus* showed significant increases in cobalt (1.4-fold) and nickel (1.5-fold) in NME, and copper (5.2-fold) and selenium (3.3-fold) in CCME compared to reference. There were no significant increases in metal concentrations in female fathead minnow tissues (body, liver, gonads, gills) in any of the treatments, suggesting that metals may not have been bioavailable. Despite the lack of a clearly defined exposure pathway, the study did show significant increases in cumulative number of eggs per female per day (+122%) after exposure to NME, and significant decreases (-39%) after exposure to CCME when compared to reference. Further characterization of the exposure pathways is needed to help identify the potential causes of egg production differences among fish exposed to complex mining mixtures.

Potential sources of metal mine effluent reproductive effects: Fathead minnow *(Pimephales promelas)* exposures to copper at metal mine effluent concentrations in an artificial multi-trophic stream system.

OUELLET, J. [1], NIYOGI, S. [1] and DUBE, M. [2]

[1]University of Saskatchewan, [2]Saskatchewan Research Council.

Metal mine effluents (MMEs) are discharged into waterways with the potential to cause environmental damage. Although treatment mitigates impacts on the receiving ecosystem, effects on invertebrates and fish in areas exposed to MMEs have been reported. To determine the contribution of trophic-transfer to effluent toxicity on fish reproduction, multi-trophic artificial stream systems were used. We performed a 21-day multi-trophic exposure using *Pimephales promelas* with *Chironomus dilutus* as a food source to compare effluent-matched copper exposures to environmentally relevant concentrations of a treated metal mine effluent (CCME). Reproductive endpoints were not statistically different in either copper or CCME exposures compared to the reference group (e.g., mean daily egg production, mean brood size, or cumulative egg production). Copper levels were higher in livers of the CCME exposed females ($P<0.05$) but not the copper treatment exposed females. Copper levels were also higher in *C. dilutus* in both the copper and CCME treatments compared to the reference ($p<0.001$). Future work will compare other single metal exposures (e.g., selenium) to determine their potential influence on effluent toxicity. Comparisons of effluent toxicity and water chemistry between different studies should help identify toxicity modifying factors and provide improved treatment options.

Challenges and opportunities when using short-term adult fish reproductive tests.

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Short-term adult fish reproductive tests have the potential to assess chemicals and waste streams for their ability to disrupt reproductive systems. A literature review found these tests had low power to detect differences in egg production among treatments, because of high variance and small sample sizes. We refined a mummichog protocol to increase power and used it to determine the effect of final treated effluent of a pulp mill

and sewage effluent (both at 30% concentrations) on egg production and gonad size. Tank pre-selection and increased sample size increased the a priori power from 7.8% to 85.5%. After exposure, power levels were 76.6%, a six-fold increase compared to studies that used the original protocol. There was high consistency in the measured endpoints compared to the original protocol; all endpoints had >75% power to detect a 25% difference among treatments. This study demonstrates that using a refinement process can address shortcomings in short-term adult fish reproductive protocols, creating a solid foundation for further standardization and possible regulatory use.

The importance of habitat matching and regional reference variation in accurately detecting and interpreting environmental effects.

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[1]Minnow Environmental Inc.

An underlying assumption of environmental study designs is that the habitat of selected reference area(s) matches the habitat of the exposure area closely enough that any chemical or biological differences among areas can confidently be ascribed to the stressor of interest. However, the acceptable level of habitat similarity between reference and exposure area(s) is typically a subjective decision. We used data collected in the monitoring program for the Faro metal mining complex, Yukon to 1) formally match each exposure site to candidate reference areas, and 2) compare the results of this small-scale Reference Condition Approach (RCA) to the results of a Control-Impact (CI) design. The habitat characteristics that were most strongly correlated to biological community characteristics were used to rank candidate reference areas from best to worst habitat match with each exposure area and to formulate a cut-off for an acceptable habitat match. The RCA sampling design was at least as sensitive as the CI design when the same suite of reference areas was used for detecting reference-exposure differences. An important advantage of incorporating more reference areas is that statistical comparisons between reference and exposure areas are more ecologically meaningful.

Développement d'un outil pour prédire les effets toxiques chroniques à partir du laboratoire: phase 1.

BUJOLD, M. [1], LEBLOND, V. [1] et LEFEBVRE, M. [1]

[1]Écotox Inc.

Certaines industries au Canada doivent effectuer des études sur les populations de poissons afin de mesurer les effets de leur exploitation dans le milieu récepteur. L'une des difficultés de la démarche est de déterminer avec précision l'étendue des effets, surtout lorsqu'il peut y avoir des facteurs confondants dans les environs. L'objectif ultime des travaux présentés ici est de pouvoir estimer l'étendue géographique des effets dans l'environnement, à partir d'essais adaptés en laboratoire sur les effluents. La première phase du projet de recherche vise à mesurer les effets toxiques des effluents et de vérifier s'il y a corrélation avec des échantillons d'eau du milieu récepteur de conductivité équivalente. Pour ce faire, un site minier abandonné a été sélectionné en

raison de l'absence de facteurs confondants et de la présence d'une toxicité très importante à l'effluent. Ce site se nomme Aldermac et est situé près de Rouyn-Noranda, en Abitibi-Témiscamingue au Québec. Les résultats de cette étude montrent que la sensibilité des essais est très variée, (*Pseudokirschiella subcapitata* est beaucoup plus sensible que *Pimephales promelas*) et que certains essais montrent une corrélation presque parfaite entre la conductivité toxique mesurée à l'effluent et la conductivité équivalente dans le milieu récepteur *(Ceriodaphnia dubia)*. Un nouvel essai adapté aux eaux environnementales (effluents et milieux récepteurs) a aussi été validé sur des cellules hormonales de truite arc-en-ciel. La seconde phase du projet visera à établir la corrélation entre les résultats des essais sur les effluents en laboratoire et les paramètres mesurés chez les poissons lors d'études environnementales.

Assessment of water quality trends contributing to cumulative effects in the Athabasca River basin using a fathead minnow bioassay.

SQUIRES, A. [1] and DUBE, M. [2]

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The Athabasca River, Alberta, Canada, is a glacial fed river system with a basin covering 157000 km^2 and is the longest (1538 km) unregulated river in the prairies. The basin holds significant cultural and economic importance, supporting more than nine First Nation groups and providing water to hundreds of industries. Previous research has quantified spatial (along the river continuum) and temporal (pre-development and present day) changes in water quantity and quality in the river. Dissolved chloride and sodium concentrations have changed significantly over space and time. Present day concentrations are in the top 10% of concentrations observed 30 years ago. Assessment of the sublethal effects of increasing salinity on fish and other aquatic life has received little attention. The objectives of this research were to 1) assess changes in fathead minnow response metrics (i.e., condition, liver and gonad size, egg production, larval survival, larval deformities) associated with increasing concentrations of dissolved chloride and sodium, and 2) to determine sublethal effect thresholds for concentrations of relevance to the Athabasca River. Three experiments (Na alone, Cl alone, NaCl) were conducted using a diluter system allowing for the dilution of a 100% test solution (based on the highest recorded concentration of the respective parameter in the river) down to 50%, 25%, 12.5%, 6.25% and a 0% control. This research contributes to method development for watershed-scale cumulative effects assessment, including development of whole river benchmarks for sublethal exposures of fish to increasing salinity, for an economically and culturally important river that is experiencing significant development pressure.

Sables bitumineux
Oil sands

Carbon dynamics, food web structure & reclamation strategies in Athabasca oil sands wetlands (CFRAW).

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[1]University of Windsor, [2]University of Waterloo, [3]University of Alberta, [4]University of Saskatchewan.

Wetlands will make up 20-50% of the final reclamation landscape of areas surface mined for oil sands in northeastern Alberta. CFRAW is a collaboration among 7 mining partners and 5 university labs to study effects of mine tailings and process waters on development, health and function of wetland communities expected to form in post-mining landscapes. Our work tests predictions about how quickly wetlands amended with reclamation materials approach the conditions seen in reference wetland systems. Supplementing constructed wetlands with stockpiled peat or topsoil is expected to accelerate succession and community development. The hydrocarbons in tailings (bitumen) and water (naphthenic acids) that occur in wetlands constructed with mine process materials are initially toxic, but may ultimately serve as a surrogate source of carbon once they degrade and/or are metabolized by bacteria. We are assessing the sources, biological uptake, pathways, and movement through the food web of materials used by the biota in constructed wetlands. Studies in progress are evaluating how productivity of new wetlands is maintained. We are monitoring net ecosystem productivity, rates of organic carbon accumulation from microbial, algal, and macrophyte production, and influx of outside materials. We are also comparing rates of leaf litter breakdown and microbial respiration to determine how constituents speed or slow food web processes of young and older wetlands. Stable isotope measurements of carbon and nitrogen in food web compartments indicate which sources are incorporated into the food web as wetlands age; how this influences community development, food web structure and complexity, and the productivity and health of fish, amphibians, and wetland birds. Flux estimates will be combined to determine whether wetlands built with peat amendments can be expected to maintain their productivity and have the potential to ultimately become true peat lands. The research will provide a conceptual model of carbon pathways and budgets to assess how the allocation of carbon among compartments changes as newly formed wetlands mature in the boreal system. Ultimately, we will recommend the materials and strategies most effective and economical in producing a functioning reclamation landscape.

Spatial and stress-related variation in benthic microbial gas flux in northeastern Alberta wetlands.

CIBOROWSKI, J. [1] and GARDNER COSTA, J. [1]
[1]University of Windsor.

The goal of this project is to study sediment microbial respiration in newly formed wetlands, as it relates to effects of oil sands process material (OSPM). Oil sands lease site reclamation in northeastern Alberta presents an opportunity to study ecosystem processes in reclaimed sites. We studied sediment gas flux (volume of gas released/m^2/day) of 10 wetlands contrasting in age and sediment characteristics. Methane gas and carbon dioxide production were low and variable compared with literature- reported averages of gas flux from boreal wetlands (Roehm, 2005). Analyses of variance were used to contrast mean wetland production of CH_4 and CO_2 with season, wetland status (reference or OSPM-affected), wetland age (<7 y old vs. >8 y old), or wetland zone (emergent-vegetated vs. unvegetated areas of wetlands). Methane production was significantly higher ($p<0.05$ $n=8$) in reference wetlands than OSPM affected wetlands. A significant interaction between status and zone of the wetland was found for CH_4 flux ($p<0.05$ $n=8$) for reference wetlands only. Methane flux was higher in unvegetated zones of reference wetlands than vegetated zones of reference wetlands ($p<0.05$ $n=8$). Carbon dioxide fluxes were low overall and not significantly different with respect to status, age, season or zone ($p>0.05$ $n=8$). Overall, the wetlands appear to be contributing little atmospheric carbon. Estimates of net gas flux and summer microbial production will be combined to estimate net benthic respiration.

Sediment oxygen demand of wetlands in the oil sands region of northeastern Alberta.

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Sediment oxygen demand (SOD) is the rate of oxygen consumption at the water-sediment interface and has the potential to drastically affect dissolved oxygen concentrations, particularly in shallow water bodies. The types of sediments used to reclaim wetlands may affect SOD rates and ultimately determine ecosystem properties. Reclaimed wetlands on the oil sands lease sites provide an excellent location to study the impacts of various reclamation sediments on SOD and, in turn, succesional processes and ecosystem trajectories. Residual chemical constituents in sediments from oil sands mine processing may increase the chemical sediment oxygen demand through oxidation of residual ammonia to nitrate. Ammonia may also react with sulfate and then bind phosphorus, making it unavailable to macrophytes. We predicted that OSPM-affected wetlands would support cyanobacterial biofilms rather than submergent macrophytes due to insufficient phosphorus levels. Sediment oxygen demand was determined by monitoring dissolved oxygen concentrations within domes resting on the sediment surface for a period of three hours. Concurrent studies that monitored gas flux and composition allowed us to quantify the biological (BSOD) component of the SOD. The chemical (CSOD) component was then determined by subtraction. Plant root simulator (PRS) probes were used to estimate concentrations of phosphorus bioavailable to

macrophytes. Submergent macrophyte surveys were conducted to compare species composition and abundance between wetland types. Preliminary results are consistent with expectations that OSPM wetlands exhibit higher CSOD and SOD than reference wetlands and support benthic biofilms rather than the submergent macrophyte communities that are characteristic of wetlands in northeastern Alberta.

Determining productivity of transferred benthic biofilms within wetlands differing in anthropogenic stressors.

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[1]University of Alberta, [2]University of Windsor.

Algal biofilms are a critical component of wetland ecosystems as a fundamental contributor to wetland productivity and to the invertebrate food web. These films are prevalent in shallow water and over large littoral zones where they maintain high turnover rates, nutrient uptake and storage capacities. We will examine biofilm transfer techniques as a method of accelerating colonization, carbon capture and plant production in newly created oil sand wetlands affected by process waters. We propose to measure a) the productivity of transferred biofilms and their abilities for accelerating succession; b) methods for transferring biofilms; and c) community composition of algae in relation to various substrates. Microcosms will be constructed and filled with four types of substrates onto which the biofilm slurries or mats will be transferred. The microcosms will be submersed in experimental trenches containing either process-affected or natural water (n=3). Dissolved oxygen (DO), chlorophyll *a*, and biomass standing crop (as a compensated analog for productivity), will be sampled at intervals through the year. Comparisons among substrate types within trenches and between oil sands process-affected water versus natural water trenches will be made using ANOVA and planned comparisons. This research will ultimately assess and quantify the contributions of benthic biofilms towards total primary productivity within wetlands as well as their utility as a reclamation tool. The results will also allow assessment of the effects of oil sands process affected material on benthic biofilm productivity, and whether biofilms will accelerate the initial carbon accumulation process in what are essentially primary succession conditions.

Investigating salt and naphthenic acids interactions in the toxicity of oil sands process water to freshwater invertebrates.

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The Athabasca oil sands deposit in northern Alberta, Canada, is the largest in the world with contents equivalent to 175 billion barrels of bitumen. The hot water extraction process used to isolate bitumen from oil sands produces a large volume of process water (OSPW) that typically contains elevated concentrations of salts and naphthenic acids (NAs), but low levels of other contaminants such as PAHs and metals. OSPW is proposed to be part of future reclamation landscapes; so, in order to develop environmentally acceptable OSPW reclamation plans, it is important to understand its

toxicity to freshwater invertebrates. This project investigated the nature of the toxic interactions between NAs and salinity on freshwater invertebrates. The toxicity of OSPW from selected water bodies was determined by performing bioassays with laboratory-cultured *Ceriodaphnia dubia*. The concentrations of NAs and salinity were elevated in the pond waters that caused a toxic response, but the concentrations of salinity ions varied greatly among ponds. This suggested that ion composition may be a factor in toxicity, not just total ionic content. The interaction between NAs and salinity was assessed by performing bioassays with mixtures representing major ion combinations present in the OSPW (carbonate, sulfate, chloride and sodium) and with NAs extracted from OSPW.

Thresholds for opportunistic disease in yellow perch *(Perca flavescens)* exposed to oil sand process-affected waters.

HOGAN, N. [1], VAN MEER, T. [2], MACKINNON, M. [2] and VAN DEN HEUVEL, M. [1]
[1]University of Prince Edward Island, [2]Syncrude Canada Ltd.

Aquatic areas reclaimed after oil sands mining operations will be influenced by natural and process affected materials containing elevated salt, naphthenic acids and PAHs. One aquatic reclamation option for most mine closures is to place mature fine tailings at the bottom of reclaimed lakes. To examine the effects of oil sands-influenced aquatic systems on fish health, perch-stocking studies were conducted during 1995-1997 and 2008-2009 using two experimental lakes influenced by oil sands process-affected waters. The first lake, the Demonstration Pond, was constructed in 1989 and contains 70,000 cubic meters of mature fine tailing capped with the same quantity of surface water. The second lake, the South Bison Pond, formed in a natural depression in an area where overburden containing unrecovered bitumen was deposited. The area surrounding the South Bison Pond was reclaimed for pasture in 1996 and is representative of an aquatic system in a lean oil sands-influenced watershed. In both sets of studies, two disease pathologies were observed in yellow perch: fin erosion caused by an as yet unidentified pathogen, and lymphocystis for which diagnostic PCR methods were conducted. In early studies (1995-1997) both pathologies were most prevalent in the South Bison Pond; however, in the recent studies (2008-2009), disease was substantially more prevalent in the Demonstration Pond. Changes in pond chemistry over the past decade allow for some inferences to be made as to causal agents and possible thresholds for disease incidence. Salinity is an unlikely cause as this has not changed in the South Bison Pond and both ponds now have equivalent salinity. Naphthenic acids have decreased in the Bison Pond from approximately 12 to less than 4 $mg \cdot L^{-1}$over the duration of studies. In contrast, naphthenic acids have increased in the Demonstration Pond from 6 to 12 $mg \cdot L^{-1}$due to tailings densification. Early studies also indicated exposure to PAHs in the South Bison Pond and PAHs have yet to be implicated or ruled out as a causative factor in disease observed in the Demonstration Pond. From these results, temporal changes in naphthenic acid levels appear to correlate with incidence of disease pathologies, and current work is focusing on measuring tissue levels of naphthenic acid as well as bile PAH metabolites.

Vegetation diversity and biomass: response to oil sand tailings disposal in Fort McMurray, Alberta.

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[1]University of Alberta.

Oil industries are producing more than 3 m^3 of processed material (tailings) for every barrel of bitumen extracted from oil sands. Covering the bottom of created wetlands with a layer of tailings has been tested as a means for its disposal. However, tailings constituents such as salt and naphthenic acids have been hypothesized to have negative impacts on wetland vegetation establishment and development. The objective of this study is to determine if created wetlands amended with a layer of tailings have a significantly lower vegetation diversity and biomass than created wetlands that are not amended with tailings. A third control contrast is by plant growth in natural wetlands. The second objective is to examine salinity and naphthenic acid effects on the vegetation in the three types of wetlands. We systematically assessed 30 wetlands in the Fort McMurray region. Preliminary results suggest that the addition of tailings negatively impacted vegetation diversity and biomass. Salinity, unlike napthenates, has been identified as at primary causal factor for these impacts.

Understanding toxicity at the watershed scale: design of the Syncrude Sandhill Fen Watershed Research Project.

WYTRYKUSH, C. [1]
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A fen is a peat-accumulating wetland having a water table at or near the surface and consists of mineral-rich ground or surface water. A large portion of the Athabasca oilsands-region landscape is fen-type wetland. However, construction of a fen wetland and its supporting watershed has never before been attempted in the post-mining landscape. Syncrude Canada´s Sandhill Fen Watershed Project is the first attempt to construct a fen in this region, and also is the first time that an entire watershed has been specifically designed to support a wetland. Another unique aspect of this project is that the wetland and its watershed will be constructed on a soft tailings deposit. Using key findings from research supported by Syncrude and consultation with expert scientists and engineers, a design basis for the fen and its watershed was developed. A major aspect of the design is to control the salinity of the landscape coming from tailings consolidation and seepage over time. The fen design, decisions made, and research undertaken to mitigate potentially toxic effects from salinity will be discussed.

RFB et autres contaminants d'intérêt émergent
BFR and other emerging contaminants

Canadian National Contaminant Biomonitoring Program: residues of BFRs in Canadian freshwater fish.

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[1]Environment Canada, [2]Indian and Northern Affairs.

Environment Canada conducts National Monitoring and Surveillance to determine concentrations of persistent organic pollutants (POPs) in whole fish collected from major watersheds in Canada to support ongoing risk assessment and risk management actions undertaken to protect ecological health. In the present study we investigated the spatial distribution of polybrominated diphenyl ether (PBDE) and non-PBDE brominated flame retardants in top predator sentinel aquatic species that were prevalent in the watershed under study. Lake trout, cutthroat trout, and walleye (n=4-20 for each location) have been collected since 2007. Lake trout in the Great Lakes and St. Lawrence River contained highest levels of sum PBDEs (site means from 22 to 65 $ng \cdot g^{-1}$ wet wt.). Seven PBDE congeners were observed as the dominant congeners in all samples. BDE-47 was found to be the dominant congener in all samples, with BDE -47 > 99 >100 >154 >153.

Brominated Flame Retardants in the eggs of four species of gulls and two terrestrial sentinel avian species spanning Atlantic to Pacific Canada.

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[1]Environment Canada.

Environment Canada's Chemical Management Plan supports a National Monitoring and Surveillance Program which focuses on monitoring of chemicals in multiple environmental media: air, water, sediment and non-human biota (fish and wildlife). For the wildlife component, upper trophic level gulls and European starlings were chosen as aquatic and terrestrial sentinel species, respectively. In the present study we investigated the Pan-Canadian spatial distribution of polybrominated diphenyl ether (PBDE) and non-PBDE brominated flame retardants in the eggs of several species of gulls that were endemic to the regions under study. Glaucous-winged gull, California gull, Ring-billed gull or Herring gull eggs (n=10 eggs for each location) were collected in 2008 from urban sites (e.g. colonies near Victoria, BC; Calgary, AB; Toronto, ON; Montréal, QC; Québec City, QC; St John's, NL) and non-urban sites (e.g. colonies on Lake Winnipeg, MB; Lake Superior, ON; Gulf of St Lawrence, QC and Sable Island, NS). In addition, in 2008 a pilot collection of starling and American kestrel eggs was made in southern Ontario. Herring gulls in the Great Lakes and St. Lawrence River contained highest levels of sum PBDEs (site means from 200 to 1100 $ng \cdot g^{-1}$ wet wt), with west coast Glaucous-winged gulls having lowest site means (80-120 $ng \cdot g^{-1}$ wet wt). A preliminary examination of the small sample of wild kestrel samples indicated that

their eggs contained a smaller proportion of the pentaBDE formulation related congeners (BDE 47, 99 and 100) relative to both the terrestrial European starling and the aquatic gull species, and a larger proportion of the hexa-congeners BDE153 and 154. Overall, the terrestrial species were an order of magnitude lower than the gulls, with site means ranging from 3 to 28 $ng \cdot g^{-1}$ and 9 to 25 $ng \cdot g^{-1}$ wet wt for starlings and kestrels, respectively.

Les polybromodiphényléthers (PBDE) dans le fleuve Saint-Laurent / Polybrominated Diphenyl Ethers (PBDEs) in the St. Lawrence River.

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Les polybromodiphényléthers sont de plus en plus présent dans le système aquatique du Saint-Laurent. Depuis les dix dernières années, les concentrations de BDE209 ont quintuplé dans les matières en suspension (MES) à Québec et doublé dans les sédiments des lacs fluviaux. À Québec, les concentrations les plus élevées dans les MES sont observées durant l'été pour le BDE209 et durant le printemps pour les congénères plus légers (PBDETOT 9). Actuellement, les sédiments les plus contaminés sont situés au lac Saint-Pierre et atteignent un niveau de contamination similaire à celui observé au lac Michigan. L'apport en PBDE au fleuve semble provenir en partie des émissaires urbains pour les PBDETOT 9, mais l'apport de BDE209 ne peut être expliqué uniquement par ceux provenant du bassin des Grands Lacs et de la rivière des Outaouais.

Polybrominated diphenyl ethers are increasingly present in the St. Lawrence aquatic system. Over the last 10 years, BDE209 concentrations have increased fivefold in suspended sediment in Québec City and doubled in sediment in the fluvial lakes. In Québec City, the highest concentrations in suspended sediment were observed in the summer for BDE209 and in the spring for the lighter congeners (PBDETOT 9). Currently, the most contaminated sediment is located in Lake Saint-Pierre, with a level of contamination similar to that observed in Lake Michigan. PBDE loadings to the St. Lawrence seem to originate in part from urban outfalls for PBDETOT 9, but BDE209 loadings cannot be accounted for solely by those originating in the Great Lakes Basin and Ottawa River.

Les polybromodiphényléthers (PBDE) dans quelques cours d'eau du Québec méridional et dans l'eau de consommation produite à deux stations de traitement d'eau potable.

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Des échantillonnages ont été réalisés dans quelques cours d'eau du Québec méridional pour y mesurer les concentrations de PBDE. Un total de 58 échantillons d'eau a été analysé pour 24 congénères de PBDE. Les concentrations mesurées varient

d'inférieures à la limite de détection pour tous les congénères analysés à 10 700 $pg \cdot L^{-1}$de PBDE totaux. Les médianes par site varient de 14 $pg \cdot L^{-1}$ à 2 530 $pg \cdot L^{-1}$. À 4 des 10 sites d'échantillonnage la concentration médiane pour la somme des congénères tétraBDE, pentaBDE et hexaBDE dépasse 300 $pg \cdot L^{-1}$, ce qui pourrait donner lieu à des concentrations trop élevées pour la faune à la suite de la bioaccumulation des PBDE dans la chaîne alimentaire. Treize échantillons d'eau présentent des teneurs en PBDE totaux supérieures à 1 000 $pg \cdot L^{-1}$, ce qui est élevé en comparaison des teneurs recensées dans la documentation scientifique. Des concentrations particulièrement fortes de PBDE ont été mesurées dans la rivière Yamaska Nord en aval de Granby. Dans l'eau, la concentration passe de 262 $pg \cdot L^{-1}$ en amont de la ville à 2 530 $pg \cdot L^{-1}$ en aval. Dans le poisson entier (meunier noir, *Catostomus commersoni*) les teneurs passent de 13 $ng \cdot g^{-1}$ à 319 $ng \cdot g^{-1}$. Des échantillonnages réalisés à deux stations de traitement d'eau potable ont démontré que celles-ci éliminaient 93 % des PBDE présents dans leur eau brute. Les congénères 47 et 99 constituent l'essentiel des PBDE trouvés dans l'eau traitée. Le décaBDE, pourtant bien présent dans l'eau brute, n'a pas été détecté dans les échantillons d'eau traitée.

Naturally occurring brominated and chlorinated organic compounds in the marine organisms of the eastern coast of Canada.

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Among actions to be undertaken against Persistent Organic Pollutants (POPs) and discussed during the fourth Conference of the Parties of the Stockholm Convention on POPs was the evaluation of halogenated chemicals (e.g. brominated flame retardants) to be added to the initial list of 12 POPs. However, adding new compounds on the list is a task complicated by confounding compounds of natural origin, especially from the marine environment from which hundreds of halogenated molecules have been reported. We present the case of a class of mixed halogenated compounds detected in the liver of a marine mammal (white-sided dolphin, *Lagenorhynchus acutus)* and two predator fish (Greenland shark, *Somniosus microcephalus* and porbeagle shark, *Lamna nasus)* caught in the Gulf of St. Lawrence, Canada. Two families of mixed halogenated cyclic compounds match the isotopic clusters observed in mass spectrometry: chloro- bromo-methyl bipyrroles and dimethyl bipyrroles. Up to twenty congeners were quantified in animals; their total hepatic concentration (lipid weight) was about 50 $ng \cdot g^{-1}$ in dolphin and up to 800 $ng \cdot g^{-1}$ in shark. Other halogenated compounds identified but not quantified in tissues are naturally occurring methoxy-polybromodiphenyl ethers that can be confounded with metabolites of technical polybromodiphenyl ethers mix. Our analytical work demonstrates how difficult is the task of toxicity evaluation of mixtures of persistent organic pollutants in marine animals; the contribution of each pollutant has to be evaluated but naturally occurring compounds of similar structure have to be well identified.

Assessing flame retardants in the Canadian environment using the National Aquatic Biological Specimen Bank (NABSB).

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Long-term systematic storage of environmental specimens has become an important component of formal environmental monitoring programs in many countries. The Government of Canada has been archiving frozen whole-body homogenates of fish and other aquatic organisms since 1977 to meet its obligations under the Great Lakes Water Quality Agreement and support more recent initiatives such as Canada's Chemicals Management Plan (CMP). The NABSB (formerly the Great Lakes Fisheries Specimen Bank) currently holds more than 37,000 samples of top predator fish, forage fish, plankton and other invertebrates collected over 32 years of environmental monitoring in Canada. Lake trout and forage species from the NABSB collected from the Great Lakes basin have historically been used to investigate temporal trends and bioaccumulation potential of both legacy and emerging flame retardants (FRs). Recently, samples have been used to set the baseline and monitor trends of novel FRs (bromo/chloro). These data will be used to investigate spatial and temporal patterns of contamination of FRs in top predator fishes in support of the CMP. Generating temporal trends for emerging contaminants, such as FRs, are possible only due to the availability of well documented and properly preserved specimens from the NABSB.

Toxicity of a brominated flame retardant (tetrabromobisphenol A) on microalgae with a multispecies bioassay.

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Due to the development of electric and electronic equipment, large quantities of brominated flame retardants (BFRs) are used by manufacturers to reduce the risk of fire initiation and propagation. Tetrabromobisphenol A (TBBPA) is one of the most used BFRs, especially in printed circuit boards (consumer electronics, computers and automobile). However, little is known about the toxic risk of TBBPA to aquatic ecosystems. Microalgae, which are the primary producers at the base of trophic-chain, are key microorganisms to investigate the toxicity of TBBPA in aquatic ecosystems. Alterations of these primary producers may strongly affect the balance and the productivity of the whole ecosystem. Single-species bioassays are usually used in ecotoxicological investigations to appraise the toxic risk of chemicals. However, if single-species bioassays are known to be highly sensitive, they do not provide realistic appraisal of chemical toxicity on natural microalgae communities. The interactions between species in communities could interfere in the response of microalgae exposed to a toxic agent. To obtain a more realistic appraisal of the toxicity of TBBPA, an

experiment was carried out with two freshwater microalgae *(Pseudokirchneriella subcapitata* and *Nitzschia palea)* in single-species and multi-species bioassays.

Changes in courtship and brood-rearing behaviour accompany reproductive changes in American kestrels *(Falco sparverius)* exposed to environmentally relevant concentrations of technical hexabromocyclododecane (HBCD).

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Hexabromocyclododecane (HBCD) is a current-use, high production-volume brominated flame retardant used in polymeric products. Like other BFRs, it leaches out of end-products into the environment and is now a ubiquitous global pollutant. HBCD is lipophilic and bioaccumulative and has been detected in many wildlife species, particularly those at high trophic levels, with effects that are still not well understood. The aim of this study was to determine the effects of chronic dietary exposure to environmentally relevant levels of technical HBCD on the reproduction of captive American kestrels *(F. sparverius).* In April 2008, 31 kestrel pairs were formed into two groups: control (N = 11) and an HBCD-exposed (N = 20). Exposed pairs were fed daily 800 $ng \cdot g^{-1}$ ww of technical HBCD dissolved in safflower oil, from three weeks prior to pairing until two days before hatching. Controls were exposed to safflower oil only. Reproductive measures were recorded, courtship and brood-raring behavioral data were collected, and chicks were measured at 5-day intervals. Compared to controls, HBCD-exposed pairs laid their eggs earlier after pairing and had larger clutches. Courtship behaviour was altered by HBCD exposure: most notably, males had decreased rates of mate-directed vocalizations and both sexes had moderately fewer nest-inspections. During brood-rearing, HBCD- exposed males entered the nest-box less while exposed females entered more often, and males performed fewer bonding and food-related behaviours. These behavioral changes were consistent with changes in nestling growth where, during their first 20 d, HBCD-exposed chicks were generally smaller and experienced slower growth rates than controls.

Changes in growth and endocrine function of nestling tree swallows (*Tachycineta bicolour*) following in ovo exposure to brominated flame retardants and other contaminants.

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Sewage treatment plants (STPs) are conduits for contaminants having potential toxicity for wildlife. As part of a larger study, this study focuses on exposure of free-ranging birds to brominated flame retardants (BFRs) and other contaminants from STPs, and possible changes in the endocrine function and growth of the birds. Colonies of tree swallows, small aerial insectivores consuming emerging aquatic insects, were established immediately downstream of STPs (N = 35 nest boxes/site): two sites near the main STP of industrial Hamilton (pop: 500,000); an STP in the manufacturing city of Kitchener (pop: 205,000); and at a rural reference site. In 2007 and 2008, freshly laid eggs were

analyzed for contaminants, and nestling morphometric measurements and fecal samples were collected. Of the PBDEs measured, the relative concentrations of the top eight congeners were (in descending order): BDE-99, -47, -100, -153, -154, -30, -28/33 and -49. Other current-use BFRs detected in these eggs included BDE-209, BTBPE, HBCD, α- and β-TBECH, and DBDPE. Overall, quantifiable BFR concentrations were highest in the Kitchener STP eggs and lowest in the reference eggs. Pre-fledging chicks demonstrated differences in weight gain, bone and feather growth measures, among the sites. Compared to the reference chicks, the STP swallow chicks had significantly lower plasma TT3 levels and plasma TT3:TT4 ratios, while TT4 levels were significantly different among the sites. The changes in nestling growth and thyroid measures were significantly correlated with in ovo concentrations of ∑PBDEs and other major BDE congeners.

Dynamics of contaminant transfer from mother to pup during lactation in harp seals *Phoca groenlandica*.

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Phocid seals have lipid rich milk, which is known to serve as a transfer medium through which persistent organic pollutants (POPs) move from mother to offspring during lactation. In March 2008, seven mother-pup pairs of harp seals (*Phoca groenlandica*) were sampled from 1 to 2 times between parturition and weaning near Magdalen Islands, Québec, Canada. The aim of this study was to examine the qualitative and quantitative partitioning of chlorinated POPs (pesticides and polychlorinated biphenyls or PCBs) and brominated POPs (polybrominated diphenyl ethers or PBDEs) in maternal blubber, blood and milk, and pup blubber and blood. The lipid-normalized concentrations in these tissues were in the ppb range (i.e. $ng \cdot g^{-1}$ lipid). PCB concentrations in harp seals from Madgalen Islands were low in comparison with previous studies suggesting a temporal decreasing, whereas ∑DDT concentrations remained stable. Lactational transfer rates were dependent on the log Kow (octanol/water partition coefficient) values of the analytes measured, less lipophilic compounds being more readily transferred to the pups by the lactational route. The pups' daily exposure to PBDEs, PCBs and hexachlorobenzene (HCB) increased during lactation period, affected by increasing lipid content in milk. While the daily burden of contaminants increased in pup seals, at the same time the daily burden in female seals decreased. This efficient generation transfer of POPs demonstrates the relative importance of lactation as an excretory route in reproductive female seals and, in parallel, as an exposure route in suckling pup seals.

Occurrence and fate of rosuvastatin, rosuvastatin-l, and atorvastatin in Canadian sewage and surface water samples.

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Rosuvastatin (RST) and atorvastatin (ATO) are prescription drugs and members of the statin family used for the treatment of elevated cholesterol levels. We have developed a method using solid-phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the determination of ATO, RST and its metabolite rosuvastatin lactone (RSTL) in sewage and surface water samples. In the LC-MS/MS analysis, electrospray ionization in the positive mode and multiple reaction monitoring (MRM) were used for the detection of the statins. In the influent and effluent samples collected from 11 sewage treatment plants located in Ontario, Canada, ATO, RST, and RSTL were detected in all samples with median concentrations of 166 $ng \cdot L^{-1}$ (influent) and 77 $ng \cdot L^{-1}$ (effluent) for ATO, 448 $ng \cdot L^{-1}$ (influent) and 324 $ng \cdot L^{-1}$ (effluent) for RST, as well as 158 $ng \cdot L^{-1}$ (influent) and 41 $ng \cdot L^{-1}$ (effluent) for RSTL. The median removal rate by wastewater treatment was 66% for ATO and 22% for RST and RSTL combined. The same compounds were also detected in a number of surface water samples at low $ng \cdot L^{-1}$ concentrations.

Trophic magnification behavior of cyclic volatile methylsiloxanes in an aquatic foodchain.

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Cyclic volatile methylsiloxanes (cVMS) have gained increased attention in recent years due to their unique behavior in the environment. The bioaccumulation of cVMS oligomers (e.g., D4, D5, D6) was studied here using a mechanistic foodchain model. The model was calibrated using laboratory measurements and then applied to a field setting (e.g., Lake Pepin, MN) to gain additional understanding of the observed trophic dilution of cVMS in aquatic foodchains. The modeling analysis revealed that cVMS are poorly assimilated via water and diet and are rapidly eliminated from fish, resulting in concentrations that are lower in larger, older fish relative to younger fish and their prey items (e.g., benthic invertebrates, small fish). This behavior collectively results in trophic transfer factors (TTF) of < 1. cVMS have unique physico-chemical properties (e.g., solubility, volatility, hydrogen bonding potential), especially when compared to PCBs and other conventional, carbon-based persistent organic pollutants, which may explain the observed (TTF = 6). The inherent concern of chemical bioaccumulation is the potential for chronic effects due to long-term exposure. Under field conditions, the measured body burdens of cVMS in fish are 100 to 1000 times lower than estimated critical tissue residue thresholds for chronic effects. The results of this study suggest that cVMS may have limited environmental risk due to bioaccumulation.

Biomarqueurs
Biomarkers

Biomarkers, metal speciation and metabolites: from scientific knowledge to regulation.

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Despite being used in ecotoxicological studies since the early nineties, biomarkers are not widely used in official biomonitoring programmes. Biomarkers provide early and sensitive responses revealing environmental stress but most of them are a source of considerable dissatisfaction due to their lack of ecological relevance. Can impairments at the infra-individual or individual levels allow assessment of effects at higher levels of biological organization? During the last decade, ecotoxicological research has proposed relevant biomarkers to forecast effects at supra-individual levels, well before environmental degradation has reached a hardly manageable degree. When such impairments are revealed, it is necessary to identify the main classes of contaminants which may be responsible for them by using a multi-biomarker approach based on the so-called "core biomarkers" – particularly biochemical markers – incorporated in integrative indices. Then the quantification of chemicals in sediments and biota will permit the validation of hypotheses based on biomarker studies. But again, the regulation focuses on global concentrations – cost effective and easy to master – whereas scientific advances have demonstrated the importance of specific fractions and metabolites. To date, passive samplers have given access to measurements of particular physicochemical characteristics of pollutants which govern their fate and bioavailability. Despite the relative maturity of some of these sampling technologies, they are still considered to be innovative, and few if any specific policies governing their use have been written into official regulations.

Variations in p53-like mRNA sequence are correlated with mussel health: a potential molecular-level tool for biomonitoring.

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Metro Vancouver initiated a program to use mussels (*Mytilus sp*.) for environment monitoring in Burrard Inlet (British Columbia). The program includes the development and validation of new molecular biomarkers. The local mussel species in British Columbia (*Mytilus trossulus*) is highly susceptible to a type of leukemia generally called haemic neoplasia, which is proposed to be associated with environmental factors. Although bivalve molluscs are popular environmental indicators, genomic tools for monitoring their health are under-developed. The p53-like gene is known to be involved in molecular processes associated with haemic neoplasia in many molluscs. We found 3 specific single nucleotide polymorphisms in p53-like mRNA sequences from *M. trossulus* that correlate with the occurrence of haemic neoplasia. Analysis of variations in all known members of the p53 family, p53, p63/73 and ΔNp63/73, showed that different

alleles are expressed in normal and neoplastic haemocytes. Based on the results of our study we propose to fully validate the particular mRNA type associated with the absence of the disease as a marker of mussel population health.

L'état du foie comme indicateur de «la santé» de l'esturgeon jaune du fleuve Saint-Laurent.

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Au Québec, l'esturgeon jaune (*Acipenser fulvescens*) fait l'objet d'une pêche commercial soutenue dans un contexte où, à l'échelle mondiale, la plupart des espèces d'esturgeons sont menacées de disparition. Si les données dont disposent les intervenants dans la gestion de cette ressource les informent sur l'état des stocks en terme de quantité, elles ne les renseignent pas sur la qualité des individus pêchés. Des doutes subsistent sur le potentiel réel de régénération et de subsistance à long terme de cette espèce. L'hypothèse principale de la recherche est que certains indicateurs biologiques pourraient signaler une atteinte potentielle à la santé des esturgeons, ce qui pourrait conduire à un examen plus approfondi des phénomènes détectés. Des relevés physionomiques (âge, sexe, indices somatiques, relevés d'anomalies morpho-pathologiques) et des analyses en laboratoire (concentrations de lipides, protéines et glycogène hépatiques, histologie du foie, cycle cellulaire des splénocytes) ont été effectuées sur des esturgeons jaunes provenant de trois stations d'échantillonnage. Deux stations se trouvent dans le fleuve Saint-Laurent (lac Saint-Louis et archipel du lac Saint-Pierre), et la troisième se trouve en Abitibi (lac Berthelot). Des analyses de contaminants environnementaux furent également effectuées (mercure, biphényles polychlorés (BPC), dioxines, furanes, éthers de diphényles polybrominé (PBDE), biphényles polybrominés (BPB)). Un indice intégré d'évaluation de la santé, traité tel qu'il le fut dans le présent travail, ne constitue pas un signal préventif adéquat. Cependant, les résultats concernant les lipides hépatiques totaux ainsi que certaines observations histologiques indiquent que le suivi de l'indice hépatosomatique constitue un signal préventif d'atteinte potentielle à la santé.

Mya arenaria used sucessfully as sentinel species in an estuarine context.

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This research aimed at using multiple physiological biomarkers to assess the health status of *Mya arenaria* in brackish waters in the Saguenay – St. Lawrence marine system. *Mya arenaria* was chosen as a sentinel organism as an infaunal bivalve integrating both the water column and sediment quality. Results showed the sensitivity of the soft shell clam to sub lethal concentrations of contaminants. Diminished responses of immune parameters were observed in anthropogenic impacted sites. An overview of physiological responses of *Mya arenaria* in different sites characterized by various contamination levels will be presented. In the Saguenay fjord, the highest impacted sites

showed decreased energy reserves, sexual maturation delays, metal contamination and high metallothionein concentrations. In the St. Lawrence maritime estuary, sexual steroid levels were decreased when clams were exposed to organotins. Since clams showed signs of endocrine disruption, recent work in our labs examined the role of neurohormones. Results showed sensitivity of serotonin and dopamine to endocrine disruptors. Evidence of links between neurohormones localization and reproduction will be provided. Finally, all these results show clearly that the feral clam, *Mya arenaria,* is a very good sentinel species and sensitive to various contaminants found in estuarine and marine environments. Research funded by NSERC and Environment Canada.

Comparison of atmospheric pressure photoionization and atmospheric pressure chemical ionization mass spectrometry for the analysis of retinoids.

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Retinoids, which include all-trans retinol (Vitamin A) and its derivatives, are low molecular weight, lipophilic molecules that are essential for a number of diverse physiological processes such as vision, reproduction, metabolism, growth and differentiation, pattern formation during embryogenesis, and overall maintenance of organism health. Sensitive and specific assays for retinoid concentrations in various biological materials are needed for continued studies to identify retinoic acid receptors, their ligands and their roles in the expression of biological responses. Atmospheric pressure photoionization (APPI) is the latest technique to couple atmospheric pressure ionization (API) to LC separation techniques. The work reported here compares the use of triple-stage LC/MS/MS with APCI and APPI for the determination of selected retinoids in biological samples.

Substances perturbatrices des systèmes neurologiques et endocriniens
Neuro and Endocrine Disrupting Compounds

Challenges with the use of biomarkers to predict reproductive impairment in fishes exposed to endocrine disrupting substances.

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Several biomarkers are often used to evaluate the potential endocrine disrupting effects of chemicals and waste streams. However, the relationship between responses in these biomarkers and population effects is not known. Short-term adult fish reproductive tests provide the opportunity to examine the relationship between functional endpoints such as egg production (a whole-organism endpoint intuitively linked with population level responses) and biomarkers that are commonly used in field studies (sex steroids, vitellogenin (VTG) and gonad size). Egg production measured in short-term adult fish reproductive tests showed weak relationships between female 17β-estradiol (E2) and relative female gonad size (gonadosomatic index; GSI), and male VTG and GSI. The

strength of the relationship between E2 and egg production was highest in the most commonly-used test species, fathead minnow *(Pimephales promelas)*. A fathead minnow population model predicted that, depending on which scenario was used, a 50% decline in E2 in females would result in a decline in population size ranging from 31% to 92% over a five-year period. These declines had wide confidence intervals, highlighting the difficulty of using the relationships between commonly-used biomarkers and egg production to quantify population-level effects. In a qualitative assessment of the relationship between biomarkers and egg production, low incidences of false positives were found (i.e., an effect in biomarkers was not followed by an effect in egg production), but there was a high occurrence of false negatives (an effect in egg production was not accompanied by a biomarker response). The occurrence of false negatives can be greatly reduced when using multiple female biomarkers. As both the occurrence of false negatives and positives is relatively low under this scenario, a suite of female reproductive biomarkers can be used as an effective indicator to screen chemicals and assess waste streams for endocrine disrupting substances.

Estrogenicity of Chilean pulp and paper mill effluents to rainbow trout.

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Endocrine disruption effects of Chilean pulp and paper mill effluent extracts (solid phase extraction, non-treated, primary and secondary treated) were evaluated using immature triploid rainbow trout (*Oncorhyncus mykiss*) in a pulse-exposure toxicity experiment (intra-peritoneal injection, corrected for individual fish weight). Disrupting effects at the reproductive level were observed in all effluent treatments. These effects include increased CYP4501a1 (EROD activity in liver) and increased plasma vitellogenin levels (VTG) after 7 days in fish injected with untreated and primary treated effluent extracts, similar to increase levels found for 17β-estradiol standard injected fish and different from the increase observed after 4 days in fish injected with secondary treated effluent extract. Additionally, differences in plasma steroids concentrations were observed in fish injected with all effluent extracts and related to the early induction of the CYP19a (aromatase) gene expression found in gonads of fish injected with pulp and paper mill extracts, estrogens, androgens and phytosterols standards. Those results were later confirmed in a second chronic exposure experiment using multiple intraperitoneal injections of juvenile natural rainbow trout, where the same differential induction patterns in EROD activity and plasma VTG levels were found irrespective of fish sex. The results indicate a significant endocrine effect due to the effluent treatments and confirm that the endocrine disruptive effects of Chilean pulp and paper mill effluents previously demonstrated in trout under both laboratory and field conditions continue to be estrogenic.

An in-situ study of the impacts of urban wastewater on the immune and reproductive systems of the freshwater mussel *Elliptio complanata.*

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The goal of this study was to examine the disruptive effects of municipal effluents on the immune and reproductive systems of freshwater mussels. For 30 days, caged mussels were immersed in the Rivière des Mille Îles (Québec, Canada), 150 m both upstream and downstream from two urban wastewater treatment plants: station F (Fabreville) and station A (Auteuil), which serve the city of Laval. Station F is 12 km upstream from station A. The immune and reproductive conditions of the mussels were thereafter determined. Though the weight/shell length ratio was not affected, the effluent induced mortality up to 60% at downstream sites. Total hemocyte counts increased, and phagocytosis and lysozyme activities were induced at station F, whereas these responses were suppressed at station A. Heterotrophic bacteria levels in mussels were negatively correlated with phagocytosis, showing the importance of this process in defending against infection. Inflammation biomarkers such as nitric oxide and cyclooxygenase activity were the same for all sites but were positively correlated with phagocytosis activity. The production of vitellogenin (Vtg)-like proteins was significantly induced at the site downstream from station A and was strongly associated with phagocytosis. This was further supported through analysis of covariance, of Vtg responses against phagocytosis, revealing that Vtg was no longer induced at the sites upstream and downstream from station A. The data support the contention that Vtg was involved, in part at least, in the immune system in mussels. Both Vtg and immune status are impacted by urban effluents and should be considered when using the Vtg biomarker to search for the presence of (xeno)estrogens in contaminated environments.

Human pharmaceuticals disrupt feeding and fuel storage in fish.

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Various human pharmaceuticals (HP) are reported at ng-$\mu g \cdot L^{-1}$ concentrations in aquatic environments. Previous studies from this lab found that lipid-lowering fibrate drugs and the selective-serotonin re-uptake inhibitor (SSRI) fluoxetine (FLX) modify levels of reproductive hormones in goldfish. Given that HP affect tissue signaling systems, this study examined the role of gemfibrozil (GEM, a fibrate drug) and FLX on fish metabolic processes. GEM injected IP into rainbow trout reduced all plasma lipids and although the proportions of the different lipoprotein classes remained unchanged, phospholipid/triglyceride ratios increased, indicating a shift to higher density lipoprotein species. Goldfish were exposed to two waterborne concentrations of FLX (540 $ng \cdot L^{-1}$, high environmental level; 54 $\mu g \cdot L^{-1}$) for 25 days with static renewal every 48 h. The exposure also included control, fasting and pair- fed groups for each exposure group. FLX especially at the higher concentration inhibited food intake, decreased body weight gain and affected body fuel composition (glycogen, protein, amino acid). Changes were also noted in metabolic enzyme activities and mRNA transcripts. These results indicate that both HP may alter the nutrient type and amount stored in tissues which could modify

energy partitioning and thus fitness of the fish in their environment. Supported by grants from NSERC, CWN and ON BEST in Science programs.

Analyses d'antidépresseurs bioaccumulés dans les tissus d'organismes aquatiques par chromatographie liquide couplée à la spectrométrie de masse en tandem (LC-MS/MS).

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De récentes préoccupations concernant la présence et le devenir de produits pharmaceutiques dans l'environnement sont à l'origine de nombreux projets de recherche au sein de la communauté scientifique. A ce sujet, des traces d'antidépresseurs ont été détectées en Amérique du nord dans les eaux de surface et les eaux usées municipales (Metcalfe et al., 2003; MacLeod et al., 2007; Schultz et Furlong, 2008; Lajeunesse et al., 2008). Étant donné l'importante influence qu'ont les antidépresseurs sur les niveaux critiques de sérotonine de plusieurs organismes aquatiques (Sundin et al., 1995; Fong, 1998; Rodriguez et Renaud, 1980), des méthodes analytiques fiables permettant d'allier sensibilité et spécificité sont désormais nécessaires afin de mieux comprendre le devenir de telles substances. Pour ce faire, une nouvelle méthode a été développée et validée pour la détermination par LC-MS/MS de six antidépresseurs et quatre métabolites dans les tissus de truites arc-en-ciel et de moules d'eaux douces. Des résultats préliminaires avec des truites exposées en mésocosmes aux effluents de la ville de Montréal indiquent un potentiel de bioaccumulation des substances étudiées.

Effects of Antidepressants on Fathead Minnows.

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Fathead minnows were exposed for a lifecycle to environmentally-relevant concentrations of venlafaxine (Effexor™) or to a mixture of the top five detected selective serotonin reuptake inhibitor (SSRI) antidepressants at concentrations similar to Canadian municipal wastewater effluent (MWWE). Exposure concentrations (nominal) were: venlafaxine 1000, 10,000 or 100,000 $ng \cdot L^{-1}$, SSRI mixture: venlafaxine 2700 $ng \cdot L^{-1}$, citalopram 300 $ng \cdot L^{-1}$, bupropion 100 $ng \cdot L^{-1}$, fluoxetine 100 $ng \cdot L^{-1}$ and sertraline 20 $ng \cdot L^{-1}$, or ten times these SSRI mixture concentrations. Measured concentrations of SSRIs in fish exposure aquaria were about 70 to 90 % of nominal concentrations, except for bupropion which was difficult to measure. Exposures began with fertilized fathead minnow eggs and fish were examined during their growth development and reproduction until 160 days post-hatch. Offspring (F1) were raised to 16 dph to assess changes in growth. Fish exposed for a lifecycle to antidepressants showed very few changes in growth or egg production. Survival and growth of F1 offspring from Venlafaxine and SSRI mixture exposures were similar to offspring from control fish. The only significant changes seen in the lifecycle exposure were alterations in male nest-defending behaviors. In behavioral assessments, venlafaxine-exposed males were more aggressive as measured by decreased time to physically contact (hit or push) a 'dummy' intruder fish held near

the nest. These observations of increased aggression in adult male fish occurred at or close to venlafaxine concentrations detected in MWWEs. Alterations to reproductive behavior may have ecological impacts in fish exposed to antidepressants in the natural environment.

Is the birth control pill an effective form of contraception for wild fishes?

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It is well known that municipal effluents contain substances that affect reproductive endocrine function in wild fishes. However, it is not well understood whether responses observed at the organism level, such as the production of the egg protein precursor vitellogenin in male fish, indicate impacts at the population level. To investigate this, a whole lake experiment was done at the Experimental Lakes Area (ELA) from 1999-2006 to understand the effects of the synthetic estrogen ethynylestradiol (EE2) on the fish populations and their supporting food web. EE2 was added continuously to experimental Lake 260 in the summers of 2001-2003 to achieve target concentrations of 5-6 $ng \cdot L^{-1}$ in the surface waters. Vitellogenin and histology samples, and population-level data were collected in Lake 260 for fathead minnow (*Pimphales promelas*), pearl dace (*Semotilus margarita*), and lake trout (*Salvelinus namaycush*) before, during and after EE2 additions, and contrasted with data from several reference lakes. During the EE2 additions, male and female fishes of some species showed induced vitellogenin production and delayed or abnormal (intersex) gonadal development. In addition, fathead minnow had recruitment failures in 2002-2005, which led to a near extinction of the species. However, in 2006 the population of fathead minnow recovered to pre-addition levels. Lake trout abundance also decreased in Lake 260 in 2003 and this response was likely related to the decline in prey species rather than direct effects of the EE2. In summary, this experiment showed that chronic exposure to low concentrations of a potent estrogen can affect the sustainability of fish populations and that the shortest-lived species are at greatest risk from these compounds. Results showed that estrogenic substances may affect fish populations both directly through impacts on recruitment and indirectly through a loss in food supply.

Réhabilitation des milieux aquatiques dégradés
Remediation of degraded aquatic environments

L'approche du MDDEP concernant l'utilisation des technologies de restauration de lacs.

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Pour contrer l'eutrophisation des plans d'eau, le Ministère du Développement durable, de l'Environnement et des Parcs (MDDEP) préconise les actions préventives et la réduction des apports de nutriments provenant du bassin versant. Toutefois, lorsque la

charge interne de nutriments est très importante ou lorsque des usages importants doivent être protégés, des interventions dans le plan d'eau peuvent être souhaitées par les riverains cherchant des moyens de réduire les manifestations de l'eutrophisation. Le MDDEP reçoit diverses demandes visant l'utilisation de technologies de restauration de lacs ou de contrôle des cyanobactéries. L'approche développée par le MDDEP pour orienter l'utilisation de ces technologies en évaluant leur efficacité et leur potentiel d'impact négatif sera présentée.

Lake restoration in a context of watershed integrated management: the Saint-Augustin Lake and its eutrophication problem.

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Effluents from industrial sources and polluted urban/agricultural runoffs have carried contaminants to water bodies, causing contamination of water and sediments in rivers, lakes, estuaries and bays. Pollutants often considered indefinitely locked within sediments pose threats because of mechanisms that trigger their release: particle resuspension, burrowing by bioturbators, upward groundwater flow through contaminated sediment layers and diffusion due to concentration gradients. Today, numerous Canadian lakes suffer from contaminated sediments and/or early eutrophication symptoms. Typical contaminants found in bottom sediments are: excess nutrients (P and N), metals (Pb, Cd, Cr, Hg) and of recent concern, road de-icing salts. These mixtures of contaminants become available to benthic organisms and disrupt the food chain through bioaccumulation, leading to reduction in biodiversity. They pose risk to human health through water/fish consumption or skin contact during recreational activities. Extreme eutrophication causes toxic algae blooms (cyanobacteria) and oxygen depletion leading to anoxic conditions. Climate warming is an accelerating factor for eutrophication. As citizens lose water uses and become aware of health/ecological risks and the negative impacts on regional economy, governments have recognized that protection and restoration of water quality and use are unattainable without INTEGRATED WATERSHED RESTORATION PLANS (IWRP) which very often will include Sediment Restoration Actions (SRA). IWRPs require mandatory control or reduction of external sources of pollution, reduction of surface run-off and restoration of vegetation buffers. These are preventive actions that need to be prioritized. The longevity and success of any in-situ remediation action depends on the achievement of source pollution reduction. In-lake remediation constitutes a curative approach where preventive measures have been proven insufficient. Indeed, research studies showed that water bodies may require a very long time to recover (from decades to centuries) after input sources are stopped. Saint-Augustin Lake suffers of extreme eutrophication with sediment contamination. In order to attain measurable improvements of water quality, one needs today to tackle the sediment problem. St-Augustin Lake is the only remaining natural lake in the urbanized Québec City and because of its location, history, and representative conditions it is an excellent site to be studied at watershed scale and used as a model for the development of innovative eco-engineering restoration techniques.

Eco-compatibility of an in lake restoration technique for the Missisquoi bay and the Saint-Augustin Lake.

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The Missisquoi bay and the Saint-Augustin Lake are prone to eutrophication and cyanobacteria proliferation which affect dramatically both their ecosystems and their beneficial uses by the neighboring human populations. A new restoration technique which combines different processes is under study to reclaim these aquatic ecosystems. Firstly, phosphorus present in the water column is immobilised by coagulation, flocculation and precipitation by the addition of alum. An active capping (limestone) is then applied to isolate contaminants retained by the bottom sediments. Sand is finally added to maintain stability of the capping and to facilitate its recolonisation by benthic organisms. Multi-species laboratory tests (microcosms) were selected to evaluate the eco-compatibility of the technique for the Missisquoi bay and for the Saint-Augustin Lake. This tool allows the simulation of the restoration scenario and the evaluation of its effects towards five species representing primary producers, *Pseudokirchneriella subcapitata* and *Lemna minor*, and primary consumers, *Daphnia magna*, *Hyallela azteca* and *Chironomus riparius*. Tests were performed in 2 liters beakers containing natural water and sediment from the two sites. The restoration technique was applied in eight replicates for each site, in addition to control systems. Microcosms were placed in an environmental chamber during an exposure time of 28 days. This presentation will focus on the principal physicochemical (pH, conductivity, NH3, Al, P) and biological (survival and reproduction of *D.magna* and *H. azteca*, population growth of *L. minor*, survival, emergence and growth of *C. riparius*, development of cyanobacteria) effects of the restoration technique.

Treatment of roads' runoff waters by active filtration combined with an adapted constructed wetland.

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Snowfall events are major problems for road security in Canada. As a consequence, road salts are spread on the entire road network in order to protect road users. Small watersheds close to these roads are sensitive ecosystems affected by road salt contamination. A good example of this is situated close to Québec City. The Saint-Augustin Lake (0.60 km^2) and its watershed (7.46 km^2) are subject each spring to high runoff of salts. The purpose of this research project is to evaluate a two-step treatment system that will reduce the contamination load contained in road runoffs which are mainly contaminated by chlorides (Cl-) and sodium (Na+). The system includes: an active filtration packed bed and an adapted constructed wetland. The packed bed uses calcareous material to adsorb P. The constructed wetland is adapted to treat Cl- and Na+ by using halophytes plants from the St-Laurence estuary which accumulate these elements in their biomass. The presentation describes the results of experiments that have been conducted on (1) chloride and sodium absorption by halophytes plants, (2) ways to

improve it by selecting optimal growing conditions and (3) greenhouse pilot scaled constructed wetlands using halophytes to treat road salts.

Total phosphorus management in the Lake Simcoe Basin.

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Managing urban growth and mitigating agricultural land use activities are significant challenges in trying to protect and sustain our water resources. Continued urban and population growth combined with impacts from existing agricultural activities have accelerated Lake Simcoe's eutrophication process. Located in southern Ontario and within an hour's drive of half the population in the province, Lake Simcoe is a fresh water lake with a surface area covering 722 km^2 and a watershed of approximately 2857 km^2. It is an extremely valuable natural and recreational resource contributing more than $200 million annually to the local economy through recreational pursuits and providing a source of drinking water for many communities. Inputs of phosphorus and other nutrients from wastewater treatment facilities, urban and agricultural runoff and other sources have more than tripled since European settlement in the 1800's. Efforts to reduce phosphorus loadings to the lake have been underway for over three decades, striving to achieve an annual 75 tonne/year target. While significant progress has been realized, increased demands for continued growth within the basin have resulted in wide ranging concern from partners and stakeholders that the phosphorus target, while achievable, could not be maintained in light of future growth. An overview of the LSRCA's phosphorus reduction program and strategy will be outlined and include overviews of some emerging techniques being explored and implemented including stormwater management innovations and the using Phoslock™ as a control.

Restoration options in lakes with internal phosphorus load.

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Internal phosphorus (P) loading as P released from bottom sediments often represents the main summer P load to lakes. Therefore, external load abatement does not necessarily lead to improved water quality. To restore lakes with high internal load, internal fluxes have to be quantified in relation to external input. If internal load abatement is necessary, several options are available that are specific to the individual lake's characteristics. In particular, restoration options are different for shallow, polymictic lakes versus deep, stratified ones, or for small versus large lakes. In this presentation the basic techniques of lake restoration will be presented and applicability, success and failure discussed for lakes from Canada, the US and Europe.

Expérimentations dans la restauration de lacs par Dessau.

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[1]DESSAU.

Dessau désire partager son expertise au niveau du cheminement de la restauration des lacs à trois niveaux, soit l'élaboration d'un projet pilote de restauration au lac Waterloo, une étude de préfaisabilité de restauration de lac par dragage de sédiments et finalement une projet soumis processus d'étude d'impact sur l'environnement d'un projet de pompage de sédiments lacustres pour le contrôle de l'envahissement par les plantes aquatiques au lac Trois Lacs. Le projet au lac Waterloo consiste en un projet pilote visant à évaluer l'efficacité de deux techniques à contrôler la prolifération des cyanobactéries, soit par pompage des sédiments ou par l'utisation des lentilles d'eau pour réduire la quantité de phosphore disponible. L'étude de préfaisabilité du dragage de sédiments lacustre a consisté à comparer les différentes techniques de dragage des sédiments afin de déterminer leur efficacité et les coûts associés. Enfin, Dessau a participé au processus d'évaluation environnementale et d'audience publique d'un projet de restauration de lac. Le projet consiste au pompage partiel des sédiments pour contrôler la prolifération des plantes aquatiques du lac Trois Lacs afin de concilier les objectifs de développement durable, soit la protection de la biodiversité, mais aussi le maintient d'activités sociales et économiques au niveau local et régional.

Contaminants anciens II – Hydrocarbures
Legacy contaminants II – Hydrocarbons

Effects of chronic produced water exposure on the expression of some immune-related genes of juvenile Atlantic cod.

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Produced water (PW) is the main discharge from the offshore oil industry. We investigated the effects of chronic exposure to PW on growth parameters, food consumption, plasma cortisol, respiratory burst activity (RB), and mRNA expression of several immune-related genes of juvenile Atlantic cod by exposing fish to 0, 100 $mg \cdot Kg^{-1}$ (10 000× dilution) or 200 $mg \cdot Kg^{-1}$ (5000× dilution) of PW for 22 weeks. No significant effects were observed in food consumption, growth, hepatosomatic index, condition factor or plasma cortisol. The RB of circulating leukocytes was significantly elevated in the 100 $mg \cdot Kg^{-1}$ group only while the RB of head kidney leukocytes was decreased in both the 100 and 200 $mg \cdot Kg^{-1}$ groups. Significant up-regulation of the mRNA expression of β-2-microglobulin, immunoglobulin M light chain and interleukins-1β and -8 was observed in fish from the 200 $mg \cdot Kg^{-1}$ group, while the down-regulation of interferon stimulated gene 15 was obvious for fish from both the 100 and 200 $mg \cdot Kg^{-1}$ groups. This results indicate that chronic exposure to environmentally relevant concentrations of PW causes modulations of the immune system of juvenile Atlantic cod,

with most immune parameters being stimulated, potentially resulting in an energetic cost that may be detrimental to fish.

The effects of Production Water, WAF or CEWAF on the fertilization success of Atlantic cod eggs.

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Activities related to the production and transport of oil have the potential to release substances to the marine environment which may affect fish. Production water (PW) from offshore platforms, oil and dispersed oil are three such substances. Eggs from Atlantic cod were fertilized in the presence of produced water collected in July of 2008 from the Terra Nova and Hibernia platforms. Preliminary results show that exposure to Terra Nova PW at 12% (V/V) or higher resulted in fertilization rates significantly lower than in controls. Exposure to 0.5% (V/V) Hibernia PW or higher resulted in significantly lower fertilization rates compared to controls. Cod eggs were also fertilized in the presence of the water accommodated fraction (WAF) and chemically enhanced water accommodated fraction (CEWAF) of Alaskan north slope oil (ANS). The dispersant used was SPC -1000. Preliminary results show that the WAF of ANS up to 25% (V/V) had no effect on fertilization success compared to controls, however at 50% (V/V) the fertilization rate was much lower than that of the controls. CEWAF significantly reduced fertilization at all concentrations tested, the lowest being 0.5% (V/V). Analysis of water samples collected during these experiments is ongoing. These results are essential to assessing the risk of these mixtures.

Uncovering the exposure mechanisms of sunken heavy oil that makes it chronically toxic to early life stages of fish.

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Nearly 150 000 L of No. 6 heavy fuel oil (Bunker C) was spilled into Wabamun Lake (Alberta, Canada) when a train derailed in August 2005. Bunker C is a residue of refining crude oil and a very complex mixture containing polycyclic aromatic hydrocarbons (PAH), particularly the 3-4 ringed alkylated forms that cause sub-lethal toxic responses in early life stages of rainbow trout (Onchorynchus mykiss). Common signs of chronic toxicity include pericardial and yolk sac edema, craniofacial deformations, and cardiovascular dysfunctioning, all characteristic of blue sac disease. Fish show such signs of toxicity when exposed experimentally to oil via mechanical and chemical dispersion, which enhance partitioning of PAH from oil to water and simulates oil spill conditions. However, heavy oil does not disperse well, and its unique feature compared to medium and light oils is a density similar to that of water, giving it the potential to sink after a spill. Nearly four years after the spill at Wabamun Lake, oil patches still persist in near-shore sediments where fish spawn. The toxicity of oil that sinks and persists in sediments is not well understood because the mechanisms of

exposure are not characterized. The objective of this research is to assess how the behaviour of heavy oil in water interacts with exposure and toxicity to early life stages of fish. Static daily renewal tests with heavy fuel oil coated on glass plates (artificial substrates) demonstrated greater oil toxicity (as mg added oil·L^{-1}) to trout embryos than oil that was mechanically or chemically dispersed. We will expand these tests using a flow-through oiled gravel column to assess whether the toxic constituents of heavy oil are transferred to water quickly enough to cause toxicity. Measuring the rate of hydrocarbon transfer into water from oil will show the role of the surface area, surface-to-volume ratios, and weathering of oil globules on exposure and toxicity to fish of sunken oil. This project will provide exposure and toxicity test methods that better reflect the behaviour of heavy oil after a spill.

Métaux aqueux ou associés à la nourriture
Waterborne and diet-borne metals

Protective role of calcium and iron against cadmium accumulation and toxicity in the green alga *Chlamydomonas reinhardtii.*

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Within the biotic ligand model (BLM), major cations such as Ca^{2+} are considered to be simple competitors for Cd^{2+} uptake into algal cells and in this manner they may offer some protection against Cd-induced toxicity. The influence of essential micronutrients (Fe^{3+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}) on Cd uptake and toxicity is considered to be negligible. However, recent studies in our laboratory using the green alga *Pseudokirchneriella subcapitata* demonstrated that Ca2+ (as well as Mn^{2+}, Cu^{2+} and Co^{2+}) did not protect against Cd^{2+} toxicity over long-term Cd^{2+} exposure in this species, whereas Fe^{3+} and Zn^{2+} were effective protectors. In order to evaluate whether the effect of these essential cations on Cd^{2+} toxicity can be generalized to another alga, we monitored Cd uptake and toxicity in a different green algal species (i.e. *Chlamydomonas reinhardtii*) after long-term exposures to a range of realistic Ca^{2+} and Fe^{3+} concentrations. We found that Cd uptake and toxicity in *C. reinhardtii* were reduced by about 2.5-fold when [Fe^{3+}] was increased 1000-fold. Increasing Ca^{2+} concentration (50-fold) also decreased Cd uptake and toxicity 2.5-fold, indicating that the interaction between Ca and Cd is strongly species-dependent. In the future, we plan to further study the protective effect of Fe^{3+} against Cd uptake and toxicity as well as the interaction of Zn^{2+}, Co^{2+}, Cu^{2+} and Mn^{2+} with Cd^{2+} and algae cells. This project will contribute to the refinement of metal uptake/toxicity models (e.g. BLM) and will lead to better predictions of metal-induced toxicity in freshwater algae growing under different chemical conditions.

Alteration of photosynthesis during cell cycle in *Chlamydomonas reinhardtii* by chromium toxicity effect.

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Effect of chromium toxicity on the photosystem II activity of the green alga *Chlamydomonas reinhardtii* was investigated when cell cycle was in different state as G0/G1, S and G2/M. Algae growth was maintain under continues light or light/dark cycle (16h/8h) to obtain homogenize cell cycle and flow cytometry method was use to determined the cycle state of algae population by the DNA content. Photosystem II activity was investigated by using fluorescence parameters indicating Photosystem II quantum yield, photosystem II antenna size, plastoquinones pool size and pH dependent non-photochemical quenching measure with Pulse Amplitude Modulated Fluoresce and Plant Efficiency Analyzer methods. Cell cycle is the regulatory factor for biomass production and the algae community in the environment. The presence of chromium can alter photosynthesis during cell cycle division and consequently it may change the biomass production and the algae community. In this study we found that chromium toxicity showed variation during cell cycle. During the cell mitosis phase chromium toxicity effect on photosynthesis appeared to be much higher. During this cycle PSII quantum yield was decrease by 80% compared to algae in G0 phases.

Uptake of lead and copper by green algae. Validation of the Biotic Ligand Model (BLM) to metal mixtures.

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The biotic ligand model (BLM) has the potential to predict biological effects and bioaccumulation in metal mixtures. In this study, interactions between Pb and Cu uptake were quantified in order to verify some of the key assumptions of the BLM. Short-term uptake by wild type *Chlamydomonas reinhardtii* was examined in a well-defined experimental medium. For exposures ranging from $5x10^{-8}$ to $5x10^{-6}$ M, internalization fluxes were measured for Pb or Cu alone in order to determine stability constants from the reciprocal of the Michaelis-Menten constants. A value of 105.8 M^{-1} was obtained for Cu, which was very similar to that obtained for Pb (105.9 M^{-1}). Nonetheless, competition experiments did not show a straightforward antagonistic competition, as would be predicted by the BLM. Surprisingly, at the lowest concentrations of Cu, Cu had a synergistic effect on Pb uptake, while only at high Cu concentrations (> 1 μM) did Cu behave as a competitive inhibitor (as would be predicted by the BLM). In fact, at these concentrations, the stability constant for the competitive binding of Cu to the Pb transport site was determined to be 105.4 M^{-1}. When Pb was present, Cu uptake also decreased at the highest Cu concentrations while it appeared to be independent of the concentration of Pb when Cu was less than 10^{-7} M. The study suggests that when predicting bioaccumulation, it will be difficult to apply the BLM to even simple metal mixtures and that it might be difficult to apply the BLM to natural samples.

Phytochelatins produced in algae exposed to selenium and the effects of sulphate.

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Phytochelatins (PCs) are short metal detoxification peptides made from the sulphur-rich molecule, glutathione. There has been little evidence to confirm the ability of selenium to induce PC production. Therefore a goal of this study was to examine whether selenium could induce phytochelatin production in algae. Furthermore, it is thought that selenate, competes with sulphate in the sulphur assimilation pathway and sulphate demonstrates a protective effect against the toxic effects of high doses of selenium in algae. Therefore the interaction of selenate and sulphate was investigated with respect to the induction of PCs. *Chlorella vulgaris* was cultured in media with either low – 3 $mg \cdot L^{-1}$ – or high – 30 $mg \cdot L^{-1}$ – sulphate. These cultures were exposed to selenate in doses of 1, 5 and 10 $\mu g \cdot L^{-1}$ for 48 h. In a separate treatment cadmium (100 $\mu g \cdot L^{-1}$) was added as a positive PC inducing control, and there was one ”no metal“ negative control. Total selenium, selenium speciation and quantification of glutathione, phytochelatins 2 and 3 were measured in each of cell digests, cell medium and cell lysates. Results showed that PCs were induced by Se and the positive Cd control. GSH also increased. In addition, there was a marked effect by sulphate whereby glutathione and phytochelatins were significantly greater for all metal treatments in the low sulphate groups and, it appeared that, phytochelatin production was inhibited in the high sulphate groups. These data support both the existence of a negative feedback system in the sulphur assimilation pathway affecting phytochelatin production when sulphate is abundant and the competition for uptake at the ion transport level between selenate and sulphate.

The Biotic Ligand Model for predicting Ni toxicity to *Lemna minor*.

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The use of the Biotic Ligand Model (BLM) approach for metal toxicity to aquatic plants is not well-established and requires cross-species extrapolation. In this study, we aim to develop a species-specific BLM for Ni toxicity to *L. minor* by examining the effect of various water chemistry factors, including major cations (sodium, potassium, calcium, magnesium), and pH. Environmentally relevant ligands such as dissolved organic carbon (DOC) and flotation reagents used in Canadian mining were also examined for their activity as modifiers of the BLM. Major cation concentrations revealed no effect on Ni toxicity to *L. minor*. However, increasing [DOC] or decreasing pH significantly reduced Ni toxicity. The effect of DOC is consistent with the BLM, as it can be explained solely by complexation of Ni^{2+} with DOC; however, modelling to take into account competition by H^+ ions at the biological surface is necessary. Nickel speciation measurements suggest that the effect of ligands on nickel toxicity can be modelled based on complexation through the reduction of free ion activity in solution alone. Further research will validate the model with data from toxicity tests in natural waters.

The effects of Natural Organic Matter (NOM) quality on copper-gill accumulation in rainbow trout *(Oncorhynchus mykiss)*.

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Natural organic matter (NOM) protects against metal toxicity in fish through NOM-metal complexation thereby reducing metal bioavailability. The degree of protection is concentration-dependent, but toxicity may also be related to differences in the molecular composition of NOM (quality). We predicted that differences in Cu toxicity in the presence of NOM of different qualities was related to variation in Cu bioavailability & Cu-gill binding. Accordingly, three distinct NOMs [terrigenous, autochthonous & sewage derived] were collected from Luther Marsh, Bannister Lake, and from local sewage effluent. Each NOM isolate was chemically characterized using fluorescence spectroscopy and by determining the specific absorption co-efficient (SAC_{340}). Rainbow trout were then exposed to increasing Cu in the presence of each NOM type (~ 4 mg $C \cdot L^{-1}$), followed by measurements of Cu-gill accumulation. Each NOM isolate was distinct; Parallel Factor Analysis (PARAFAC) of the fluorescence spectra indicated that terrigenous Luther Marsh NOM was mainly humic acid, while Bannister Lake and sewage effluent NOM had less humic acid but greater fulvic acid. Darker Luther Marsh NOM had an SAC_{340} of 37.8, 3-4 times greater than more lightly coloured autochthonous Bannister Lake (SAC_{340}=12.4) and sewage effluent NOM (SAC_{340}=9.2). At low-moderate Cu (0-60 $\mu g \cdot L^{-1}$), all NOM isolates reduced Cu-gill binding by 70-90%. However, variation in NOM quality did not cause distinguishable differences in Cu-gill accumulation with low-moderate Cu exposure. At high Cu (100-800 $\mu g \cdot L^{-1}$), NOM quality influenced Cu-gill binding with terrigenous Luther Marsh and sewage effluent NOM reducing Cu-gill accumulation by an additional 40-70% compared to more autochthonous Bannister Lake NOM. We suggest that variation in NOM quality only has distinguishable effects at high Cu concentrations. Differences in Cu toxicity at lower Cu concentrations do not appear to be related to differences in Cu-gill accumulation.

Nickel contamination assessment and dynamics in the biomonitor *Chaoborus.*

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We set out to determine if the phantom midge *Chaoborus* can be used to monitor nickel (Ni) contamination in lakewater. To achieve this goal we collected *Chaoborus* larvae and water samples from 15 lakes in the mining regions of Sudbury, ON, and Rouyn-Noranda, QC. *Chaoborus* [Ni] were highly correlated with concentrations of the free Ni ion in lakewater if competitive interactions with hydrogen ions (in acidic lakes) were taken into account. Furthermore, we were able to reproduce this competitive effect in the laboratory. To better understand the biodynamics of Ni in this insect we created a laboratory food chain using the green alga *Pseudokirchneriella subcapitata*, the cladoceran *Daphnia magna* and the phantom midge *Chaoborus flavicans*. We found that Ni uptake by *Chaoborus* is mainly from water (2/3) but that some Ni also enters via its

prey (1/3). The lack of importance of food as a Ni source is due in part to the fact that Ni assimilation (~12%) from food is inefficient and is not dependent of the proportion of Ni localised in prey proteins. We used our measurements of Ni uptake and loss by *Chaoborus* to estimate and explain its [Ni] in various lakes. Our results suggest that *Chaoborus* larvae would be a useful component of risk assessment strategies designed to evaluate the Ni exposure of lake-dwelling organisms.

Dietary and waterborne toxicity of Cd and Cu to to *Hydra Attenuata*.

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The objectives of our studies have been to understand the sublethal effects of Cu and Cd on *Hydra attenuata*, to develop a biotic ligand based prediction model for the effect of water chemistry on toxicity and to understand the relative importance of waterborne and dietary exposure routes. Effects were assessed in standardized 96-h tests using reproduction (budding), morphology (tentacle regression and clubbing) or lethality as endpoints. Hydra cultures were established in reconstituted soft water (Ca of 170, Mg of 140, Na of 150, Cl and K of 30 (all units in $\mu mol \cdot L^{-1}$) and a pH of 7.0) and water chemistry manipulations included adjustments in Ca, Na, Mg and Cl as well as natural organic matter. In tests with Cd, efforts were made to understand the influence of solution pH on toxicity, but Hydra proved to be very sensitive to alterations of the pH. Clubbed tentacles in Hydra proved to be a very sensitive endpoint, EC20 values in culture water were 3.8 $\mu g \cdot L^{-1}$ (60 nM). In datasets where there was a significant reduction in toxicity due to an altered water chemistry parameter, binding constants were calculated and used to develop BLM constants. Results for Cu exposures are compared to Hydra responses to Cd exposure where cationic competition with Ca was the only mitigating influence on acute responses and complexation by organic matter did not appear to reduce toxicity. As well, in tests with Hydra fed on Cd contaminated *Daphnia pulex*, the dietary exposure route was shown to be less sensitive than waterborne. Funding for this project was provided by a NSERC Strategic Grant and Rio Tinto Alcan.

Modeling cadmium bioaccumulation and toxicity in the freshwater amphipod *Hyalella azteca* from a natural periphyton diet: implications for water quality guidelines.

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Diet can be an important source of metal exposure for aquatic invertebrates though current water quality guidelines do not directly account for this exposure route. In this research we aimed to develop a bioaccumulation model to predict the relative contributions of cadmium (Cd) from water and a natural periphyton diet in the metal sensitive freshwater amphipod *Hyalella azteca* and link this to toxic effects. We conducted 28-d experiments with juvenile *Hyalella* consisting of Cd in water and periphyton separately and combined over a full response range of concentrations. Survival, growth and Cd bioaccumulation in *Hyalella* were recorded. Non-linear

regression modeling predicted that Cd in a periphyton diet contributed an average of 40 – 55% of the total bioaccumulated Cd in *Hyalella*. Cadmium in periphyton and water were estimated to contribute approximately equally to lethality emphasizing the important role that diet plays in predicting aquatic toxicity. Results of applying this model to the field and the implications for water quality guidelines will also be discussed.

Development of the Metal Effects Addition Model (M.E.A.M.).

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The current Canadian Water Quality Guidelines for the Protection of Aquatic Life (CCME, 1999) contain individual numerical limits for environmental stressors, which were developed to protect all forms of aquatic life and all aspects of the aquatic life cycles. However, the guidelines do not provide limits for mixtures of environmental stressors. The Environmental Protection Service of Environment Canada does outline the Toxic Units or Concentrations Addition method for the prediction of mixture toxicity. This method is based on the assumption that the toxicity of individual chemicals is additive (i.e., the mode of action for each chemical is similar). Another method, effects addition or response addition, is based on the assumption that the toxicities of individual chemicals are independent and hence the predicted response by the organism to each individual chemical in the mixture can be added to predict the response to the total mixture. The development of the model for the aquatic invertebrate *Hyalella azteca* will be demonstrated, from individual metal bioaccumulation to effects models through to the combined metal mixture bioaccumulation and effects model (M.E.A.M.). The M.E.A.M. will be compared to the Toxic Units approach in the evaluation of a toxicity test conducted with a full concentration series of a 10-metal mixture.

Waterborne and dietborne metals: Can the relative importance of each be determined under field conditions?

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Tightly controlled laboratory studies have demonstrated that both water and diet are important sources of metals such as cadmium to filter-feeding organisms. Application of two-compartment kinetic models also indicate the importance of both as routes of metal uptake. However, trying to discern the relative importance of each as a source of metal under field conditions continues to be a challenge. Field studies employing stable isotopes have demonstrated that for filter-feeding bivalves such as the Pacific oyster, recently deposited and suspended sediments are not important diet components and hence not important sources of cadmium to the organism. Other studies have demonstrated that different size fractions mechanically filtered from the water are poor predictors of bivalve cadmium concentrations. These findings could lead one to conclude that under field conditions diet is not an important source of metal to these organisms. However, like the mussel, the oyster is highly selective in its feeding, filtering organic rich particles from the water column. Our recent studies have used gut contents to assess if this is a better

predictor of cadmium exposure to the oyster via the diet. We also applied stable isotope analysis, $\delta^{13}C$ and $\delta^{15}N$, to determine the origin of ingested organic material (from terrestrial to oceanic). Under field conditions diet could account for at least 40% of observed oyster cadmium residues but was region dependent. Oysters from the most marine influenced sites contained gut and tissue cadmium residues of 30.4 ± 3.08 (S.E.) $\mu g \cdot g^{-1}$ and 6.0 ± 0.6 $\mu g \cdot g^{-1}$, respectively, and a stable isotopic signature typical of marine phytoplankton. In contrast, oysters sampled from regions influenced by coastal processes contained significantly greater concentrations of cadmium, 43.0 ± 2.4 $\mu g \cdot g^{-1}$ and 10.2 ± 0.68 $\mu g \cdot g^{-1}$, gut and tissue respectively, with isotopic signatures representative of terrestrial organic matter. Presumably, the remaining 60% variation in oyster cadmium concentrations was uptake via waterborne sources; however, this route could also be region-dependent, being more or less important depending on prevailing physico-chemical conditions. Field studies where uptake via food and water is contrasted across a number of differing physico-chemical conditions as well as different food sources need to be implemented such that we have a better understanding of how the relative importance of the two routes of uptake vary in nature. Parallel controlled laboratory experiments that better reflect actual field situations would also help determine the relative importance of each to cadmium concentrations in bivalves under natural conditions.

Application of metal bioavailability and impact models to predict biological recovery of metal contaminated lakes in the Sudbury Area.

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Lakes surrounding the metal mining and smelting sites in Sudbury, Ontario have been enriched in copper, zinc and nickel, and acidified by sulphuric compounds associated with industrial emissions. Contaminant loading peaked in the 1960s after which large emission reductions occurred. Over the last three decades an intensive monitoring program has collected chemical and biological data from a number of the affected lakes around Sudbury and improvements in water chemistry have resulted in biological recovery. Using existing chemical data for both contaminated lakes and reference lakes from the Sudbury area, a biotic ligand model (BLM) based approach was used to assess metal bioavailability and predict impacts as estimates of biological recovery. Acute BLMs for Cu, Ni and Zn and application of acute/chronic ratios were used to calculate the overall toxicity of the water using the toxic units (TU) approach, thus the contribution of each metal to the overall toxicity of the water was determined through time for each lake. TU significantly correlated to indices of zooplankton recovery in the contaminated lakes ($p < 1.0$); the zooplankton community is approaching, but has not achieved, the status representative of lakes unaffected by acidification or metal impaction.

Restoration of *Daphnia* species in metal stressed lakes: do digestive alterations contribute to their lack of success?

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The metal concentrations in Sudbury lakes have declined over the past thirty years because of reduced local emissions; however, ecological recovery in the lakes has been slow and incomplete. Daphniids, a group of metal sensitive microcrustaceans, are lacking in many lakes. Almost no assessments of metal toxicity exist for Daphniid species native to Canadian Shield Lakes. To determine if the current copper and nickel concentrations in the Sudbury lakes are affecting daphniid recovery, five lakes ranging in metal contamination were selected to test the performance of four species of native soft water Daphniids collected from reference lakes on the Canadian Shield. Two groups of fourteen day static with renewal bioassays were performed: one adding Cu and Ni mixes reflecting the five Sudbury lakes to lake water from a reference lake (Blue Chalk Lake), and the other with water from the actual five lakes. Two of the lakes selected were limed in the past, and now receive salt runoff from winter highway maintenance. Digestive track alterations were found in the Daphniids: lack of digestion of the ingested algae and/or lack of food intake. The laboratory bioassays showed that the presence of sodium and calcium diminished the alterations in the digestive tracts of the Daphniids at metal concentrations that were otherwise lethal. The findings suggest that sublethal effects are operating at the digestive tract level, impairing the Daphniids' ability to cope with physiological demands and environmental stress, and that those effects are diminished by sodium and calcium levels seen in nature.

Mercury in Lake St. Francis Walleye: the roles of historical contamination, mercury bioavailability and food chain transfer.

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Due to past industrial inputs of mercury, Lake St. Francis was designated as an Area of Concern under the International Joint Commission's Great Lakes water quality program. Although direct industrial inputs of mercury to the lake have been eliminated since 1995, mercury concentrations are still above guidelines in both sediments and walleye. The Ontario Ministry of the Environment has issued advisories about the consumption of walleye from Lake St. Francis because they contain almost twice the concentration of mercury as walleye found upstream. The goal of this study is to determine if the high concentrations of mercury in walleye are due to transfer from contaminated sediments, from past industrial inputs, or other uncontrolled sources. Sediment, pore water, amphipods and yellow perch were collected from 27 sites around Lake St. Francis to describe the geographical distribution of mercury and methyl mercury as an indicator of potential sources, and to assess the pathway and extent of mercury biomagnification. Preliminary results indicate that sediment mercury concentrations are highest in the area just downstream of the previous industrial inputs and the City of Cornwall. Due to prior relationships found among mercury concentrations in sediment, amphipods and yellow perch, areas with the highest sediment mercury concentrations are areas where we expect to find biota with the highest mercury concentrations.

Has mercury biomagnification in large lake fish food webs changed since the early 20th century?

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Many aquatic food webs have been changing throughout the last century due to the impacts of exotic species, fisheries practices, and other disturbances. Mercury (Hg), a neurotoxic chemical, bioaccumulates in food webs, and its distribution in aquatic ecosystems is partly determined by the structure of those webs. Therefore, it is hypothesized that structural changes in fish food webs will affect the Hg burden of top trophic level fish. However, data related to historical pre-disturbance food webs and Hg contamination prior to the 1970s are extremely limited. In this study, we are investigating the utility of archived museum ichthyology collections as a means to extend our databases to the early 20th century by analyzing Hg, stable N and C isotopes. To account for the effects of chemical preservatives, we have conducted 1-year preservative experiments with periodic measurement of Hg and isotope values of fish tissue. Our results show that preservatives cause an increase of ~0.6 ‰ in $\delta^{15}N$ and 0.5 $mg \cdot kg^{-1}$ in Hg concentrations in the first few weeks of preservation; these changes stabilize over time. Our food web analyses of Lakes Nipigon, Simcoe, and Champlain reveal some major changes in food web structure since the 1920s, and we are now assessing possible shifts in Hg food web transfer. The results of this research will enhance our knowledge of historical contamination levels and biomagnification trends as we better seek to mitigate the impacts of bioaccumulating contaminants today.

Effect of selenium on early life-stage development of westslope cutthroat trout.

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As part of on-going efforts by the Elk Valley Selenium Task Force to characterize benchmarks for selenium that are protective of environmental quality, the effect of egg selenium burden on early life-stage development of westslope cutthroat trout was investigated for fish from the Elk Valley, BC. The investigation was designed to provide data to derive a Tissue Residue Guideline for selenium for this species and region, and to resolve apparent differences in results from prior studies. Eggs collected from adult fish from lentic and lotic habitats were reared to 28-days beyond swim-up to establish whether habitat type resulted in differences in expression of selenium-related effects, including larval survival, rates of deformities and growth. Speciation of selenium in eggs from lentic and lotic environments was measured, and effects on study results assessed. The study demonstrated that larval mortality was the primary adverse effect of elevated selenium, with deformities generally limited to surviving fry from egg batches that also elicited significant mortalities. A sharp dose-response curve was obtained, with no evidence of differing sensitivities for eggs obtained from fish from lentic and lotic habitats. The EC10, EC20 and EC50 values for larval survival were 19.0, 22.8 and 29.9

$\mu g \cdot g^{-1}$ Se (egg, dry weight). Speciation of selenium was shown to be similar in eggs from lentic and lotic sites, although analytical complications prevented an assessment of the relative concentrations of individual seleno-amino acids present.

Nickel and thallium accumulation and toxicity in fathead minnows (*Pimephales promelas*): influence of the route of exposure and prey type.

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In order to assess the ecological risk of metals in aquatic ecosystems, one must characterize their source, their behaviour and their potential toxicity. Our project aimed at contributing to a better understanding of nickel (Ni) and thallium (Tl) accumulation and toxicity in fish, final consumers of aquatic food webs, using environmentally realistic laboratory conditions and fathead minnows (*Pimephales promelas*) as a model species. In fish, early-life stages generally show a greater sensitivity to metals. Thus, we evaluated the accumulation and toxicity of Ni and Tl in embryos and larvae. The chorion partially protected the embryos from Ni, but not Tl, accumulation. Exposure to the highest aqueous Ni concentration decreased time-to-hatch and increased biosynthetic capacities, suggesting that aqueous Ni exposure stimulates metabolism in early-life stages. To our knowledge, no previous study examined the relative contribution of water and prey as Ni and Tl sources in fish. We addressed this question for larvae and juveniles. Water was the major source of metal for larvae whereas water and prey contributed almost equally to metal accumulation in juveniles. Because fish take up metals from prey, it is important to measure factors controlling the trophic transfer of metals. Juveniles assimilated dietary Tl more efficiently than Ni, regardless of prey type, and the proportion of metal bound to subcellular fractions considered trophically available in prey had no significant influence on the efficiency with which fish assimilated Ni or Tl. We measured a significant effect of prey type on the activities of two enzymes and toxic effects of Tl on two others. In the context of ecological risk assessment, our results show the importance of considering the contribution of prey as a source of metal to fish and also suggest that it is important to examine each life stage and to consider the influence of prey type on the endpoints selected.

The use of field-based mesocosms to assess uranium mill effluent exposure pathways to fathead minnow (*Pimephales promelas*).

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Metal mining and milling effluents have been shown to cause increased levels of metals in the aquatic environment. Using a field-based mesocosm system at Key Lake Mine, SK, Canada, a 21-day fathead minnow (*Pimephales promelas*) reproduction assay was used to evaluate the interactive effects of treated milling effluent and contaminated sediment. Fish were allocated to one of four treatments: 1) reference water/reference sediment 2) reference water/contaminated sediment 3) 25% effluent/contaminated sediment 4) 25% effluent/reference sediment. Trios of fathead minnow (1M:2F) in eight

replicate streams per treatment were used to evaluate survival (adult and 5 day larval), larval deformities, reproductive effects (egg production, spawning events) as well as metal tissue burdens (muscle, gonad, eggs and larvae) in female fathead minnow. Egg production significantly increased in the effluent treatments compared to the reference water treatments by approximately 40%. Effects were solely effluent-mediated with no contribution observed due to the presence of contaminated sediments. For the effluent treatments, although egg production increased, fewer eggs hatched, fewer larvae survived and there was a significant increase in skeletal deformities in 5 day old larvae. Total selenium significantly increased in the 25% effluent, algae, female muscle, gonad, eggs and larvae. Findings indicate that the biofilm/algae component appears to be key in the transfer of available Se into the system and a source of dietary exposure in fish and possibly invertebrates.

Critères et normes pour l'eau et les sédiments
Water and sediment standards and criteria

Federal environmental quality guidelines developed for the Chemicals Management Plan for the protection of aquatic life.

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Environment Canada is developing Federal Environmental Quality Guidelines (FEQGs) for use in risk assessment and risk management of substances evaluated under the Chemicals Management Plan. These FEQGs are developed in a manner consistent with national protocols published by the Canadian Council of Ministers of the Environment (CCME) but with added low, moderate, and higher risk categories to aid in the interpretation of monitoring data and in the development of risk management strategies. These federal guidelines may be tabled with the CCME for consideration as national values. Case studies e.g. chlorinated paraffins, will examine similarities and differences with exsiting national and federal approaches.

Evaluation of exceedance of water quality based limits for the effluents of 37 pulp and paper mills in Québec.

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Through its Industrial Waste Reduction Program, the Québec Ministry of Sustainable Development, Environment and Parks has evaluated the impact of 37 pulp and paper mill effluents on their receiving waters. A preliminary sampling of 103 parameters has been used to select a group of 40 parameters with the potential of adverse effects on aquatic life or other water uses. Water quality based effluent limits were developed for each parameter selected for a more detailed sampling at each effluent. In order to verify the compliance with these limits, low detection limits were used for the analysis of most parameters. Following the quality assessment of the data, the results for

formaldehyde, silver and mercury were discarded. Other data were also discarded on a case by case basis. On average 3.7 parameters exceeded the water quality based limits at each mill. The exceedance was from low to high for PCBs, dioxins and furans and phosphorus, and from low to moderate for hydrogen sulfide, suspended solids, copper and zinc. Thirteen other parameters including chronic toxicity bioassay for a fish and an alga showed a low or uncertain exceedance. Based on these results and the best available technology, additional requirements were set in the renewable permit (depollution attestation) of the concerned mills.

Development of a water quality objective for chloride at the EKATI Diamond Mine.

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[1]Nautilus Environmental, [2]Rescan Environmental Services.

Acute and chronic toxicity tests were conducted using nine freshwater species to evaluate the sensitivity of these organisms to chloride as part of efforts to develop a site-specific water quality objective for chloride at the EKATI Diamond Mine. Acute-to-chronic ratios (ACRs) from these tests indicate that the ACR of 7.59 employed by the USEPA in deriving the 1988 water quality guideline for chloride of 230 $mg \cdot L^{-1}$ may be overly conservative; a revised ACR of 3.63 was calculated on the basis of the new and existing data. Data from chronic toxicity tests presented here, as well as by other investigators, were used to calculate a water quality objective for long-term exposure to chloride using a species sensitivity distribution approach. The 5th percentile from the distribution was calculated as 325 $mg \cdot L^{-1}$ chloride and proposed as the water quality objective. Cladocerans were the most sensitive species to chloride in the dataset, and one cladoceran, *Ceriodaphnia dubia*, was used to evaluate the relationship between water hardness and sensitivity to chloride. A strong relationship was observed and was used to establish a hardness-related equation to modify the water quality objective on the basis of water hardness, resulting in proposed objectives ranging from 67 $mg \cdot L^{-1}$ chloride at 10 $mg \cdot L^{-1}$ hardness to 411 $mg \cdot L^{-1}$ chloride at 160 $mg \cdot L^{-1}$ hardness (as $CaCO_3$).

What's a nice metal like you doing with a guideline like that? A review of molybdenum water quality standards.

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Abstract

Molybdenum water quality guidelines in several jurisdictions were significantly influenced by very low values reported in early studies; values which have not been replicated in more recent studies. In 2008, after an extensive review of data now available, the State of Nevada revised its Molybdenum Aquatic Life Water Quality Standard from 19 $\mu g \cdot L^{-1}$ to an Acute Criterion of 6.16 $mg \cdot L^{-1}$ and a Final Chronic Criterion for Molybdenum of 1.65 $mg \cdot L^{-1}$. Short term toxicity data for molybdenum were used to derive a Type A – Short Term Exposure Guideline under CCME Protocols. The calculated Short Term Exposure Guideline for protection of aquatic life (5th percentile of the cumulative species sensitivity distribution), was 92.9 $mg \cdot L^{-1}$ with 95% confidence

limits of 76.5 and 110 $mg \cdot L^{-1}$. Molybdenum chronic toxicity data were insufficient for a SSD approach. The Nevada review of molybdenum toxicity calculated a geometric mean of 7.5 as the acute to chronic ratio for molybdenum, a value only slightly below the 'safety factor' of 0.1, used in the 1999 CCME guideline for molybdenum. Accordingly, multiplication of the short term exposure guideline of 92.9 by a factor of 0.1 results in a long term exposure guideline value of 9.3 $mg \cdot L^{-1}$.

Introduction

Molybdenum (Mo) is a naturally occurring Group 6 element with the atomic number 42. Like other transition metals, molybdenum has several common oxidation states (+2 to +6). Although most molybdenum compounds have low water solubility, Mo(+6) will form the soluble molybdate ion MoO_2^{-4} if Mo-containing minerals are in contact with free oxygen and water (Eisler 1989). Environmental sources of molybdenum include natural deposits and mining, and combustion of fossil fuels. This element is a trace constituent of coal and because of the high temperature of combustion, molybdenum in flyash is largely in the hexavalent molybdate form. In the Great Lakes, total molybdenum concentrations generally do not exceed 2 $\mu g \cdot L^{-1}$, but levels as high as 500 $\mu g \cdot L^{-1}$ (0.5 $mg \cdot L^{-1}$) have been reported in inland waterways downstream of mining discharges (Fletcher et al. 1998).

Molybdenum is an essential micronutrient, present in more than 20 enzymes. However, high concentrations of molybdenum can interfere with Cu homeostasis producing Cu deficiency (Eisler 1989). Molybdenum is of relatively low toxicity when compared with other industrial metals and the present Canadian Water Quality Guideline value of 73 $\mu g \cdot L^{-1}$ is appreciably greater than the values for cadmium and arsenic (0.017,and 5 $\mu g \cdot L^{-1}$) but considerably lower than the value for iron (300 $\mu g \cdot L^{-1}$).

This paper reviews relevant data on molybdenum toxicity to freshwater aquatic life, including reviews of peer-reviewed scientific literature, searches of on-line toxicology databases such as EPA's ECOTOX and contacts with other researchers, with particular emphasis on more recent data. The acute and chronic toxicity data obtained were then compiled and the acute toxicity values analysed using methods outlines in the CCME Protocols (CCME 2007).

Chronology and Rationale of History of Molybdenum Water Quality Guidelines

Molybdenum water quality criteria or guidelines have been determined for several jurisdictions in Canada and the USA since the mid 1980's and include. A chronological listing of the guidelines and their derivation follows.

1986 - British Columbia - Water Quality Criteria – 30 d average < 1 mg·L-1, max 2 mg·L-1. Scientific information used in deriving the guideline was not documented.

1994 Ontario - Provincial Water Quality Objective – 40 µg·L-1 (Interim). Lowest measured adverse effect value, the chronic toxicity value of 0.730 mg·L-1 for embryo/larvae of rainbow trout (Birge 1978) divided by a final uncertainty factor of 16.

1999 CCME - Canadian Water Quality Guidelines for the Protection of Aquatic Life - 73 µg·L-1. Lowest chronic toxicity value (28-d LC50 of 0.73 mg·L-1 for rainbow trout - Birge 1978), multiplied by a safety factor of 0.1.

2006 - State of Nevada molybdenum water quality standard – 19 µg·L-1. Value was determined as the geometric mean of Adverse Effect Level (519 µg·L-1 - geometric mean of toxicity values for rainbow trout (120 µg·L-1 – Birge 1980); narrow mouthed toad (960 µg·L-1 – Birge 1978) and the NOEC for Daphnia magna (1150 µg·L-1 – Kimball 1978) and US national ambient background concentration of 0.68 µg·L-1.

2008 - State of Nevada Revision of Molybdenum Aquatic Life Water Quality Standard – Acute Criterion - 6.16 mg Mo·L-1; Final Chronic Criterion - 1.65 mg Mo·L-1. Final Acute Value (FAV) was determined from the geometric mean of the Genus Mean Acute Values for the 4 most sensitive species (Ceriodaphnia, fathead minnows, Euglena and Tubifex). The Criteria Maximum Concentration (CMC) was then calculated by dividing the FAV by 2 and the Final Chronic Value or Criterion for Continuous Concentration was calculated by dividing the CMC by the acute to chronic ratio of 7.5.

Acute toxicity

Species mean acute values (SMAV) for molybdenum range from 29 to > 10,000 $mg \cdot L^{-1}$ (Table 1). These values were drawn from a recent comprehensive review of aquatic life water quality criteria for molybdenum in Nevada (Tetra Tech 2008) which compiled acute toxicity data for 20 freshwater aquatic species, with data for 17 species deemed suitable[1] for establishing molybdenum water quality criteria. These values are conservative, as the LC_{50} was greater than the highest concentration tested for 5 of the 17 species listed. All species used in the Nevada review of aquatic water quality criteria for molybdenum also occur in Ontario. In addition, the OMOE toxicology lab is presently conducting molybdenum toxicity tests of arctic charr, lake trout, lake whitefish, round whitefish, arctic grayling and rainbow trout. Initial acute values are greater than 3,000 $mg \cdot L^{-1}$ (D. Poirier – pers. com).

Chronic toxicity

Mean chronic molybdenum concentrations of several species for which acceptable chronic aquatic toxicity studies are available are summarized in Table 2. The growth and development of egg and larval fish was affected by chronic molybdenum concentrations as low as 164 $mg \cdot L^{-1}$, while reproduction of zooplankton was affected by chronic molybdenum concentrations as low as 60 $mg \cdot L^{-1}$ and growth of algal growth was affected by concentrations as low as 15 $mg \cdot L^{-1}$. In reviewing molybdenum toxicity data for the State of Nevada, Tetra Tech (2008) determined that sufficient acute and chronic toxicity data were available for five species, two cladoceran zooplankton, *Daphnia magna* and

[1] Studies deemed unsuitable for establishing criteria were rejected for one or more of the following reasons:
1. Performance of control organisms was not documented and no confidence intervals were provided
2. Control mortality was greater than 10%
3. No effect at a Mo concentration too low to be toxicologically relevant

Ceriodaphnia dubia, and three fish species, fathead minnow, white sucker and rainbow trout. The calculated acute to chronic ratios (ACR) ranged from 2.6 to 22.9 for each species, with a final geometric mean ACR of 7.5 for molybdenum.

Nevada Water Quality standard. These early studies of molybdenum toxicity to rainbow trout and *Daphnia magna* were not used because of the consistent discrepancy between the values reported in these studies and those obtained by later studies. Specifically, Davies et al. (2005) were unable to replicate Birge's 1978 and 1980 results with rainbow trout, and both Diamantino et al. (2000) and Caton et al. (2007) obtained EC_{20} and IC_{25} values for *Daphnia magna* that were more than an order of magnitude greater than those reported by Kinbal (1978).

Application of CCME Protocols to derive suggested Molybdenum Water Quality Guidelines

The methods outlined in the CCME Protocol for the Derivation of Water Quality Guidelines for the Protection of Aquatic Life (CCME 2007) were then applied to derive water quality guidelines for molybdenum. As the species mean acute toxicity values for molybdenum summarized in Table 1 met the CCME criteria for acute toxicity data, they were compiled to generate a cumulative Species Sensitivity Distribution (SSD) plot (Figure 1). The resulting curve was analyzed statistically, with the best fit for the data was obtained using a saturated growth model, in which the percent of species affected is described in terms of SMAV by the equation

Percent affected = 124.03*SMAV/ (2347.0+SMAV)

As recommended by the CCME, the Type A – Short Term Exposure Guideline value for protection of aquatic life was estimated as the 5^{th} percentile of the SSD. For molybdenum, the 5th percentile of the SSD was 92.9 $mg \cdot L^{-1}$ with 95% confidence limits (CL) of 76.5 and 110 $mg \cdot L^{-1}$.

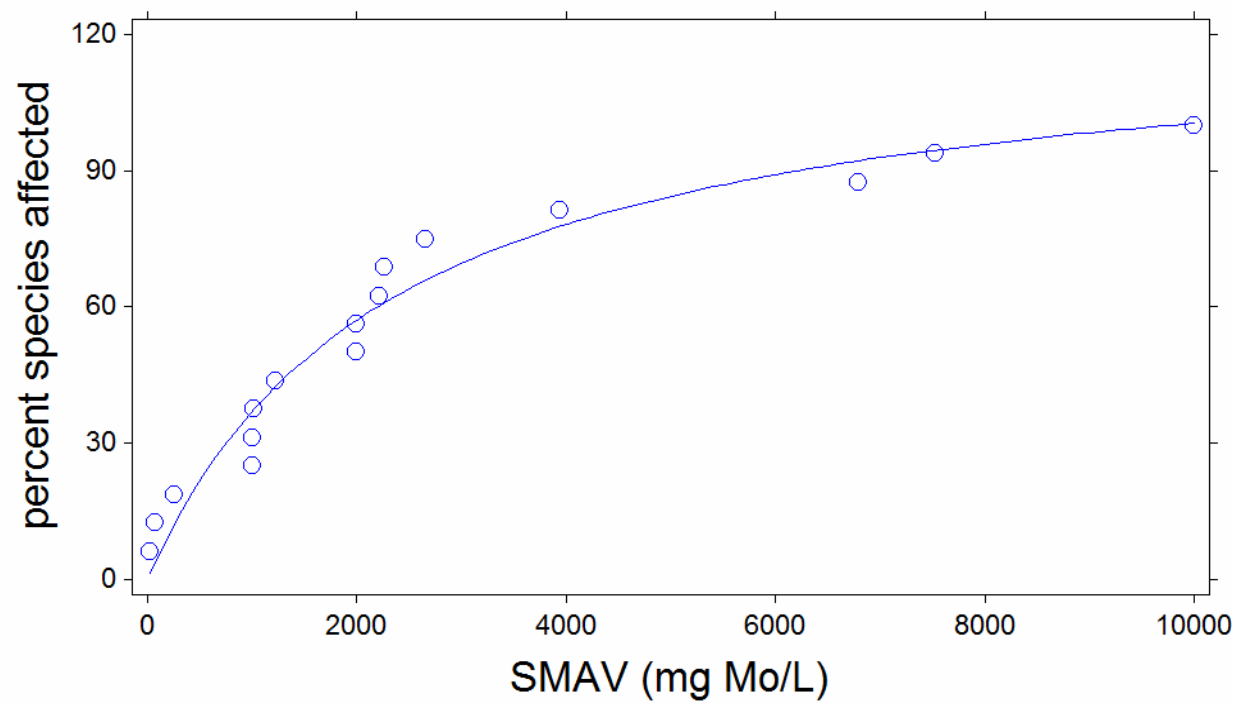
It is particularly noteworthy that the papers of Birge (1978, 1980) and Kimbal (1978) which have largely determined Ontario, CCME and pre-revision Nevada molybdenum water quality guidelines were not included in the data used in the present review.

Table 1. Summary of Species and Species Mean Acute Values (SMAV) for which acceptable aquatic molybdenum toxicity studies are available.

Species	SMAV	References and notes
Ictalurus punctatus	>10,000	< 50% mortality up to value (Bionomics 1973)
Chironomus tentans	7,533	LC_{50} (Canton et. al. 2007)
Lepomis macrochirus	6,790	96 h TL_{50}[a] (Bionomics 1973)
Gammarus fasciatus	3,940	48 hr TL_{50} (Bionomics 1973)
Crangonyx pseudogracilis	2,650	LC_{50} (Martin and Holmic. 1986)
Oncorhynchus mykiss	2,269	4,050, 6,970 and 7,340 LC_{50} at 148, 154 and 290 mg $CaCO_3 \cdot L^{-1}$ (Bionomics 1973); 800 & 1,320 LC_{50} for 25 & 55 mm fish; < 50% mortality of larval and juvenile fish up to 1,000 and 1,190 $mg \cdot L^{-1}$ (Pyle 2000)
Daphnia magna	2,218	48 h LC_{50} (Bionomics 1973; Diamantino et. al. 2000)
Oncorhynchus nerka	> 2,000	< 50% mortality up to value (Reid 2002)
Catostomus commersoni	> 2,000	< 50% mortality up to value (Pyle 2000)
Girardia dorotocephala	1,226	LC_{50} (Canton et. al. 2007)
Ceriodaphnia dubia	1,015	LC_{50} (Canton et. al. 2007)
Oncorhynchus kisutch	> 1,000	< 50% mortality up to value (Hamilton and Buhl. 1990)
Oncorhynchus tshawytscha	> 1,000	< 50% mortality up to value (Hamilton and Buhl. 1990)
Pimephales promelas	254	96 h LC_{50} (Bionomics 1973; Pyle 2000)
Euglena gracilis	72.3	Geo.mean of control and concentration which completely stops growth (5.44 & 960 $mg \cdot L^{-1}$) (Colmano 1973)
Tubifex tubifex	28.9	96 h EC_{50} (immobilization) (Khangarot 1991)

[a]TL_{50} is the median tolerance limit which represents the concentration at which 50% of the test organisms survive and is essentially equivalent to LC_{50}.

Figure 1: Cumulative distribution of the revised molybdenum Species Mean Acute Values.



Although acute toxicity data were sufficient for a SSD analysis to derive a Short Term Exposure Guideline, the molybdenum chronic data were insufficient for this approach. Alternative approaches to estimating chronic guidelines and the values obtained include the following:

- Lower 95% CL of the 5th percentile of the SSD function - 76.5 mg Mo·L^{-1}
- Short Term Exposure Guideline divided by Acute to Chronic Ratio – 12.4 mg Mo·L^{-1}
- Short Term Exposure Guideline divided by Safety Factor of 10 – 9.3 mg Mo·L^{-1}

Bioaccumulation and field studies

Like many other metals which are essential micronutrients, algae readily take up molybdenum from solution. Under experimental conditions, Sakaguchi et al. (1981) observed that various species of green algae accumulated molybdenum to concentration ratios between 756 and 2,321 (concentration in algae/concentration in solution), with higher uptake under various other atypical environmental conditions (e.g., high temperature). However in field studies, lower concentration ratios were generally observed at higher trophic levels. Robertson and Liber (2007) reported molybdenum

bioaccumulation and biota-sediment accumulation factors of 2.7 to 24.3, respectively in *H. azteca* caged in Saskatchewan streams receiving uranium mine and mill effluents. Similarly in a study of the San Joaquin River, California, Saiki et al. (1993) determined that molybdenum accumulated in tissues at all levels of the foodweb, but did not increase with trophic level, and that tissue in invertebrates and fish and were normally lower than in algae.

Overall, these studies indicate that molybdenum does not appear to biomagnify significantly, suggesting that biota can regulate non-toxic molybdenum tissue burdens. Consequently, it does not appear that molybdenum presents additional toxic hazards, which are not accounted for by the criteria based on aquatic toxicological studies. This conclusion is consistent with the two available field studies in which the effects of elevated molybdenum on aquatic life in receiving systems were evaluated. In their studies of Saskatchewan streams receiving uranium mine and mill effluents, Robertson and Liber determined that molybdenum concentrations as high as 4.95 $mg \cdot L^{-1}$ in the surface water and 8.25 $mg \cdot L^{-1}$ in the pore water were not related to observed reductions in the survival of *H. azteca*[28]. Similarly, in an evaluation of the impacts of discharges from a molybdenum mill and mine on benthic macroinvertebrate communities of receiving streams in British Columbia, Whiting et al. (1994) observed no impacts to the benthic macroinvertebrate communities at any of these sites relative to conditions in reference sites. Among these sites was one with mean and maximum molybdenum concentrations of 24.8 $mg \cdot L^{-1}$ and 32.5 $mg \cdot L^{-1}$, respectively.

Table 2. Summary of mean chronic molybdenum concentrations to aquatic species

Species	Mean chronic value ($mg \cdot L^{-1}$)	References and notes
Oncorhynchus mykiss	1,425	EC_{20} of 32 d egg – larval exposure (Davies et al. 2005), unable to replicate Birge (1978) and Birge et al. (1980)
Pimephales promelas	163.5	Geo. mean of duplicate EC_{20} values (Caton et al 2007)
Daphnia magna	97.0	Geo. mean of duplicate EC_{20} reproduction values of Caton et al. (2007) and the Geo. mean of LOEC and NOEC values (Diamantino et al. 2000)
Ceriodaphnia dubia	60.4	Geo. mean of duplicate EC_{20} reproduction values of Caton et al (2007) and reproduction IC_{25} (Naddy et al.1995)
Chlorella regularis	50	Significant 96-h growth effect (Sakaguchi et. al. 1981)
Chlorella vulgaris	15	Lowest concentration significantly affecting growth in 3 month study (Den Dooren De Jong 1965)

Conclusions and Recommendations

In reviewing aquatic toxicity data and the both the Ontario Provincial Interim Water Quality Guideline and CCME water Quality Guideline for molybdenum, it was apparent that these guidelines were significantly influenced by the very low values reported in the early studies – values that have not been replicated in more recent studies. When the CCME Protocol for the Derivation of Water Quality Guidelines for the

Protection of Aquatic Life was applied to the expanded data on the aquatic toxicity of molybdenum now available, the calculated values were more than two orders of magnitude greater than the present Ontario and CCME Guidelines.

Further, molybdenum does not appear to biomagnify significantly, suggesting that biota can regulate non-toxic molybdenum tissue burdens. Consequently, molybdenum does not appear to present additional toxic hazards which are not accounted for by the criteria based on aquatic toxicological studies.

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Derivation of no-effect values for metals in sediment and the influence of benthic community effect criteria on no-effect value derivation.

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Using uranium operations in northern Saskatchewan, Canada as a case study, a series of no-effect (considered "safe" at the site-specific level) and reference ("background") values for metals in sediment were derived based on different types of benthic invertebrate community data (different community metrics). To calculate these values, paired data sets of benthic community composition and bulk sediment total-metal concentrations were obtained from previous monitoring reports. The no-effect datasets were identified based on different groupings of benthic invertebrate community metrics (i.e., different combinations of abundance, richness, evenness, and similarity). Bulk sediment concentrations of As, Cr, Cu, Pb, Mo, Ni, Se, U, and V from each of the datasets evaluated were plotted and a logistic model fit to the data. The no-effect (or reference) value for each metal was interpolated at the 90th centile of the data distribution. It was concluded that no-effect values for Cr, Cu, Ni, Pb, and V did not change with an increase in the number of benthic community metrics used to determine if an effect was observed. With the exception of Ni, the no-effect values for these metals were very similar to derived reference values. For As, Mo, Se and U, the no-effect values generally decreased (became more conservative) with an increase in the number of metrics used to identify an effect on the benthic invertebrate community and were generally higher than the derived reference values. The findings from this study have implications for the redevelopment and/or use of sediment quality guidelines.

Pesticides
Pesticides

Pesticides in surface waters of Ontario.

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Pesticides are heavily used in agricultural production and in urban areas of southern Ontario. Since 2003, the Water Quality Monitoring and Surveillance Division of Environment Canada in Burlington has been sampling a number of surface water sites for a variety of pesticides. This program is part of a national initiative by Environment Canada and has evolved from sampling a standard suite of currently used pesticides such as neutral herbicides, acid herbicides and organophosphorous insecticides to now include pesticides such as sulfonyl urea and related herbicides, carbamate pesticides and methoprene. This has allowed for the development of a surface water pesticide database with over 900 samples collected from over seventy locations in southern Ontario representing pesticide inputs from corn and soybean growing areas, fruit growing areas and areas influenced by urban activities. The most commonly detected pesticides include atrazine, metolachlor, dicamba, mecoprop, 2,4-D and diazinon. Many samples contained multiple detections of pesticides. These results will be discussed in relation to aquatic life guidelines, pesticide use information and implications for analytical methods development.

Pesticides dans l'eau des régions agricoles du Québec.

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Les activités agricoles laissent une empreinte sur l'environnement et particulièrement sur les milieux aquatiques. Environ 79 % des pesticides employés au Québec sont utilisés en agriculture. Leur usage a un impact non négligeable sur la qualité des cours d'eau des régions agricoles. Durant la saison de culture, la plupart des rivières du sud du Québec montrent la présence de plusieurs pesticides. La nature et la complexité de ces mélanges varient en fonction de plusieurs facteurs tels que les précipitations, les conditions propices à l'émergence des ravageurs, l'utilisation des pesticides et le type de cultures présentes dans le bassin versant. Au Québec, le suivi environnemental des pesticides est structuré par type de cultures. Dans les zones à dominance de maïs et de soya, cultures qui couvrent les plus grandes superficies au Québec, les herbicides tels que l'atrazine, le métolachlore, le glyphosate, le bentazone et le dicamba sont les produits détectés le plus souvent. Dans les petits cours d'eau drainant les champs en cultures maraîchères on trouve une grande diversité de pesticides détectés, notamment les herbicides métribuzine et linuron, les insecticides chlorpyrifos, malathion, phosmet, carbaryl et imidacloprid ainsi que le fongicide chlorothalonil. Les petits cours d'eau près de vergers de pommiers, montrent la présence des insecticides azinphos-méthyl, phosmet, cyperméthrine, carbaryl, diazinon et chloprpyrifos ainsi que le fongicide captane. En aval

des fermes de canneberges on trouve la présence de l'insecticide diazinon et des herbicides dichlobénil et napropamide. Finalement l'herbicide hexazinone est détecté dans les rivières drainant des zones de bleuetières

Effect of mesotrione on energy dissipation processes of phytoplankton

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Mesotrione, a relatively new selective herbicide for use in maize, is a member of the benzoylcyclohexane-1,3-dione herbicides and acts as a HPPD (4-hydroxyphenyl pyruvate) competitive inhibitor. HPPD converts tyrosine to plastoquinone (which is an essential cofactor for phytoene desaturase involved in carotenoid biosynthesis) and α-tocopherol. In higher plants, the effects of mesotrione, such as bleaching symptoms are well caracterized, but are less known for phytoplankton. Therefore, our aim was to evaluate the effect of mesotrione on photosynthetic energy dissipation processes and pigment content of phytoplankton. *Chlamydomonas reinhardtii* (green alga) was acclimated to two different light intensities (60 and 600µE/m2/s) and exposed for 7 days to four concentrations of mesotrione. The pigment content (HPLC), the growth rate (cell density) and photosynthetic activity (PAM and PEA fluorometry) were measured. The results showed that the higher concentrations of herbicide used affected growth and pigment content. Moreover, partial inhibition of photosynthetic activity was shown by a decrease in the operational PSII quantum yield without apparent PSII destruction (since no change in maximum PSII quantum yield was observed). We noticed also that photochemical and non-photochemical energy dissipation processes were also affected. Under steady-state electron transport condition, PSII appeared to be highly reduced (UQFrel increased up to 100% at 5000µg·Kg^{-1} of mesotrione compared to control). High light intensity appeared to enhance the observed mesotrione effect seen under low light intensity.

Ecological risk assessment of pesticides using realistic Canadian mixture scenarios.

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The ecological risks posed to aquatic ecosystems by the occurrence of insecticide mixtures (dimethoate, chlorpyrifos, imidacloprid) was investigated under simulated risk scenarios. Acute and chronic exposure tests in the laboratory indicated that dimethoate was much less toxic to *C. tentans* than chlorpyrifos or imidacloprid. Antagonism was observed whenever dimethoate was present in acute exposure scenarios. Chronic exposure to the study insecticides resulted in fewer females emerging from experimental chambers, producing a skewed sex ratio for this species. In addition, we undertook a pilot study in mesocosms to examine the potential for nutrient enrichment to mask the effects of low dose exposure to insecticides. Our preliminary findings indicate that oligotrophic (N:P~1:3) nutrient addition may be sufficient to mask some of the effects of sublethal pesticide exposure (control versus treatment). Additionally, mesotrophic levels of nutrient addition (N:P ~1:1) appeared to boost system productivity further despite

exposure to sublethal concentrations of insecticide. These results suggest a tipping point between mesotrophic and eutrophic habitats relative to the effectiveness of nutrients to mask sublethal exposure to pesticides. More definitive tests are needed to fully elucidate these patterns.

Pesticide runoff from agricultural fields – ensuring exposure and hazard assessment times are appropriate.

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Pesticide runoff from potato fields in Atlantic Canada has been shown to be substantive, and have been the documented cause of fish kills in adjacent watercourses. Monitoring of streams has been conducted over a number of years and a strategy has evolved to measure the highest concentrations during runoff events with the belief that those are the most important times of exposure to aquatic organisms. In some instances, those concentrations have been shown to be well above current Canadian Water Quality Guidelines for the Protection of Aquatic Life, however they are relatively short lived , with elevated concentrations not generally exceeding 6hr from the initial production of runoff. Short-term pulse bioassays ranging from 1h to 10h, were undertaken for a number of high use pesticides to determine how toxic thresholds changes with reduced exposure periods which more closely match those documented in the field. The acute toxicity of the pesticides tested was reduced compared with conventional continuous exposure periods, however those reductions vary substantively and are most likely related to the physical and chemical characteristics of each pesticide. The concentrations measured during runoff events are compared to the short-term toxicity of the pesticides to determine more realistic hazard assessments.

Pesticide use at Expo '67: would Rachel Carson have approved?

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In 1967, the City of Montreal, Québec, hosted the World Exhibition or “Expo ’67,” attended by millions of international visitors. The exposition site was St. Helen’s Island in the St. Lawrence River near downtown Montreal. During the planning stages, event officials quickly identified the millions of emerging shadflies as a nuisance and potential threat to the event success. An insect abatement scheme was developed which included repeated applications of the larvicide DDD directly to the River upstream of St. Helen’s Island, totaling more than 30 000 lbs over a two-year period. Post application shadfly emergence was recorded to have decreased by up to 98%. We compared a sediment core in downstream Lac Saint-Pierre to a core from upstream Lac Saint-François to determine whether a pesticide signal corresponding to the Expo ’67 DDD applications can be detected, and the years in which peak concentrations occurred. Pesticide residues were analyzed by gas chromatography and normalized to total organic carbon to remove dilution effects of sand associated with high sedimentation rates caused by bank erosion and tributary contributions. Similarly, potential distortions in lead-210

dating by bank erosion were assessed by measurements of cesium-137, which peaked in 1963 due to controls on nuclear weapons testing. DDD:DDT ratios will demonstrate whether elevated concentrations of DDD are specific to a spike in DDD, or if they represent degraded DDT, which was once widely applied terrestrially for insect control.

Presence of two common herbicides in the South Nation River watershed and their relation to macrophyte species composition.

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Intensification of agriculture over the last several decades has been characterized by an increase in habitat loss and fragmentation as well as an increase in the use of agrochemicals such as herbicides and fertilizers. Herbicides and fertilizers are used on close to 75% of all farms and are applied to a similar area of land each year in Canada (25,000,910 ha and 24,014,813 ha respectively).

In agricultural watersheds, water bodies such as ditches, streams, wetlands and rivers are intimately associated with croplands and may be inadvertently exposed to agrochemicals, such as herbicides and fertilizers, mainly through run-off and leaching. Macrophytes may potentially be affected by agrochemicals due to their immobility and taxonomic similarity to the intended targets. While the effect of herbicides on macrophyte communities has been poorly established, the effects of excess nutrients have been better characterized. Nutrient loading may result in excess macrophytes growth, leading to the impairment of watercourses. However, nutrient loading may also lead to increased algal growth that may eventually shade out macrophytes and lead to a decline in macrophytes diversity, particularly for submerged species. The objective of this study was to characterize the presence of two common herbicides in an agricultural watershed in relation to the macrophytes community structure at sites with differing levels of agricultural impact.

This study was conducted in the South Nation River watershed located in Eastern Ontario. The river is 177 km long and the watershed comprises 3919 km^2 of land. The watershed is predominately agricultural (57% of land use) with crops of corn, soybean and wheat most commonly grown. Twenty-six sites located throughout the watershed were selected due to their varying levels of agricultural impact. Sites were paired along tributaries with sites with low levels of agricultural impact located upstream of sites with high levels of agricultural impact. Overall, sites were characterized as having high in-stream concentrations of nutrients, particularly for nitrate. Average springtime phosphate concentrations ranged from 0.002 to 0.050 $mg \cdot L^{-1}$ while nitrate concentrations ranged from 0.02 to 6.66 $mg \cdot L^{-1}$ (average of 0.02 and 2.02 $mg \cdot L^{-1}$ respectively).

A total of 216 plant species were identified along stream banks and within the channels. The number of species per site ranged from 31 to 86. Sites with higher levels of agricultural impact tended to have slightly fewer species with a higher percentage of non-native species. A number of species common to sites with low levels of agricultural impact, such as *Potamogeton natans*, *Ludwigia palustris* and *Eupatorium maculatum*, were uncommon at sites with high levels of agricultural impact.

Glyphosate in-stream concentrations (taken from two sampling events in June 2008) were low, ranging from 0.10 to 0.50 $\mu g \cdot L^{-1}$ with an average of 0.21 $\mu g \cdot L^{-1}$. Variation between replicates was often high and no difference in glyphosate concentrations was observed between sites with different levels of agricultural impact. In contrast to glyphosate, atrazine in-stream concentrations were slightly higher. Concentrations ranged from 0.05 to 1.6 $\mu g \cdot L^{-1}$ with an average concentration of 0.37 $\mu g \cdot L^{-1}$. Atrazine concentrations were higher at sites with high levels of agricultural impact.

Principal coordinates analysis (PCoA) was used to characterize the 26 sites in terms of their macrophyte species composition. Axis 1 and 2 represented 17.6 and 12.8% of the variation in species composition respectively. Stepwise multiple regression was then used to determine if any measured physical and chemical parameters explained differences in species composition between sites. Variation in species composition along Axis 1 was explained by bank slope, stream width and in-stream pH whereas variation in Axis 2 was explained by nitrate, phosphate and stream depth. Although no direct relationship was observed between herbicide concentrations and species composition, atrazine and nitrate concentrations were highly correlated (R^2= 0.791).

Agriculture was shown to have an overall negative effect on macrophyte communities. In-stream concentrations of two common herbicides, glyphosate and atrazine, were low. However, peak herbicide concentrations following run-off events are expected to be higher than what was observed for grab samples and may pose a risk, particularly for atrazine. Although no direct effects of herbicides on macrophytes community structure were observed, nitrate and atrazine were strongly correlated and may have a compounding influence.

In situ exposures of *Hyalella azteca*: A tool to assess the impacts of pesticide use on freshwater ecosystems.

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[1]Environment Canada.

Previous studies have shown that *in situ* exposures of *Hyalella azteca* can be used to evaluate the impacts of pesticide use in freshwater streams. This study examines sites in southern Ontario where acetylcholinesterase-inhibiting pesticides (organophosphates, carbamates) are consistently measured in surface waters. One-week exposures were conducted in 2008 at two agricultural sites (Two-Mile and Vineland Creeks), one urban stream (Indian Creek), and one reference site (Spencer Creek). Exposures were conducted monthly during the growing season: once pre-pesticide application (May), four times during peak pesticide application (June-September), and once post-pesticide application (October). Significant mortality occurred during periods of peak pesticide usage: at Two-Mile Creek, 100% mortality occurred in June, August, and September, and survival was significantly decreased in July; and at Vineland Creek, 100% mortality occurred in June and survival was significantly impaired in July, August, and September. Acetylcholinesterase activity in July was 55% of controls at Two-Mile Creek and 17% of controls at Vineland Creek, and preliminary results from pesticide analysis of surface waters indicate that organophosphates and carbamates were consistently measured at

these sites. Exposures were also conducted in 2009 with the addition of two sites: Richardson Creek and Bailey Bridge on Twenty-Mile Creek. Initial results from these exposures will be presented. The validity of using these methods to evaluate pesticide impacts on freshwater ecosystems will be discussed.

The ecotoxicological significance of non-regulatory performance standards in the application of watershed-scale Beneficial Management Practices to reduce surface water contamination by agricultural pesticides.

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A methodology to interpret watershed-scale, agricultural pesticides, achievable performance standards (APSs) in terms of ideal performance standards (IPSs) was developed. The Environment Canada Pesticide Project of the National Agri-Environmental Standards Initiative (NAESI) Program focuses on these non-regulatory water quality standards. IPSs are either based on aquatic species sensitivity distribution (SSD) curves or any other pertinent ecotoxicological data to identify the desired level of concentration needed to maintain ecosystem health. APSs represent a set of standards that could be achieved using Beneficial Management Practices (BMPs). There is a need to link APSs, predicted pesticides concentrations, to their potential biological impact in the river. We developed a hydrological modelling framework to define APSs for pesticides. The Cumulative Distribution Frequency curves (CDFCs) for priority pesticides concentrations were simulated and used to define APSs, that is, the 90th percentile of a CDFC. The simulations take into account various on-farm BMPs which reduce the pesticide concentrations in the river (e.g., buffer strips of various widths). This talk will provide a discussion on how to interpret APSs in terms of IPSs, that is in terms of ecotoxicology when SSD curves are available. Hence, the discussion will both: (i) further our understanding of the ecotoxicological meaning of APSs and (ii) compare some impacts of BMPs in reducing pesticide concentrations (APSs) and their chronic effect.

Evaluation of best management practice for reducing environmental risk from sprayer track rows in potato production.

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Previous Environment Canada work suggests that compacted sprayer track rows (i.e. the rows a tractor passes over numerous times a season for pesticide applications) in potato fields may lead to concentrated flow and compromise the filtering function of grass buffers along streams. A study was undertaken in 2007 to investigate the contribution of runoff from sprayer track rows and evaluate whether a best management practice (mulch treatment) can reduce the risk from these rows. Nine sample collectors (3 per treatment: bare sprayer track, mulch-treated sprayer track, non-compacted furrows) were deployed in the study field. Following each rainfall-induced runoff event, runoff volumes, toxicity to *Daphnia magna*, sediment, pesticide and nutrient loads were measured for each collector. Fourteen runoff events occurred between 2007-2008 field

seasons resulting in approximately 120 toxicity, pesticide and water quality analyses each. Mulch-treatment resulted in statistically significant reductions in nitrate-nitrogen, total suspended solids, aqueous chlorothalonil concentrations and sediment-associated dithiocarbamate and metribuzin concentrations. The study site selected for Year 2 was less than adequate for research purposes and resulted in inconsistent results compared to Year 1. Year 3 analyses are underway. If they are found to be consistent with Year 1 results, mulching in sprayer track rows may become a recommended practice for reducing the risk from these rows in potato production.

The development and use of novel toxicity tests with native species to evaluate pesticide risks in Atlantic Canada.

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Adverse effects to native species resulting from exposure to pesticides from agriculture and aquaculture usage are a concern. There is limited data available evaluating the toxicity of these chemicals to native marine organisms. It is difficult to establish guidelines or "safe levels" for the protection of native populations as their general sensitivity to contaminants is not well known. Environment Canada in Moncton, NB in collaboration with several partners, has been refining methods for testing larval stages of the American lobster (*Homarus americanus*) and the sand shrimp (*Crangon septemspinosa*) in order to evaluate risk to these important native species. Test methods are being established for acute and chronic toxicity tests with these marine species. Several experiments were conducted to investigate the influence of diet, temperature, and water quality in order to establish a methodology. These methods have since been used to evaluate the toxicity of chemicals of interest with these two species. For the lobster larvae testing, endpoints include: survival, molting success, histology and behavioural response. For the sand shrimp testing, endpoints include growth and survival. We are also investigating toxicogenomic endpoints for both of these species using DNA microarrays. Results will be described from tests with high-use and/or high-risk pesticides from the Atlantic region.

Relationship between pesticide properties and absorption in turtles eggs from treated soil.

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Our knowledge about pesticide exposure via treated soil to nontarget organisms, particularly vertebrates, is relatively sparse. Terrestrial vertebrates can be exposed to pesticides through direct exposure during application of pesticides, ingestion of treated soil, or through exposure from treated soil. Reptiles often nest within agricultural fields and thus developing embryos can be potentially exposed to pesticides. We tested the hypothesis that snapping turtle eggs are impervious to absorption of pesticides, or alternatively that turtle eggs will absorb pesticides from treated soil. We exposed eggs to

soil treated with a mixture of 10 pesticides (atrazine, azinphos methyl, captan, carbaryl, chloropyrifos, chlorothalonil, dimethoate, endosulfan, metolachlor, simazine), at two concentrations (1.92 kg/ha and 19.2 kg/ha of a.i). We sampled eggs after both 1 and 8 day post-exposure, and measured residual pesticides or metabolites in the egg contents. We also attempted to predict which physical and chemical properties of the pesticides (Fugacity ratio, solid vapor pressure (Pa), liquid vapor pressure (Pa), water solubility (g/m3), octanol-water partition coefficient (log KOW), Henry's Law constant (Pa·m3/mol), organic carbon sorption partition coefficient (log KOC) and molecular weight) were the best predictors of absorption into the eggs. Pesticides were analyzed either using HPLC or GC-MSD. Chlorothalonil was the only pesticide that was not absorbed in turtle eggs in any of the treatments. Generally, the pesticides that had the highest absorption rates were those that had the lowest absorption coefficients to either organic matter (metolachlor, simazine, atrazine, dimethoate) or lipids (e.g. simazine, dimethoate), or both. In addition, these pesticides were amongst the more soluble pesticides tested. Although endosulfan sulphate, a metabolite of endosulfan, was measured at fairly low concentrations in the soil, and only at the highest treatment, it was the compound that had the largest concentration in the eggs relative to the soil. We speculate that the prime route of exposure in eggs to pesticides from treated soil may be through the vapour, not dissolved, phase, similar to water exchange in turtle eggs. Our results indicate that reptilian eggs will readily absorb pesticides from treated soil.

Toxicologie des amphibiens et toxicologie faunique I
Amphibians and Wildlife Toxicology I

Effects of the pesticide carbaryl on skin peptide defenses in amphibians.

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Amphibians are suffering unprecedented declines on a global scale. One of the most serious threats is the chytrid fungus, *Batrachochytrium dendrobatidis* (Bd), which causes the disease chytridiomycosis. Environmental chemicals have the potential to inhibit immune defenses and increase susceptibility to disease. Ongoing studies demonstrate that antimicrobial peptides (AMPs) produced in the skin are an important component of immune defenses against Bd. Therefore, we initiated studies to determine whether the pesticide carbaryl can alter AMP defenses. When exposed to Bd, newly metamorphosed *Rana boylii* lagged in growth but did not die. Carbaryl-treated frogs (0.48 $mg \cdot L^{-1}$) secreted less total skin peptides 3 days after treatment than controls, but Bd-induced mortality was not increased. When day 3 samples were examined for AMP signals by mass spectrometry, 3/4 of control samples showed AMP signals, but none of the samples from carbaryl-treated frogs did. At seven days after carbaryl treatment, induced peptide concentrations had increased to control levels in pesticide-treated frogs, and expected AMP signals were detected. In adult *Rana pipiens*, carbaryl-treatment resulted in increased skin peptide recovery at 3 days post exposure in comparison with norepinephrine-induced controls. Thus, carbaryl exposure can alter AMP defenses which

may predispose amphibians to increased susceptibility to disease. Funding: Rose Foundation and NSF 0619536.

Assessment of beneficial use impairments in a model amphibian, northern leopard frogs, in the St. Clair Area of Concern.

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The St. Clair River between Sarnia and Lake St. Clair and the territory of the Walpole Island First Nation (WIFN) has been designated by the International Joint Commission as an Area of Concern (AOC), due to high levels of contamination in the sediments from present day and historical industrial activity raising questions about potential impacts to wildlife health. Potential beneficial use impairments (BUIs) to wildlife in the St. Clair AOC include wildlife deformities and reproductive impairments and degradation of wildlife populations as measured by contaminant body burdens. Northern leopard frogs were used as a representative amphibian species, to assess these BUIs at sites within and upstream of the St. Clair AOC in 2006 and 2007. Survivorship was high and deformity rates were low in leopard frog eggs raised in water from both AOC and upstream sites. Rates of deformities of young of year frogs exceeded 10% at one site within the AOC. Contaminant body burdens or mercury, PCBs and organochlorine pesticides were relatively low and were below guideline levels for the protection of fish eating wildlife. Testicular ovarian follicles (TOFs) were found in the testes of some male frogs both within the AOC and upstream sites. Rates of TOFs were significantly higher in AOC sites, and were present in over 50% of frogs at some sites.

Sexual differentiation and development of the reproductive system of the bullfrog (*Rana catesbeiana*) tadpole in an agricultural environment.

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Over the past 20 years, concentrations of pesticides in the Yamaska river drainage basin have surpassed the Canadian Water Quality Guideline up to 20-fold. 43% of the 4784 km2 of the surrounding land is used for agriculture, mainly corn and soybeans. Agricultural contamination is suspected to contribute to declines in frog population. The objective of this study was to characterise the effects of agricultural run-off on the sexual differentiation and gonadal development of the American bullfrog, a good aquatic sentinel species. Tadpoles were collected at 6 strategic sites in the drainage basin, with low, medium and high agricultural intensity, during the summers of 2008-2009. Morphometric measures were taken and nephro-gonadal complexes were collected and processed for histological analyses. There were significant differences in body weight, body length, tail length and width between reference sites and contaminated sites. There were tendencies for impaired or delayed gametogenesis in both genders of 2008 samples. While sex ratios were not different from the expected 50-50, greater ratios of undifferentiated animals were observed in contaminated sites than reference sites. Also,

oocytes were observed in testes from 2 sites with high agricultural intensity. These results indicate alterations in reproductive development, which could impact bullfrog populations in the future. Results will contribute to the characterization and a better understanding of the consequences of pollution on reproduction of the bullfrog.

Immune status of the bullfrog (*Rana catesbeiana*) exposed to agripesticides.

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Since many years, the amphibian populations decline and this condition is alarming. The reasons explaining this reality are numerous but the increase of infections is particularly worrying. This is the reason why, we have chosen to study the impact of agripesticides on the frog immune system. Having in mind that pesticide could impair the immune system and reduce resistance to infections. The immune system of amphibians is composed of several kinds of cells, among them we can mention macrophages and lymphocytes. Moreover, skin peptides play a critical role in protecting the frogs against infections. To evaluate the consequence of a chronic exposure to agripesticides, bullfrogs were sampled at six different sites around the Yamaska River in Québec during two years (2008, 2009). At some sites, the level of metolachlore and atrazine, are particularly high. The analyses of three peptides found on the skin (ranatuerine 1, 2 and 6) were made by HPLC-MS-MS. The immune biomarkers that we have looked at using the spleen are: viability, cellularity, phagocytosis and oxidative burst. The data collected up to date show that the presence of peptides is modulated by agripesticides as well as viability and phagocytosis.

Application of a native North American species, *Pseudacris regilla*, to the amphibian metamorphosis assay for assessing thyroid active substances.

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The amphibian metamorphosis assay has been identified by the Organization for Economic Co-operation and Development as a relevant test system for the detection of thyroid active substances in vertebrate species. This assay entails exposing premetamorphic *Xenopus laevis* tadpoles to waterborne toxicants for 21 days and measuring various treatment-related changes in metamorphic development. The aim of the present study was to examine the utility of this assay for detecting thyroid active substances using the Pacific tree frog (*Pseudacris regilla*), a species native to North America. To this end, premetamorphic (Gosner stage 28-29) Pacific tree frog tadpoles were exposed to dilution water, solvent control (0.05% 50 mM NaOH), and thyroxine (0.75 $\mu g \cdot L^{-1}$, 1.5 $\mu g \cdot L^{-1}$, 3.0 $\mu g \cdot L^{-1}$ and 6 $\mu g \cdot L^{-1}$) treatments for 21 days. Growth-related parameters (whole body length, snout-vent length and wet weight) decreased after 7 and 21 days with thyroxine treatments, typical of tadpoles advancing through developmental stages. Thyroxine induced a concentration dependent increase in developmental parameters as expected; hind limb length after 7 and 21 days, and developmental stage

after 21 days. In contrast to previous thyroid hormone exposures in frog larvae, a reduction from 5 to 4 hind limb digits was observed in response to the thyroxine treatments in a concentration dependent manner. The present findings suggest that the Pacific tree frog is a candidate model species for the amphibian metamorphosis assay that could be applied to assess the impacts of thyroid active contaminants in wild frog species native to North America.

Cyanobactéries
Cyanobacteria

Estimation des apports de nutriments vers la Baie Missisquoi: rôle du phosphore, de l'azote et de la température sur la prolifération des cyanobactéries.

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Au Québec, les cyanobactéries font la une des journaux depuis plusieurs étés. Ces cyanobactéries, pouvant être toxiques, prolifèrent dans les plans d'eau soumis à de forts apports en nutriments. D'autres facteurs climatiques interviennent aussi et influencent leur prolifération. Dans ce contexte, cette étude a pour objectif de comprendre l'interaction existante entre ces différents facteurs et son influence sur le développement des cyanobactéries à la Baie Missisquoi. Le modèle hydrologique SWAT (Soil and Water Assessment Tool), après calibration et validation, a permis de déterminer l'apport en nutriments des terres amont à la Baie. Les données utilisées au niveau de la Baie ont été collectées pendant 15 ans. Ces données comprennent les concentrations de phosphore, d'azote, de chlorophylle *a*, et de la température de l'eau. L'analyse des données a permis d'appréhender la relation complexe existante entre ces facteurs héliothermiques et nutritionnels et la prolifération des cyanobactéries. Par ailleurs, le suivi de la culture de l'espèce *Microcystis aeruginosa in situ* a permis de comprendre la dynamique de cette espèce sous différentes concentrations des nutriments. Les résultats préliminaires indiquent que la chlorophylle *a* est corrélée avec le phosphore total ($R^2 = 0.37$). Cependant, l'établissement d'une régression linéaire multiple en intégrant l'azote et la température a permis d'améliorer le résultat avec un R^2 de l'ordre de 0.67. Ainsi, la combinaison de cette relation et les sorties du modèle SWAT permettront de développer un outil de suivi de la qualité de l'eau ainsi que la détection de la croissance des cyanobactéries à la Baie Missisquoi.

Effects of microcystins on chlorophyll *a* fluorescence of *Scenedesmus obliquus, Chlamydomonas reinhardtii, Pseudokirchneriella subcapitata* and *Navicula pelliculosa*.

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Cyanobacterial blooms occur widely in freshwater aquatic ecosystems around the world. These toxin-producing blooms can have important repercussions on recreational and commercial activities, but also on the health of animals and humans. One of the most frequently found cyanotoxins is microcystin, a hepatotoxin which counts more than 70 variants. Therefore, development of rapid tests for detection of microcystins is needed. Since photosynthesis is one of the major processes governing algal growth, and that growth was shown to be affected by microcystins, our goal was to evaluate the effect of microcystins on algal fluorescence signal. Chlorophyll fluorescence based bioassays are known to be effective to evaluate photosynthetic activity. However, sample coloration may have an important impact on the interpretation of results obtained by this method. Therefore, we need to develop a procedure to minimize the effect of the extract color. Light and acid were used to bleach extracts obtained from a toxic strain of *Microcystis aeruginosa*. We evaluated the effect of bleaching treatments on microcystin content (analysis underway). Four algal species (*Scenedesmus obliquus*, *Chlamydomonas reinhardtii*, *Pseudokirchneriella subcapitata* and *Navicula pelliculosa*) were then exposed to these extracts for up to 96h. Chlorophyll *a* fluorescence was measured with PAM (Pulse Amplitude Modulated) and PEA (Plant Efficiency Analyzer) fluorometers. We showed that the extract coloration has an impact on result interpretation and that bleaching treatment is absolutely needed for fluorescence based bioassays. Also, the toxic effect of microcystins on photosynthetic processes was shown to be more important after short exposure time than after longer exposure.

Biochemical responses after exposure to microcystins in young trout.

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Microcystins (MC) are usually the predominant cyanotoxins in natural water associated to cyanobacterial blooms. In the present study, young trouts (*Oncorhynchus mykiss*) were exposed to methanol extracts of *Microsystis aerugunosa* taken along *Microsystis aerugunosa* growth. The extract represented various doses of MC-LR. The four experimental conditions were formulated to represent natural conditions: the control (cyanobacteria free); Day 11 ($5\mu g \cdot L^{-1}$ of MC-LR); Day 32 ($1.8\mu g \cdot L^{-1}$ of MC-LR) and Day 49 ($0.75\mu g \cdot L^{-1}$ of MC-LR). Young fish were exposed for 4 days in these cyanobactrial extracts in static conditions. Interestingly, protein phosphatase measured in the liver showed a significant increase when exposed to MC-LR indicating a defense mechanism against protein phosphatase inhibition. No increase of glutathione-S-transferases (GST) activity was observed and lipid peroxidation remained unaltered in both liver and brain tissue exposed to the cyanobacterial extracts. Protein phosphorylation in acetone-fractionated proteins was lower in brain's trout exposed to MC-LR. Unexpectedly, acethylcholinesterase activity in brain's trout was altered when exposed to

the highest MC-LR concentration suggesting a slowdown neural activity. The results showed that the classical biochemical disturbances in the liver and brain of young trout were induced without triggering biotransformation processes or detoxification of GST. MC-LR concentration above the 1 $\mu g \cdot L^{-1}$ as recommended by the World Health Organization, suggests brain damage in trout.

Extraction et détection simples de microcystines de tissus de poisson.

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Les cyanobactéries sont des organismes aquatiques dont une quarantaine peut être toxique pour la vie aquatique comme pour l'homme. Au cours des dernières années, on a pu observer une augmentation de leurs efflorescences notamment à cause de l'eutrophisation des lacs et rivières. De plus, la composition, la forme et la quantité de ces cyanotoxines est fonction de l'environnement et plus particulièrement de la température, de la lumière et de la disponibilité des nutriments (Sivonen et Jones, 1999; Duy et coll., 2000). Il s'agit donc d'un problème de santé publique difficile à cerner de part la diversité des cyanotoxines et la diversité des réponses des cyanobactéries à leur environnement. Dans cette étude, nous nous sommes intéressés aux microcystines LF, LR, RR et YR. Il s'agit de cyanotoxines hépatotoxiques très stables et donc persistantes dans l'environnement. Les microcystines peuvent ainsi s'accumuler dans la chair et les organes des poissons et ainsi se répandre le long de la chaine alimentaire (Chorus, 2001; Vasconcelos, 1999). Ce sont des molécules particulièrement néfastes puisqu'elles inhibent l'activité des protéines phosphatases ce qui entraine la destruction des hépatocytes (Van Apeldoorn et coll. 2007). Dans cette étude, nous avons voulu mettre au point une méthode d'extraction à partir de tissus de poissons et une méthode de détection simples et rapides des microcystines LF, LR, RR et YR. Nous nous sommes plus particulièrement intéressés au foie et aux muscles de poissons. Le foie est l'organe cible des microcystines et le muscle est couramment la seule partie consommée par l'homme. Nous avons aussi déterminé s'il est nécessaire de lyophiliser les tissus avant de procéder à l'extraction.

Dans cette étude, des échantillons de foies et de muscles ont été placés pendant 4 heures à 4°C avec agitation en présence d'ajouts dosés des standards de microcystines. Pour chaque type de tissus une moitié des échantillons a été lyophilisée et l'autre a été congelée en attendant l'extraction. Nous avons choisi une extraction « solide-liquide » dans du méthanol. D'abord les échantillons sont mis en présence de 1.5 mL de méthanol 100% puis le tout est mis 15 min au bain à ultrasons et centrifugé 5 min à 5000 rpm. Le surnageant est récupéré. Puis on fait une nouvelle fois l'extraction avec 1.5 mL de méthanol 80% et une troisième fois avec 1 mL de méthanol 100%. Les 3 surnageants sont regroupés puis évaporés environ 2 heures sous un jet d'azote jusqu'à ce qu'il ne reste que environ 100 µL. L'extrait ainsi concentré est remis en suspension dans 500 µL de méthanol 100% pour être conservé jusqu'à la détection. La méthode de détection sélectionnée est un couplage chromatographie liquide/spectrométrie de masse (HPLC-MS/MS). La chromatographie se fait dans un gradient acétonitrile : eau de 30:70 à 95:5 (7 min) sur une colonne C18 et au niveau du spectromètre (Agilent 6400) nous avons

choisi une ionisation par ESI par une source MMI (Multimode Ionisation) et une détection en MRM (transitions m/z LF : 986→135, LR : 995→135, RR : 1038→135, YR : 1045→135 et NOD : 826→135). Comme le gradient de la chromatographie commence à 30% d'acétonitrile, les extraits doivent être dilués pour atteindre une concentration de 30% de méthanol (+ 0.1% d'acide formique) afin d'optimiser la détection. Le standard interne, de la nodularine (NOD), est aussi ajouté et l'ensemble est filtré (filtre de cellulose régénérée à 0.2 μm) afin de purifier l'extrait avant l'injection dans le système de détection.

Table 1 : pourcentages (%) de récupération moyen pour les microcystines YR, RR, LR et LF en fonction du tissu et de si il y a eu ou non lyophilisation.

		MC-YR	**MC-RR**	**MC-LR**	**MC-LF**
FOIE	**non lyophilisé**	77,21 ± 5,57	83,44 ± 18,65	104,02 ± 10,76	59,00 ± 1,55
	lyophilisé	48,53 ± 4,75	43,54 ± 8,42	67,92 ± 6,59	44,42 ± 10,90
MUSCLE	**non lyophilisé**	83,19 ± 4,16	124,60 ± 8,59	94,18 ± 8,33	85,92 ± 5,58
	lyophilisé	26,64 ± 0,62	39,01 ± 7,30	31,01 ± 1,68	36,77 ± 0,24

La méthode mise au point permet un taux de récupération élevé d'au moins 77% pour les microcystines YR, RR et LR et de 59% pour la microcystine LF pour les extractions faites à partir du foie non lyophilisé. A partir des muscles non lyophilisés, le pourcentage de récupération est d'au moins 83% pour toutes les microcystines. Dans le cas des extractions faites à partir des tissus lyophilisés, la récupération est moindre et varie entre 43 et 67% pour le foie et entre 26 et 39% pour le muscle (table 1). Par ailleurs, l'effet matrice moyen pour le foie est de -22% avec en moyenne un effet de -19.24% pour le non lyophilisé et –24.76% pour le lyophilisé. Dans le cas du muscle, cet effet matrice est très faible (en moyenne 2.17%) puisqu'il n'est que de 3.33% pour le non lyophilisé et 1.00% pour le lyophilisé. La méthode que nous avons mise au point permet donc une extraction des microcystines simple, rapide et efficace par rapport à d'autres travaux. Par exemple, la méthode de Smith et Boyer (2009) nécessite la préparation d'un standard interne spécifique, alors que nous pouvons utiliser une solution du commerce, ainsi que la précipitation des protéines pour des taux de récupération comparables à ceux que nous avons obtenus. Ou encore, même si la méthode de Bogialli et coll. (2005) ne se fait qu'avec de l'eau acide à haute température, elle nécessite la préparation et l'installation d'un système de filtration à base de sable relativement contraignant et les taux de récupération sont inférieurs à ceux que nous avons obtenus.

Cette méthode d'analyse sera utilisée pour étudier l'accumulation des microcystines dans les tissus de la truite et la perchaude. Ceci permettra ultimement de mieux estimer l'exposition humaine aux microcystines par l'alimentation.

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Toxicologie des amphibians et faunique II
Amphibians and Wildlife Toxicology II

The effects of the herbicide atrazine and the prostate cancer drug finasteride on 5-beta-reductase in two species of frogs.

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Endocrine disrupting chemicals (EDCs) such as pesticide and pharmaceuticals can upset amphibian development and may contribute to worldwide amphibian population declines. The enzyme 5-beta-reductase (SRD5β) is important for vertebrate development and biological functions (e.g. sexual development, bile biosynthesis and erythropoiesis) and may be a potential target of EDCs. We investigated the effects of Aatrex© (ATZ; atrazine) and the prostate drug finasteride (FIN; known as 5-alpha-reductase inhibitor in humans) on 5-beta-reductase during anuran larval development. Chronic exposures to 0.1 and 1.8 $\mu g \cdot L^{-1}$ ATZ on Rana pipiens were performed in semi-controlled mesocosms, while short-term (25, 50 and 100 μM FIN) and chronic (25 μM FIN) exposures on *Silurana tropicalis* were done under laboratory settings. Exposures altered SRD5β activity in the two species of frogs. Both ATZ and chronic FIN treatments induced a female-biased sex ratio. Real-time RT-PCR analysis showed that FIN treatments affected SRD5β and other sex-related genes mRNA levels. These results suggest that a disruption of SRD5β can induce morphological and physiological changes in developing frogs.

Impact of agriculture on retinoids and oxidative stress: Comparison of responses between bullfrogs (*Lithobates catesbeianus*), yellow perch (*Perca flavescens*) and brown bullheads (*Ameiurus nebulosus*).

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As the tendency toward intensive agriculture increases, the risk of effects in local freshwater fish and wildlife populations is heightened. In the Yamaska River drainage basin, Québec, approximately 70% of land area is associated with agricultural production, the proportion of intensive agriculture varying between sub-watersheds. In this study we evaluate oxidative stress and vitamin A (retinoid) status in three aquatic vertebrate species. Retinoids are essential for many physiological functions (e.g. reproduction, embryonic development, growth) and demonstrate anti-oxidant properties. Specimens were captured in the Yamaska sub-watersheds of Boivin (low agricultural activity), Pot-au-Beurre (Baie Lavallière characterized by moderate-to-intense agriculture) and Lake Saint-Pierre which receives the outflow of the Yamaska River. Retinoids are currently being analyzed in liver and plasma whereas the oxidative stress is being evaluated by anti-oxidant enzymatic activities (catalase and glutathione peroxidase), an anti-oxidant molecule (glutathione) and an indicator of lipid peroxidation (thiobarbituric acid reactive substances assay, TBARS). The biochemical markers of retinoid status and oxidative stress will be compared with morphometric and population data.

Contaminants and biomarker responses in St. Lawrence wildlife: a review of signs of impairment.

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Studies on contaminants in wildlife in the St. Lawrence system began 40 years ago, not long after the effects of PCBs and DDT on bird populations were observed in the Great Lakes. Since then, levels of contaminants have declined, but organisms are still exposed to a multiplicity of chemicals as well as to other stressors. Contaminant-induced sub-lethal effects may have important population consequences without causing death; these include the limiting of physiological processes; impairment of behavior, growth or other functions; and reduced capacity to tolerate subsequent stress. Monitoring early signs of impairment with biomarkers should improve our ability to detect significant effects of contaminants at an earlier stage (Fox 1993). The objective of this presentation was to review studies conducted on effects of chemical pollution in wildlife in the St. Lawrence River to determine if cause-effects linkages could be identified. The studies chosen were conducted either in or around the Cornwall-Massena Area of Concern (under the Remedial Action Plan of the Great Lakes), or elsewhere along the St. Lawrence River (conducted for the development of a monitoring network established to assess the state of the river, a component of the St. Lawrence Action Plan; see Table 1). Based on epidemiological criteria for causation such as probability, time order, strength of association, consistency, and coherence (Fox 1991), clear signs of impairment were observed in the studies evaluated, including evidence of endocrine disruption, decreased

reproductive success, stress response and developmental anomalies. However, it was difficult to determine whether the St. Lawrence wildlife populations are impaired or disabled in relation to their exposure to specific measured contaminants. Evidence for a causal linkage between endocrine-disrupting chemicals and effects in free-ranging wildlife are limited, due to logistical difficulties in studying wild animals, and the challenge of extrapolating data generated *in vitro* and in laboratory animals to highly diverse free-ranging species (Rattner 2009). We conclude that more research and surveillance is needed to better understand this large and complex ecosystem that supports a rich wildlife assemblage.

Table 1 Effects observed in various species in the St. Lawrence River

Species	**Effect**	**Cause-effect linkage**	**Reference**
Mudpuppy	Disruption of HPI axis	Possible	1
	Decreased recruitment	Possible	2
Snapping turtle	Developmental abnormalities	Possible	3
	Alteration of secondary sexual characteristics	Not in Akwesasne	4
Tree swallow	Depressed vit. A	Possible	5
	Increased EROD	Possible	
	Reduced fledgling rate	Not clear	6
	Increased EROD	possible	
Herring gull	Vitellogenin in adult male	Yes	7
	Depressed corticosterone response in juveniles	Possible	
Black-crowned night-heron	Depressed vit. A in plasma and liver	Not clear	8
Great blue heron	Depressed vit. A in plasma and liver	Possible	8, 9
	Retinoids in eggs	Possible	10
	T3 and T4	Not clear	8,9

1 : Gendron et al. 1997; 2 : Gendron 2001; 3 : Bishop et al. 1998; 4 : de Solla et al. 2002; 5 : Bishop et al. 1999; 6 : Légaré et al. 2005; 7 : Hughes (in prep); 8 : Champoux et al. 2002; 9 : Boily et al. 1994; 10 : Champoux et al. 2006.

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Interspecific comparison of organochlorines, brominated flame retardants and mercury in 6 seabird species breeding in the Gulf of St. Lawrence.

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Organochlorines (OCs) and mercury (Hg) have been shown to pose a threat in ecosystems because most of these contaminants are persistent in the environment, they bioaccumulate in organisms, biomagnify in food chains and are toxic. There is evidence that brominated flame retardants (BFRs) have similar properties. We measured concentrations of Hg, OCs and BFRs in eggs of 6 seabird species breeding in the Gulf of St. Lawrence with different feeding ecology and life cycle. Stable nitrogen (δ15N) and carbon (δ13C) isotopes were used as ecological tracers to measure trophic level and connectivity with benthos, respectively. We found that concentrations varied significantly between species. The sum of polychlorinated biphenyls (∑PCBs) was the most important OC group measured in all seabird species followed generally by the sum of chlorinated hydrocarbons (∑CHCs). ∑BFRs were generally lower than ∑CHCs followed by total Hg (THg). ∑PCBs were highly correlated with ∑CHCs. We also found that ∑PCBs, ∑CHCs and ∑BFRs increased with δ15N, whereas THg did not. None of the compounds seemed to increase with a higher connectivity with the benthos.

Levels of heavy metals, PCBs, pesticides and flame retardants in tissues of Barrow's Goldeneyes (*Bucephala islandica*) wintering in the St. Lawrence marine system.

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[1]Environment Canada.

The eastern North American population of Barrow's Goldeneye (*Bucephala islandica*), a sea duck species, is designated "of special concern" by the COSEWIC. During six months per year the majority of this population at risk estimated at 6 000 individuals winters within only 300 km of non contiguous coastline in the St. Lawrence marine system. Large numbers of individuals congregate for prolonged periods of time in severely contaminated areas where they feed on benthic invertebrates, mostly molluscs. Obviously, this clustered distribution pattern and the feeding habits of the species make this small population highly vulnerable to contamination. Our objective was to determine the level of contaminants in tissues of Barrow's Goldeneyes wintering in the St. Lawrence marine system. Specimens were collected in the regions of Charlevoix (Upper Estuary), Manicouagan (Lower Estuary) and Chaleur Bay and were analyzed for hepatic PCBs, chlorinated pesticides, brominated flame retardants, and hepatic and renal Hg, Cd, Se, As, and Pb. Metals were detected in most of the specimens. Pesticide and flame

retardant levels were generally low. DDE contributed for most of the total DDT detected. Specimens from Chaleur showed the highest Se and total DDT levels. Specimens from Charlevoix had highest pesticides (other than DDT) and Cd levels, and specimens from Manicouagan had highest PCBs and Hg levels. Baie-des-Anglais in Manicouagan is known to be heavily contaminated by PCBs and juveniles collected there showed similar PCB levels than adults from the same region.

Évaluation des risques environnementaux
Environmental risk assessment

The role of environmental risk assessment in the regulation of nuclear facilities under the Canadian Nuclear Safety and Control Act and Regulations.

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[1]Canadian Nuclear Safety Commission.

With the coming into force of the Nuclear Safety and Control Act and its regulatory authority the Canadian Nuclear Safety Commission in 2000, Canada's nuclear regulator was given two new responsibilities: 1. direct protection of the environment; and 2. regulatory responsibility for hazardous substances in addition to nuclear substances. The Act and regulations contain numerous references to protection of the environment. Two key requirements are the obligation to: 1. *prevent unreasonable risk to the environment* …, and 2. *make adequate provision for the protection of the environment* … These are inherently risk based statements. Hence, the CNSC adopted environmental risk assessments (ERA) as the core environmental risk management tool throughout the complete life-cycle and regulatory cycle of a facility. The approach is outlined for the integration of ERA from the original EA, through multiple licensing stages and periods as one of the core tools in the environmental management of a facility. The use of ERA for developing the monitoring program, the testing of performance predictions with monitoring results and the ongoing use of ERA as an adaptive management tool continuously informed through the accumulating site-specific data is discussed. The benefits, growing pains and remaining difficulties in this use of ERAs will be discussed both theoretically and through case examples.

Use of the SALE weight of evidence approach to assess environmental risk at a former uranium mine site.

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A uranium mine and mill site was in operation from 1952 to 1982, after which time it was decommissioned until 1985. A licence was issued by the Canadian Nuclear Safety Commission for the reclamation, monitoring and maintenance of the site. Various special environmental and human health studies were completed between 2005 and 2008. A "sequential analysis of lines of evidence" (SALE) approach was used to assess risks at

the site and to integrate the information (Hull and Swanson, 2006; Chapman, 2007). CNSC staff considered five main lines of evidence in determining ecological risk:

- the hazard vs. environmental contamination
- the level of organization (individual vs. population) where the effect was observed
- the scale of effects (local vs. regional)
- the timeframe of effects (single generation vs. several)
- the relationship of the impacted population to other communities in the food chain.

The five criteria used to assess ecological risk compared the exposure site to:

- reference site conditions
- environmental quality objectives/guidelines or toxicity thresholds
- predicted hazards from other environmental effects assessments
- gradient responses from the near-field to the far-field
- temporal trends

In addition, the influence of uncertainties on the weight associated with each line of evidence was evaluated as was the need for additional studies to resolve any remaining environmental issues.

Using TerraSys™ to identify biodiversity indicator species: Application to eight industrial sites.

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[1]Sanexen Environmental Services inc.

Biodiversity conservation is becoming a focus for programs both in government and in business. For industry, developing a biodiversity conservation plan requires knowing what impacts each installation might have on biodiversity. Functional indicators of biodiversity are then needed to monitor whether the conservation actions taken are having the desired effects. However, a simple field survey of biodiversity cannot discriminate the impacts on biodiversity of a single source. Thus, there is a need to identify potential impacts on biodiversity that result from a specific source. Mathematical modelling is proposed as a solution. The software program TerraSys™ can be used to model both the fate and effects of multiple chemical substances in complex ecosystems, allowing identification of species that are specifically at risk in relation to a given industrial facility. The value of this user-friendly tool in identifying specific indicators of potential impacts on biodiversity is demonstrated with a case study of eight (8) Canadian industrial installations. The development of conceptual models representing the ecosystems under study is discussed. After modelling the fate and effects of the contaminants of concern at each site, the species present are ranked according to their sensitivity to the facility's releases. Modelling identified a total, over the eight sites studied, of 35 different vertebrate species that could be useful indicators of changes in biodiversity due to specific industrial activities.

The physical toxicity side of oil and gas development: the question of seismic surveys.

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The question of impacts of seismic surveys on fish mortality and morbidity continues to be of concern for the fishing sector as well as for industrial and management interests in relation to oil and gas development offshore. Sublethal effects can be most difficult to address since serious physiological and anatomical damage may be occurring at unknown depths in the water column. There is a major knowledge gap in this area. The studies we have carried out over the past few years on the effects of seismic on lobster, snow crab and monkfish larvae will be reviewed. Some studies have extended from observations on gene expression through to observations on feeding, histopathology and delayed mortality. The results point to the need for more comprehensive studies regarding the potential for seismic surveys to produce sublethal effects in fish and shellfish.

Development of an ecotoxicological risk assessment (ERA) approach for the sediments of the St. Lawrence River.

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As a result of the Third St. Lawrence Action Plan, the current vision of a sustainable management of this river and its main functions entails the need of sound risk-based assessment approaches. More specifically, the implementation of the Sustainable Navigation Strategy implies to develop a detailed risk assessment framework for sediments, encompassing the range of the available management options. An ERA approach using a chemical characterization in the initial stage and bioassays in the subsequent stages is currently under development. The first management option addressed in this perspective is the discharge of dredging sediments in open water. Thus, assessment endpoints will account for three kinds of potential impacts: short term survival of pelagic organisms, deposit re-colonisation by benthic species, or spawning success of sensitive fish species. This presentation will describe the various stages of the proposed ERA and the measurement tools considered, as well as the next steps in the development of this approach.

Geographical analysis of sediment contamination in the traditional territory of the Oujé-Bougoumou Cree Community.

OLSON, J. [1]

[1]CJB Environnement Inc.

The traditional territory of the Oujé-Bougoumou Cree community, an area of nearly 10 330 km² located in central Québec, contains several rivers and large lakes whose sediments have been adversely affected by mining. Within the context of a screening level environmental risk assessment covering this territory and making use of pre-existing contaminant measurements in soil, sediment, water, fish and mussels, a large scale analysis of sediment contamination was conducted. This analysis focussed on the bodies of water most directly affected by past mining activities (Lake Aux Dorés, Lake Chibougamau, the Nemenjiche River and the Obatogamau Lakes), included over 160 sediment samples and covered 27 different potential contaminants (mostly metals). To assess the risks linked to the quality of these sediments, several benchmark values were developed, including two methods for determining a regional natural background concentration. The first based on concentrations in lakes not directly affected by human industry and the second based on deep, pre-industrial, sediments. Other benchmarks used were ecotoxicological risk thresholds taken from sediment quality criteria (for risks to benthic organisms) or based on a multitrophic exposure model we developed (for risks to birds and mammals). We also compared the levels in sediments to those in local mine tailings. The sediment concentrations were mapped and compared to the benchmarks using GIS software, giving a good visual representation of the extent of the contamination. This analysis allowed us to conclude that several metals associated to mining in the area were present at levels sufficiently high to cause a risk.

Science and Policy Integration I: Chemical assessment choices and their policy implications in Dredged Material Management

APITZ, S. [1] and AGIUS, S. [2]

[1]SEA Environmental Consulting, [2]Environment Canada.

Science has provided a number of possible options to assess sediment chemistry and to predict the bioaccumulation and biomagnification potential of sediments. For example, various types of sediment quality guidelines, analytical chemistry methods, and biomagnificication assays and models exist. Applying these tools in a regulatory context can be challenging, however, as the appropriateness of each scientific tool varies in the context of legal and policy objectives that have to be met. This talk will examine the use of chemical analysis tools in the early tiers of a potential regulatory framework for assessing sediments proposed for disposal at sea. Consideration will be given to how different scientific tools might change the extent to which legal and policy objectives are met at each step of the assessment process, and when interacting together in the assessment as a whole.

Science and Policy Integration II: Biological assessment choices and their policy implications in Contaminated Dredged Material

APITZ, S. [1] and AGIUS, S. [2]

[1]SEA Environmental Consulting, [2]Environment Canada.

In a similar fashion to the talk titled "Science and Policy Integration I", this talk will examine the utility of various scientific tools at meeting legal and policy objectives. However, the focus here will shift to the biological assessment of sediments in the early and upper tiers of a potential sediment assessment framework for dredged materials proposed for disposal at sea, and in monitoring done to ensure that regulatory decisions were correct. The legal and policy objectives at each level of assessment and in subsequent monitoring differ, and provide interesting examples of how different criteria can be applied to the selection of biological assessment tools intended to support differing legal and policy goals.

Génomique, protéomique et métabolomique
Genomics, proteomics and metabolomics

OMICS – Essential risk assessment tool or Rube Goldberg contraption?

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Ecotoxicogenomics promises much for ecological risk assessment but its practical utility remains unproven. It is not clear if and when the technology will become more widely available to risk assessors and what role genomics will play in the regulator's tool box. We will use examples from our own work and other group's to highlight some of the pressing issues about the use of ecotoxicogenomics in risk assessment. Some of the questions to be addressed include whether the role of ecotoxicogenomics will be limited to providing insight into mode of action or whether genomic endpoints can be used for regulatory decisions. We will examine the current data on the use of genomics applied to field situations and compare the findings with laboratory studies. The use of NOTEL (No Observed Transcriptional Effect Level) has been suggested as a possible endpoint and we discuss the current evidence and potential utility of NOTEL in risk assessment

LC-based proteomics approach to study steroid receptor agonist in the fathead minnow.

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Toxicoproteomics is an emerging field that is greatly enabled by LC-based quantitative proteomics for biomarker discovery and characterization for endocrine disrupting chemicals. Using iTRAQ (isobaric tagging for relative and absolute

quantitation), we quantified a diverse range of proteins in the liver and brain (telencephalon) of fathead minnows (*Pimephales promelas*) (FHMs) in response to an androgenic (17β-trenbolone; Tb) and an estrogenic (17α-ethinylestradiol; EE2) pharmaceutical. In female FHMs exposed to 5 μg $Tb \cdot L^{-1}$ for 48 hours, we detected altered hepatic proteins that were involved in metabolism (e.g., glyceraldehyde 3-phosphate dehydrogenase), general stress response (e.g., heat shock protein 60), and translational regulation (e.g., ribosomal proteins). Cell processes such as growth rate, cell division, differentiation, and glycolysis were candidate processes regulated via androgen receptor signaling. In male FHMs exposed to 5 ng $EE2 \cdot L^{-1}$ for 48 hours, we identified altered proteins that were involved in cell structure (microtubule), metabolism (lactate dehydrogenase), and cell signaling (calmodulin). In addition, EE2 affected proteins involved in oxidative stress, synaptic plasticity, and long-term potentiation. Exposure to both steroid receptor agonists affected cellular metabolism and stress responses. Gene expression analysis in the liver and brain of FHMs revealed that changes in mRNA levels may not always correlate to protein levels. Thus, the challenge of integrating proteomics and genomics data will need to consider temporal and regulatory relationships between genes and proteins for improved predictive power in risk assessment. We demonstrate the utility of LC-based proteomics methods in aquatic toxicology and show that steroid receptor agonists rapidly alter the teleostean proteome.

Combined effects of temperature and copper exposure on gene expression in fathead minnows.

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Literature about effects of high temperature on the physiology of fish is abundant. Much is also known about the mechanisms of Cu toxicity in fish and other animals. However, we know very little about the effects of combinations of natural and anthropogenic stressors. In the context of climate change, where events of extreme weather including hot spells are expected to increase in frequency, a better knowledge of the combined effects of temperature and contaminants is essential to improve our capacity to protect wildlife from human activities. In this experiment, young adult fathead minnows (*Pimephales promelas*) were maintained for 28 days at either 20 or 32˚C (the latter approaching lethal upper temperature for the species) and either kept in clean conditions, exposed to aqueous Cu or fed Cu-contaminated prey (*Lumbriculus variegatus*). Muscle samples were then analyzed for the differential expression of genes using a commercial 15K fathead minnow microarray. Using this technology, only about a hundred genes were statistically differentially expressed among the treatments, and of these, only 38 had a known biological function. At 20˚C, both routes of Cu exposure modified gene expression to a similar extent, but at 32˚C, diet had a larger influence than water on gene expression. Further, the normal genomic response to high temperature was largely inhibited by aqueous and dietary Cu exposure. Genes most affected by Cu and temperature were those related to protein metabolism and ion transport. Comparison of gene expression profile changes with physiological endpoints such as enzyme assays will

complete this study. Taken together, the results of this study will greatly improve our understanding of the combined effects of temperature and metal contamination in fish.

Transcriptional responses to environmental metal exposure in wild yellow perch (*Perca flavescens*) collected in lakes with differing metal levels (Cd, Cu).

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To investigate the mechanisms involved in metal stress in wild fish, yellow perch (*Perca flavescens*) were collected in four lakes of the Rouyn-Noranda region (Canada). Due to mining and smelting activities, this region presents a broad contamination gradient in metal concentrations (Cd, Cu and Zn) and offers a unique research opportunity to investigate relationships between metal bioaccumulation and resulting deleterious effects in indigenous biota chronically exposed to metal mixtures. The expression level of genes encoding for proteins involved in metal detoxification (metallothioneins, mts), protein protection (heat shock protein-70, hsp-70), growth (insulin growth factor-1, igf-1), aerobic energy metabolism (cytochrome c oxydase, cco-1) and protection against oxidative stress (Cu/Zn superoxide dismutase, sod-1) were assessed in fish liver and muscle in association with protein and enzymatic assays for cytochrome c oxidase (CCO). Bioaccumulation of both Cd and Cu increased in response to higher ambient metal concentrations, but the two metals clearly have different modes of action. For Cd, changes in gene expression levels were more marked in the liver than in the dorsal muscle, whereas for Cu the opposite trend was observed. Hepatic Cd accumulation was linked to decreased cco-1 and sod-1 gene expression, whereas Cu accumulation was associated with a decrease in CCO enzymatic activity and an increase in total protein concentration and in cco-1, mts and hsp-70 gene expression levels. By coupling gene expression to biochemical and physiological endpoints, this work provides new insights into the mechanisms involved in metal stress and the adaptive response of fish chronically exposed to metal mixtures.

Evaluation of nanometal effects on frogs using toxicogenomic and biological endpoints through "frogSCOPE".

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Nanometals, such as nanosilver, quantum dots, and nanozinc oxide, are substances manufactured at particle sizes with diameters in the nanometer range. Their uses are increasing in a variety of consumer and health products because of desirable properties they possess. These nanometals are deliberately or inadvertently introduced into aquatic environments yet there is a lack of information regarding potential effects of these materials. Amphibians are regarded as environmental sentinels and demonstrate an exquisite sensitivity to thyroid hormone action; a hormone that is essential for human

health. Through frogSCOPE (frog Sentinel species Comparative "Omics" for the Environment), we have examined the potential effects of these nanometals on *Rana catesbeiana* tadpoles using a combination of morphological and toxicogenomic endpoints. Environmentally-relevant concentrations (0.1-10 nM) of nanosilver and quantum dots altered the expression of transcripts linked to thyroid hormone- and stress-mediated pathways while nanozinc oxide had no effect. Other forms of silver or cadmium telluride (such as ionic or micron) did not have the same effect as the nanomaterial. Recent progress of the systems-based approach using transcriptomics (QPCR and cDNA array), proteomics (isobaric tags for relative and absolute protein quantitation; iTRAQ) and metabolomics (high-field Fourier-Transform Ion Cyclotron Resonance; FTICR) and their linkage to biological endpoints will be discussed.

Global profiling endocrine function and disruption: comparative and meta-analysis reveals novel mechanisms of action of contaminants.

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[1]University of Ottawa.

We have developed microarrays and proteomic approaches to study brain function in the goldfish. Using commercial oligonucleotide arrays, we have also studied the effects of pulp mill effluents on fathead minnows. *In vivo* effects of estrogens, neuroleptic chemicals, and methylmercury on hypothalamic gene expression has been assessed. Profiles of the effects dopamine and Prozac on brain function are basic mechanistic studies that have provided the framework required to determine the effects of endocrine disrupting chemicals and human pharmaceuticals that pose a risk to teleosts. An "omics" approach has uncovered novel mechanisms and pathways of action, especially for pharmaceuticals, that would be impossible to have predicted using conventional methods. Construction of large datasets derived from fundamental analysis of neuroactive pharmaceuticals can be used to characterize the transcriptional signature of a pollutant or a mixture, and to test hypotheses of their mechanisms of action. Advancement of the use of transcriptional and proteomic methods for risk assessment requires advancement in bioinformatics to accommodate meta-analysis within a comparative genomics framework. Supported by NSERC-Canada.

Contamination dans le parc marin Saguenay- Saint-Laurent
Contamination in the Saguenay-St. Lawrence Marine Park

Long-term chemical contamination of the Saguenay Fjord: A review of past and present situations.

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The Saguenay Fjord has been subject to industrial contamination since the 1940s when aluminum smelters and pulp and paper mills were erected along the Saguenay River. First reports about water and sediment contamination of Saguenay Fjord by trace metals and polycyclic aromatic hydrocarbons (PAHs) appeared in the 1970s. High level

of mercury contamination was first observed in shrimp (*Pandalus borealis*) in 1972 and led to major changes in industrial practices to reduce Hg discharges in the River. Similarly, new regulations were implemented in the early 1970s to reduce PAHs emitted by Soderberg aluminum reduction cells. This presentation will review available data on chemical contamination of the Saguenay Fjord and evaluate temporal changes that occurred in the last 40 years. In addition to trace metals and PAHs, the Saguenay Fjord has been impacted by organotin compounds such as tributyltin (TBT) used until recently in anti-fouling paints for large commercial vessels. We observed in early 2000s a general contamination by butyltin compounds of the Fjord food web up to the beluga whale. Also subject to recent concerns are petroleum-derived hydrocarbons often released by commercial and pleasure navigation. Risks related to these new sources of contamination will be discussed and actions for a cleaner Saguenay Fjord will be proposed.

Developing an iterative process to assess and manage multiple stressors at the regional scale of Saguenay St. Lawrence Marine Park, Canada.

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An ecological risk assessment is being conducted at the regional scale of the Saguenay St. Lawrence Marine Park in Eastern Canada. The objectives are twofold: (1) to assess cumulative risks of multiple stressors on management endpoints and (2) to determine the applicability of the relative risk model (RRM) in an iterative process to assess and manage ecological risks at the regional scale of the Marine Park. In a complex and multi-stressed ecosystem, traditional framework for ecological risk assessment cannot be used. We develop an iterative process based on the RRM framework to integer stressors multiplicity and their potential impacts over the whole marine ecosystem. Methodology consists in building a conceptual model to list stressors and link them from their sources to the habitats and endpoints they could affect. We quantify sources and habitats by relative ranks, and pathways to endpoints by exposition and effect filters to finally integrate them in cumulative risks calculation. Critical to this assessment is the acquisition of field data. In two-year field campaigns we collected required data about sewage discharges and vessel traffic contamination to complete the stressors database (microbiology, nutrients, heavy metals, organic pollutants). First results confirm the general RRM design and point sewages distributed along the shores as a significant source of stress. The highest concentrations of coliforms were found in sediments, water surface and shellfish sampled in plume discharge of effluents. We analyzed high concentrations of iron, cupper, zinc and arsenic in those shellfish. Mollusks and worms showed lead contamination in docking areas and marinas.

The Saguenay-St.Lawrence Marine Park: the challenge of establishing a marine protected area downstream of urban and industrial North America.

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Born from the Great Lakes and headwater regions, the St.Lawrence River becomes the world's largest estuary as it flows from freshwater to the ocean. Life abounds here, yet, as in other parts of the Great Lakes – St.Lawrence watershed, human activity exerts pressure in the form of pollution, coastal development, intensive navigation and fishing. To enhance conservation and to promote public awareness, efforts were initiated in 1970's to establish the Saguenay – St. Lawrence Marine Park, one of Canada's first marine protected areas. The fate of the threatened St.Lawrence beluga whale population, impacted by contaminants coming mainly from upstream, triggered the establishment of the Marine Park in 1998 after decades of planning and negotiations. Furthermore, the park is located at the confluence of two rivers which are also major industrial waterways, therefore allowing the inflow of contaminants and making the area vulnerable to environmental spills. The presentation will touch upon the evaluation of the state of the Marine Park 10 years after its establishment and issues concerning species and risk, ecosystem heath and sustainable use in the marine protected area.

The state of the St. Lawrence hydrographic system. Fluvial and marine ecosystems restoration. Implementation of a legal and regulatory regime for water management. Contribution of science to a better knowledge of the system.

DUFOUR, J. [1]

[1]Comité de coordination du Saguenay-Saint-Laurent.

Based on the last reports published by Environment Canada and other government agencies on the subject, such as the St. Lawrence Action Plan 1988, the Report "20 Years of Action" and the Canada-Québec Agreement on the St. Lawrence 2005-2010, and according to several scientific publications, this presentation deals with the situation that prevails today, examining the recent evolution of the different action plans and programs (science, restoration, rehabilitation, resources management, protection and monitoring) which are underway. It also provides views on the main prospects for the progressive elimination of sources of degradation and pollution.

Connectivity between Saguenay Fjord populations and those of the Gulf of St. Lawrence.

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[1]Ministère des Pêches et des Océans, [2]Environment Canada, [3]Parcs Canada.

L'analyse des marqueurs microsatellites et d'allozymes chez différentes espèces de poissons de fond (morue, flétan du Groenland et sébaste) et de crustacés (crabe des neiges et crevette nordique) montre que les organismes du Saguenay et du Saint-Laurent appartiennent aux mêmes populations. La seule différenciation génétique est observée au

locus Pan I chez la morue. Cette différenciation pourrait toutefois être causée par la sélection, qui agirait dans le fjord du Saguenay, plutôt que par l'isolement génétique de la population. Les données complémentaires disponibles pour les poissons de fond (composition élémentaire des otolithes, morphométrie et faune parasitaire) montrent que les individus capturés dans le Saguenay diffèrent de ceux du Saint-Laurent. Ces différences suggèrent que les individus du Saguenay et du Saint-Laurent passent la majeure partie de leur cycle vital dans des environnements différents. Considérant la très faible survie larvaire observée dans le fjord, cette revue suggère que les populations de poissons de fond du Saguenay constituent des populations puits, dont le recrutement dépend de l'apport de juvéniles depuis le Saint-Laurent. Une fois les individus installés dans le Saguenay, ils y passent la majorité de leur vie. Même si nous ne possédons pas de données complémentaires pour les crustacés, il est possible que le même mécanisme opère chez ces espèces. Ces résultats peuvent aider à mieux comprendre le niveau et la variation de la contamination des spécimens capturés dans le Saguenay.

La laimargue atlantique (requin du Groenland) : résident ou touriste du fjord du Saguenay?

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A l'hiver 2006, un requin du Groenland (*Somniosus microcephalus*) pêché sous la glace dans le fjord a fait la nouvelle. Huit mois plus tard, en septembre, deux autres grands requins ont été capturés accidentellement au large de Godbout sans grande couverture médiatique; un autre requin du Groenland et un requin taupe-commun (*Lamna nasus*). Grâce à ces trois spécimens et à l'analyse de leur tissus hépatique, notre équipe a tenté de répondre à la question maintes fois posée: le requin du Groenland hante-t-il en permanence les eaux du fjord une fois l'embouchure du Saguenay franchie? Il semblerait que oui, selon quelques indices chimiques. D'abord les niveaux de mercure, arsenic et cadmium sont plus élevés chez le requin du fjord que ceux observés chez les requins de l'estuaire. Ensuite, la concentration des polybromodiphényléthers (PBDE), des substances ignifuges d'origine industrielle, est plus faible chez le requin du fjord que chez les requins exposés aux contaminants provenant des Grands Lacs et de la Côte Atlantique Est. Finalement, la mesure des isotopes stables de l'azote (15N) chez les trois spécimens, un indice naturel de position trophique, montre que le requin du fjord occuperait un réseau trophique différent que celui du requin du Groenland de l'estuaire. Ces informations tendent à prouver qu'une fois dans le fjord, le requin du Groenland semble s'y plaire. Paradoxalement, c'est la pollution caractéristique du fjord qui contribue à comprendre un peu plus la place qu'occupe la laimargue atlantique dans l'écosystème marin du Saguenay.

Contaminants anciens III – Organiques et métaux
Legacy contaminants III – Organics and metals

The Îles de la Paix, Lake Saint-Louis: a monitoring area for dioxins and furans in the sediment of the St. Lawrence River/ Les îles de la Paix, lac Saint-Louis : une zone de surveillance des dioxines et furanes dans les sédiments du fleuve Saint-Laurent.

PELLETIER, M. [1], DESROSIERS, M. [2] and MARTEL, L. [2]
[1]Environnement Canada, [2] Ministère du Développement durable, de l'Environnement et des Parcs du Québec.

Dans le cadre du Programme de monitoring et de surveillance de la qualité de l'eau au Québec, Environnement Canada a ciblé les Îles de la Paix comme une zone de surveillance de la qualité des sédiments au sud du lac Saint-Louis. Cette zone est connue depuis de nombreuses années en raison de la présence de fortes contaminations de mercure et d'hydrocarbures aromatiques polycycliques (HAP) provenant d'apports industriels locaux. Les récents travaux de surveillance ont montré la présence de substances réglementées comme les biphényles polyclorés (BPC), les BPC planaires et les dioxines (PCDD) et furanes (PCDF) à des concentrations dépassant les critères de qualité dans les sédiments de surface. Cette présentation portera sur une approche mixte utilisant à la fois l'hydrodynamique, la dynamique sédimentaire et la géochimie. Nous présenterons aussi une analyse statistique des résultats afin d'identifier l'origine potentielle de ces contaminants.

As part of the Québec Water Quality Monitoring and Surveillance Program, Environment Canada has targeted the Îles de la Paix like a sediment quality surveillance area in of the southern part of Lake Saint-Louis. Higher levels of mercury and polycyclic aromatic hydrocarbons (PAHs) concentrations in that area have been known for several years because of the presence of local industrial plans. Given that those substances have been regulated for many years, recent sediment monitoring has found polychlorinated biphenyls (PCBs), planar PCBs, polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) at concentrations that exceed sediment quality criteria in surface sediment. This presentation will look at a blended approach incorporating hydrodynamics, sedimentary dynamics and geochemistry. We will also present a statistical data analysis to identify the potential origin of the contaminants.

Diminution des concentrations de plusieurs substances toxiques dans la rivière Yamaska Nord à la suite d'interventions d'assainissement industriel à Granby.

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[1]Ministère du Développement durable, de l'Environnement et des Parcs du Québec.

Des études réalisées en 1995 ont révélé que la municipalité de Granby était une source importante de substances toxiques pour la rivière Yamaska Nord. Devant ce constat, le MDDEP et des organismes collaborateurs ont élaboré et mis en œuvre le Plan d'action Granby. Dans le cadre de ce plan, 66 entreprises de la municipalité ont été visitées pour y vérifier la conformité à la réglementation environnementale et, le cas échéant, pour y faire diminuer les rejets de substances toxiques. Afin d'évaluer les effets du Plan d'action Granby sur la rivière Yamaska Nord on y a mesuré les concentrations d'un grand nombre de substances toxiques. Les échantillonnages ont été réalisés en amont et en aval de la ville au début du plan, en 2002, et après que ce dernier fut complété, en 2006. Plusieurs contaminants affichent une diminution des concentrations de 2002 à 2006 à la station d'échantillonnage située en aval de Granby. Les diminutions de concentrations sont substantielles et statistiquement significatives, par exemple: 39 % pour les BPC, 50 % pour les dioxines et furannes, 67 % pour les HAP du groupe 1 (cancérigènes), de 6,4 % à 90 % pour de nombreux autres HAP, de 43 % à 99 % pour certains composés organiques volatils ou semi-volatils, 84 % pour les nonylphénols éthoxylés, de 40 % à 85 % pour certains acides gras et de 8,4 % à 67 % pour plusieurs métaux.

Mecanisms of phenols removal in woodwaste leachate by trickling biofilter.

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Selon des rapports internes du Centre de Recherche Industrielle de Québec (CRIQ), ainsi que des rapports du ministère du Développement durable, de l'Environnement et des Parc, le Québec est au prise avec une quantité considérable de résidus de transformation du bois (poussières de bois, rejets de tamisage, sciures) et d'écorces produits par les scieries et les usines de pâtes et papiers. Étant donné que l'industrie du papier est l'un des leviers économiques les plus importants, il faut développer des méthodes de gestion et de traitement des déchets générés suites aux activités des scieries et de papeteries. Les résidus de bois produisent un volume important de lixiviat généré par percolation des résidus par les eaux pluviales ou par les eaux de procédés suites aux activités de production. Ce lixiviat contient des concentrations élevées de composés phénoliques dont la gestion et le traitement s'imposent pour réduire les impacts environnementaux. Avant de traiter ces rejets, il fallait développer des méthodes de détection et de quantification fiables, rapides et efficaces pour l'analyse de ces composés dans de telles matrices. Deux biofiltres pilotes ont été conçus ensuite pour le suivi des mécanismes d'enlèvement des phénols en continu. Les paramètres cinétiques de biodégradation des phénols ont été déterminés par respirométrie et la validation des modèles décrivant le processus a été effectuée. Un bon rendement épuratoire a été obtenu pour la DCO, la DBO et les composés phénoliques.

Woodwaste produces large volumes of leachate, which often contains high concentrations of phenolic compounds. These compounds are environmental contaminants whose proper management and treatment are mandated to reduce associated environmental impacts. Quality diagnostic and treatment efficiency assessments necessitate the development of rapid, accurate, and reproducible methods of detection and analysis to accurately quantify phenolic compounds before the process treatment. Two trickling biofilters were designed for continuous mechanisms study. Biokinetic parameters determination was carried out by respirometric measurements during phenols biodegradation in the batch reactor; Haldane model described well the biodegradation of phenols and a good efficiency for COD and BOD elimination was reached.

Suivi des métaux à l'état de trace dans les rivières du Québec.

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Entre 2004 et 2007, les concentrations de métaux traces ont été documentées dans une trentaine de rivières du Québec. Les échantillons étaient acidifiés sur le terrain et analysés en salle propre par ICP-MS après décantation. Cette méthode présente des différences par rapport aux méthodes reconnues dans la littérature. En 2008, un suivi a été réalisé dans 10 rivières afin de comparer 5 méthodes de prétraitement des échantillons: métal dissous avec filtration sur le terrain, métal dissous avec filtration en laboratoire, métal soluble à l'acide avec filtration, métal soluble à l'acide avec décantation et métal extractible total. Pour la plupart des métaux, la filtration sur le terrain et en laboratoire donne des résultats comparables. La méthode « soluble à l'acide avec décantation » donne des résultats analytiques supérieurs en moyenne de 20 % par rapport à la méthode « soluble à l'acide avec filtration » et inférieurs en moyenne de 15 % par rapport à la méthode « extractible total ». En 2008, pour tous les métaux à l'exception de l'aluminium, du fer, du béryllium, du cuivre et du plomb, il n'y a eu aucun dépassement des critères de qualité pour la protection de la vie aquatique et ce, peu importe la méthode de prétraitement des échantillons. Entre 2004 et 2007, la méthode « soluble à l'acide avec décantation » a engendré des résultats similaires.

Spatio-temporal evaluation of heavy metal concentrations in mussels (*Mytilus edulis*) from the Baie des Chaleurs, New Brunswick.

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Since the seventies, high concentrations of lead, cadmium and mercury have been reported in sediments and biota from sites of the Baie des Chaleurs. However, no ecosystemic study looking at the overall state of contamination in fish has been conducted in this region. The objective of this study is to establish a spatio-temporal distribution of heavy metals in mussels from the Baie des Chaleurs. In the summers of

2006 and 2008, we sampled blue mussels *(Mytilus edulis)* in various sites along the New Brunswick coastline of Baie des Chaleurs. Results show high mercury and lead levels in mussels near the industrial centers, which exceed the maximum levels allowed for commercial sales (0.5 $\mu g \cdot g^{-1}$ ww for both metals). Mussels from Belledune have concentrations of cadmium (4.15 $\mu g \cdot g^{-1}$ ww) and zinc (41.5 $\mu g \cdot g^{-1}$ ww) up to four times higher than in other sites. Concentrations of lead (28.9 $\mu g \cdot g^{-1}$ ww) are up to ten times higher than in the other sites. Moreover, mercury concentrations in mussels near the chloralkali plant in Dalhousie (0.92 $\mu g \cdot g^{-1}$ ww) are forty times higher than at the other sites. Cd concentrations in mussels sampled in 2006 are higher than those fished in 2008 in all sites. Other metal concentrations in mussels are comparable to other regions in Canada.

Examination of Metal Contamination within the UNESCO Designated Rideau River Waterway.

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[1]Queen's University.

The Rideau Canal is a UNESCO World Heritage Site comprised of a series of lakes connecting Ottawa to Kingston, ON. In 2006, a preliminary study evaluated element concentrations in sediment and water from nine of these lakes. The mean sediment concentrations exceeded Canadian Federal guidelines (in brackets) for Cd (0.6 $mg \cdot Kg^{-1}$), Hg (0.17 $mg \cdot Kg^{-1}$), Pb (35 $mg \cdot Kg^{-1}$) and Zn (123 $mg \cdot Kg^{-1}$) within each of the studied lakes and Cu (35.7 $mg \cdot Kg^{-1}$) within all but one lake. The mean concentrations for Cd, Hg, Pb and Zn, within the lake with the highest overall concentrations in 2006 (Indian Lake) ranged between 0.4 - 3.5 $mg \cdot Kg^{-1}$; 0.02 - 0.52 $mg \cdot Kg^{-1}$; 5.2 – 327 $mg \cdot Kg^{-1}$; and 30.3 - 306 $mg \cdot Kg^{-1}$, respectively, for surface sediment samples. Utilizing paleolimnological techniques, historical element records indicate that THg peaked in the mid-1980s, at concentrations almost five times background concentrations, above the Federal Probable Effect Level (PEL). Cd, Pb and Zn were all found to peak around 1979, at concentrations ranging from 4.1 to 17.2 times background levels. While peak enrichment ratios for Indian Lake fall within the normal range for most elements, the enrichment of Pb is particularly high. The objective is to examine the spatial and temporal distributions of anthropogenic elements to a headwater lake. With the high rate of recreational use, especially for sport fishing, it is imperative to recognize the rate of metal influx to the system in order to protect both human and aquatic health.

Mercury and element accumulation in a freshwater food web of a Chinese reservoir.

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[1]Queen's University.

Freshwater fish are an important constituent of the Chinese diet, and the use of reservoirs for the capture and culture of fish is widespread in China. This study focused on an important reservoir in the Eastern Plains Lake Region of China, where elevated concentrations of mercury in top trophic predators such as yellow catfish (*Pelteobagrus*

fulvidraco) were found. Preliminary trace element results indicate that wild and farmed fish species had markedly different concentrations. In this study, we will examine food web biomagnification trends for mercury and several elements for both wild and farmed fish. In 2008 and 2009, fish of several feeding guilds were collected from markets and from fishermen. Stable isotope results indicate distinct isotopic patterns among some fish species collected at different markets, suggesting some of the wild fish species brought in the market may not be from the reservoir, thereby providing a useful measure not only of food web structure, but also confirming the origin of wild-caught fish. Mercury and element bioaccumulation trends, as well as the importance of fish origin will be discussed. This study contributes essential risk assessment data that is presently lacking for many Chinese fish species, and will help to evaluate the importance of the residual effects of reservoir creation in freshwater environments.

Impact of the Sainte-Marguerite 3 hydroelectric reservoir on the mercury exposure of local fish consumers.

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The Sainte-Marguerite-3 hydroelectric generating station, with an installed capacity of 882 MW, required the impoundment of a 315 km^2 reservoir, of which 262 km^2 consisted of flooded land. As expected, 7 years after the impoundment of the SM 3 reservoir, fish mercury levels increased by factors ranging from 4 to 8 according to the main fish species. Total mercury concentrations reached 0.78 $\mu g \cdot g^{-1}$ (ww) in 400-mm Lake Whitefish and 1.85 $\mu g \cdot g^{-1}$ (ww) in 700-mm Northern Pike. In partial fulfilment of the authorization conditions, Hydro-Québec, in collaboration with Santé Québec and Health Canada, carried out fish consumption and mercury exposure surveys in 1997, before impoundment and in 2006, 9 years after flooding. Compared to pre-impoundment conditions, non-native fishers consumed significantly more local fish on a monthly basis, while native Innu fishers consumed significantly less fish, for reasons unrelated to the mercury issue. The average hair mercury concentration remained unchanged for the non-native fish consumers at 0.9 $\mu g \cdot g^{-1}$, while it decreased significantly from 0.9 to 0.5 $\mu g \cdot g^{-1}$ for native fish consumers. For both native and non-native women of child-bearing age, the average hair mercury levels remained unchanged at 0.3 $\mu g \cdot g^{-1}$.

Indicateurs au niveau de la communauté
Community Level Indicators

Le suivi de la qualité de l'eau des rivières à l'aide de l'Indice Diatomées de l'Est du Canada (IDEC).

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Les diatomées sont des algues microscopiques qui vivent en suspension dans l'eau ou attachées sur le fond des lacs et des rivières. De part leur sensibilité aux nutriments (surtout le phosphore et l'azote) et à la matière organique, les diatomées sont un excellent indicateur du niveau d'eutrophisation des cours d'eau. L'indice Diatomées de l'Est du Canada (IDEC) fut développé afin de comparer le niveau de dégradation des cours d'eau et de mesurer la « distance écologique » qui sépare un cours d'eau pollué d'un cours d'eau de référence. Les classes de l'IDEC ont été délimitées à partir de seuils écologiques. Le passage d'une classe à l'autre signifie que des changements importants se sont produits dans l'écosystème qui ont eu pour effet de modifier en profondeur la communauté de diatomées. L'échelle de l'indice reflète toute la gamme des perturbations rencontrées dans les rivières et les ruisseaux de l'Est du Canada (Québec, Ontario et Maritimes). Entre 2005 et 2009, l'indice fut utilisé pour effectuer le suivi de la qualité de l'eau dans une quinzaine de bassins versants du Québec. Les organismes qui ont commandés ces études sont majoritairement des organismes de bassin versant, des comités de restauration et des institutions de recherche. Dans le cadre d'un programme de restauration d'une rivière et de son bassin versant, le calcul de l'IDEC avant et après les interventions permet de mesurer l'impact réel du programme de restauration sur la qualité de l'eau en général et sur le niveau d'eutrophisation en particulier.

La surveillance biologique basée sur les macroinvertébrés benthiques au Québec.

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Les macroinvertébrés benthiques sont les organismes les plus couramment utilisés comme indicateur biologique. Ils sont reconnus pour être de bons indicateurs de l'état de santé global des écosystèmes aquatiques. Puisque ces organismes sont en lien direct avec la qualité de l'eau et de leur habitat, ils intègrent les effets cumulatifs et synergiques à court terme (quelques années) des multiples perturbations physiques (modifications de l'habitat), biologiques et chimiques des cours d'eau. En se basant sur l'expérience des États-Unis, du Canada, de la Nouvelle-Zélande, de l'Australie et de l'Europe, un projet pilote a été initié au Québec en 2002. Ce projet vise à développer une méthodologie rapide (échantillonnage, traitement des données, indice biologique, etc.) s'appliquant à des petits cours d'eau. Fondé sur les conditions de référence, ce projet pilote nécessite la recherche de stations de référence pour définir les conditions dites « normales » pour les

communautés de macroinvertébrés, c'est-à-dire celles qui ne subissent pas d'effets de l'altération de leurs habitats ou de la qualité de l'eau. Cette nouvelle approche rapide est développée dans l'optique de la mise en place d'un réseau de surveillance biologique des macroinvertébrés benthiques permettant l'évaluation de l'intégrité écologique des écosystèmes aquatiques et de fournir un outil de diagnostic pour l'analyse des bassins versants. Une évaluation de la faisabilité de mettre en place un programme québécois de surveillance volontaire des communautés de macroinvertébrés benthiques (SurVol Benthos) est également en cours à la Direction du suivi de l'état de l'environnement (MDDEP). Cette initiative est motivée par les demandes des organismes de bassin versant et communautaires pour des outils simples visant une caractérisation générale de la santé des cours d'eau.

Assessing toxic sediment pollution in streams using bio-ecological traits of benthic macroinvertebrates.

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Faunal and chemical data from mountain streams in France were analysed to examine the accuracy of a multimetric approach based on biological and ecological traits of benthic macroinvertebrate communities to assess toxic sediment pollution in streams. A toxic quality class (from 'high' to 'bad') was pre-assigned to each site using the French water quality assessment system based on toxic substances (metals, PAH, PCB) in sediment. Each trait was described in multiple categories. The affinity of macroinvertebrate taxa for the different categories of a trait was described using a fuzzy coding procedure. The relative abundance of trait categories was calculated for communities at each site. A non-parametric multiplecomparison statistical procedure was used to compare relative abundances of trait categories between groups of sites assigned to different quality classes and to identify the combinations of trait categories that best separated sites between adjacent toxic quality classes. Based on such sets of trait categories, we propose a statistical procedure to allocate sites to toxic quality classes from the attributes of their benthic macroinvertebrate community. This study shows that biological criteria based on macroinvertebrate traits could provide new methods for biological assessment. This is the first step towards an *in situ* functional tool of stream sediment contamination assessment at community level. However, we need to increase the number of sites to improve the tool's precision, extend the procedure to additional stream types and validate the design at a larger spatial scale.

Utilisation de la méthode « Pollution Induced Community Tolerance (PICT) » pour évaluer les effets de contaminants: Application aux biofilms naturels.

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L'adaptation des communautés microbiennes aux toxiques peut être caractérisée par leur tolérance à ces toxiques, exprimée par des valeurs de CE50 relatives à un contaminant et basée sur un indicateur biologique donné. Nous avons appliqué l'approche PICT sur des biofilms aquatiques drainant un bassin versant viticole et exposés à des pesticides. Quelle que soit la saison, les CE50 montrent une tolérance microbienne croissante d'amont en aval, liée à l'augmentation des concentrations en pesticides dissous. Ceci suggère une augmentation progressive des taxons tolérants et une extinction des taxons les plus sensibles. Des analyses de diversité et structurelles de la communauté microbienne confirment les changements de la composition taxonomique. L'approche PICT a permis d'évaluer la résilience du biofilm, par transfert de biofilms d'un site contaminé vers un site amont propre, en caractérisant l'évolution de la tolérance du biofilm. Selon le paramètre, jusqu'à 9 semaines sont nécessaires pour obtenir une résilience partielle. Cette durée, plutôt longue pour des microorganismes, a été associée à la persistance de contaminants (Cu) adsorbés dans le biofilm. La tolérance des biofilms serait alors principalement sous le contrôle des concentrations internes en contaminants.

Can aquatic biomonitoring detect nutrient-masked, sublethal effects?

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Run-off from agricultural drainages contains complex mixtures of sediments, nutrients and contaminants entering streams as complex mixtures. Although we know that pesticide inputs can reshape the structure and function of aquatic food webs, the role of nutrients as a modifier of pesticide effects is poorly understood. In response to this knowledge gap we are examining the interactions of an insecticide with respect to a nutrient addition gradient. In a series of mesocosm experiments, we explored a gradient of concentrations of an insecticide (imidacloprid) on freshwater invertebrates. Using classic approaches, we examined the responses of benthic macroinvertebrate communities with respect to nutrients regime and insecticide dosing. We found that metrics of benthic community structure were insensitive to the sublethal effects of insecticide exposure; whereas the abundance of adult insects emerging from treatments streams and the body size of mayflies (*Ephemeroptera*) were excellent predictors of insecticide and nutrient treatment. The trophy of the system (oligotrophic to mesotrophic gradient) was also found to be important driver in the improved success of sensitive species under sublethal exposure regimes. These findings indicate that existing approaches are insufficient to detect nutrient-insecticide interactions, particularly in the sublethal effect range where, presumably, most effects occur.

Relationships between taxon-specific tolerances of cadmium and profundal macrobenthic community structure in cadmium-contaminated lakes.

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Information on functional traits such as taxon-specific tolerances of metals can be of use in accounting for spatial variation in the composition of benthic invertebrate communities in contaminated lakes, thereby linking ecological disturbance with particular anthropogenic stressors. Profundal benthic communities from 10 lakes ranging in degree of metal contamination from a smelter in Abitibi, Québec, were assessed for variability in taxon densities. Variability among sites was analyzed in relation to concentrations of cadmium and other metals in sediment and overlying water, multi-species responses to lake sediment in laboratory toxicity tests, and generic-level Cd sensitivity data from published studies. Benthic communities in the two most contaminated lakes were distinctly impoverished compared to those in less contaminated lakes. The comparatively Cd-tolerant midge *Chironomus* dominated in the contaminated lakes, whereas the more Cd-sensitive amphipods, mayflies and sphaeriids, were reduced or absent. Although the oligochaete worm *Tubifex* is also indicated as tolerant to Cd, based on published studies and toxicity tests with sediment from the contaminated lakes, other tubificid genera native to the study area were notably absent from the contaminated lakes. Many taxa had "limiting factor"-type relationships to concentrations of Cd in sediment among sites. These results show how functional traits such as taxon-specific ecotoxicological responses to stressors can help understand impacts of human activities on benthic communities.

Influence of environmental factors at various scales on littoral benthic communities of Lake Saint-Pierre, St. Lawrence River.

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Benthic communities occurring in littoral areas are directly exposed to the conditions of the environment and to pollution-related stresses. However, the effects of the environment on the structure of benthic communities in the littoral ecosystems of the St. Lawrence fluvial lakes are not well understood, mainly due to the tremendous complexity of these ecosystems. Our study is the first step in describing the health of the macroinvertebrate community in Lake Saint-Pierre, with a view to establishing a long-term monitoring program. Sampling covered both the north and south shores of the lake, which are characterized by water masses with contrasting physical and chemical properties: mineralized water from the Ottawa River and loaded with humic acid on the north shore, and low mineralized water from the Great Lakes on the south shore. The spatial distribution of our sampling stations takes account of their exposure to known anthropogenic stresses such as agricultural runoff, and also of natural stresses like seasonal water level. The effects of environmental variables acting at various spatial and

temporal scales (e.g. nutrients, heavy metals and landscape) on the density and taxonomic composition of benthic invertebrate communities were explored.

Relating macroinvertebrate community structure to environmental characteristics and sediment contamination at the scale of the St. Lawrence River.

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There is still no assessment of the impact of sediment chemicals and environmental conditions on macroinvertebrate community at the scale of the St. Lawrence River continuum. This study aims to characterize the community structure of macroinvertebrates in the fluvial section of the St. Lawrence River, including the three fluvial lakes and the Montreal harbour, using several taxonomic levels (genus and family) and data (abundance, presence/absence and indicator taxa). The goal of the study is to relate changes in macroinvertebrate community to sediment chemical conditions and environmental characteristics of habitats. Furthermore, this study also aims to determine the taxonomic level and the data suitable for a good assessment of the relative contribution of sediment chemical conditions and environmental characteristics of habitats using variance partitioning. Four different macroinvertebrate assemblages were found distributed along environmental gradients in the fluvial continuum when using both abundance and presence/absence data at the genus and family levels. Specific indicator taxa were characteristics of the different macroinvertebrate communities and were associated with the contamination gradient. Environmental characteristics of the habitats (water masses, sulphur and DOC in sediments) had higher influence on macroinvertebrate assemblages than sediment chemical conditions, with strong interactive effects. However, together they explain less than 30% of the total variance in macroinvertebrate communities. Our study confirms that family level can give comparable information to genus level using presence-absence or abundance of macroinvertebrates; however, a higher number of indicator taxa were detected at the genus level.

Functional traits of benthic macroinvertebrates as alternative tools for ecological risk assessment (ERA): the case of the St. Lawrence River.

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The St. Lawrence River is an essential waterway for North America and is exposed to many anthropogenic stresses such as industrial and municipal wastewater and agricultural activities. This study is a part of a large research project aiming at developing

an ERA tiered framework for sediment management, in the context of integrated management of contaminated sediment, site restoration and sustainable navigation. The purpose of this part of the study is to assess sediment quality by exploring the relationships between chemical contamination and benthic community structure using functional trait approaches. During falls 2004-2005, macroinvertebrates were collected in 59 sites in the St. Lawrence River, especially in its three fluvial lakes and in the harbour zone of Montreal. Organic (PCBs, PAHs, petroleum hydrocarbons) and inorganic (As, Cd, Cu, Cr, Hg, Ni, Pb and Zn) contaminants and sediments characteristics (e.g. grain size, metal-binding phases, nutrients) were measured in whole sediment. The functional trait approach based on the benthic macroinvertebrate compartment was adapted for the St. Lawrence River. To build the matrix of traits, we conducted bibliographical research on the biological traits (e.g. size, morphology, life cycle) or ecological traits (e.g. preferences for different habitat characteristics) that can potentially inform of ecosystem functioning, at the genus level, for all the benthic macroinvertebrates of the specific ecozone of the Québec region. The traits were coded by taking into account regional peculiarities of climate and the type of ecosystem. The relative influence of the chemical contamination and the environmental characteristics on the structure of the bioecological traits was estimated by multivariate analyses. The results of station clustering according to the traits, significant indicator traits according to the clusters and their relations with the explanatory variables will be presented.

Tests de toxicité
Toxicity Tests

Luminotox: Development of an algal test battery for water quality monitoring.

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A biosensor based on the measurement of photosynthetic activity, the LuminoTox, has been developed by Lab-Bell and successfully incorporated into field assessment for the detection of toxic molecules in water. The photosynthetic electron transport chain is the target of several toxic molecules found in water, affecting the growth of phytoplankton and aquatic plants. This characteristic makes the photosynthetic process very effective in the detection of water pollutants. A handheld fluorescence apparatus, included in the LuminoTox test, was designed to measure photosystem II photochemical yield. The presence of toxic agents altering the photosynthetic electron transport chain is measured by the decrease in the photochemical yield given an impact index of the chemical contamination in the sample (ICC). Good results are obtained to manage wastewater treatment in municipalities and industries by the LuminoTox with PEC, made with photosynthetic enzyme complexes extracted from spinach. The PEC is not sensitive enough to new problematic molecules to insure water quality monitoring. However the SAPS, algae, were seen to be more sensitive to HAP, pesticides, metallic cations etc. than PEC. The use of algae broadens the kind of molecules detected if not to a lower

concentration. Furthermore, by using different species of algae we detect other type of molecules including pharmaceutical derivatives and endocrine disruptors. Comparative results are presented using photosynthetic enzyme complexes (PECs), isolated from spinach; freshwater green algal species *Chlorella vulgaris* and *Chlamydomonas reinhardtii*; cyanobacteria *Anabaena flos-aquae*. Different protocols (incubation time, illumination conditions, pH, temperature, algae concentration) were tested to improve toxicity detection.

The impact of calcium and food upon the reproduction and survival of *Daphnia pulex*: an *in situ* bioassay.

CAIRNS, A. [1] and YAN, N. [1]

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The decline in aqueous calcium (Ca) is a newly recognized stressor in freshwater ecosystems in regions with historically high acid deposition, especially when coupled with multiple logging cycles. Daphniids have high Ca demands due to their heavily calcified exoskeleton and regular moult cycle, and rely on aqueous Ca for the majority of their Ca uptake. Reproductive delay in *D. pulex* has been detected in the laboratory at 1.0-1.5 mg $Ca \cdot L^{-1}$, and 60-100% mortality is observed at 0.1-0.5 mg $Ca \cdot L^{-1}$. We chose the same *D. pulex* clone to determine if lab-defined thresholds could be replicated in the field. We ran 16-day bioassays in 4 lakes at nominal concentrations of 1.0, 1.3, 1.5, and 2.5 mg $Ca \cdot L^{-1}$. Individual *D. pulex* were held in 50mL vessels open to lake water. We added lab-cultured algae (1 mg $C \cdot L^{-1}$) to one treatment and exposed another to raw lake water and natural food levels only. Reproduction occurred in the food treatment only. Cox proportional hazard models showed an effect of Ca upon daphniid survival when results were pooled; however, when our food treatment was analyzed separately, Ca did not significantly affect survival. In the raw lake treatment, survival was not possible below 1.3 mg $Ca \cdot L^{-1}$. We hypothesize that food-supplemented daphniids use the extra energy to compensate for increased metabolic rates at low Ca. The field bioassays demonstrate that food will affect the response of *D. pulex* to Ca, and may confound experimental results.

Sensitivity and precision of acute *Daphnia magna* tests performed with organisms from laboratory cultures of hatched from dormant eggs

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The presentation brings forward and discusses the major facts and figures of an extensive review on the sensitivity and precision of the acute *Daphnia magna* test, elaborated by nine ecotoxicologists from different countries.The acute *D. magna* assay is one of the most internationally used bioassays for toxicity screening of chemicals and for toxicity monitoring of effluents and contaminated waters. Standard methods have been developed for this assay which are now prescribed by national and international organisations for application of this toxicity test within a regulatory framework.

Historically, the organisms used for the acute *D. magna* assay are taken from live stocks which are cultured in the laboratory, usually on live micro-algae and mostly with laboratory specific "recipes". The standard protocols of this assay therefore differ to a certain extent in different countries on some procedural aspects.

Because of the technical and biological problems inherent in year-round culturing and availability of the biological material, as well as the culturing/maintenance costs of live stocks, the application of this very popular bioassay is nevertheless restricted worldwide to a relatively low number of highly specialised laboratories. Attempts have been made to bypass the former bottle-necks and have led in the early 1990s to "culture/maintenance free" aquatic microbiotests. These assays with species of different phylogenetic groups were developed at the Laboratory for Environmental Toxicology and Aquatic Ecology at Ghent University in Belgium and were given the generic name "Toxkits". The latter microbiotests are unique in that they employ dormant eggs of the test species, which can be stored for long periods of time and "hatched" at the time of performance of the assays. One of these microbiotests, the Daphtoxkit F magna, makes use of *D. magna* neonates hatched from the dormant eggs and is now used in many laboratories worldwide for research as well as for toxicity monitoring.

Since the acceptance (or possible non-acceptance) of assays is intrinsically based on "sensitivity" and "precision" criteria, a review has recently been elaborated to address these questions, for assays performed with either *D. magna* test organisms obtained from lab cultures or hatched from dormant eggs. This extensive document with many tables and figures is based on the scientific literature on one hand, and on data obtained from various laboratories on the other, and the major findings and conclusions of the review are briefly outlined here.

Comparison of quality control (QC) data on the acute *D. magna* toxicity test revealed that virtually all the results from assays performed with Daphnias taken from lab cultures or with *Daphnia* microbiotests are within the acceptability range set by ISO standard 6341 for the reference chemical potassium dichromate, and that the mean 24h EC50s of the *Daphnia* microbiotests performed in different laboratories are within the range of the mean EC50s of the assays based on lab cultures, with similar variation coefficients. In addition, it was also found that the (in house) precision of the quality control *Daphnia* microbiotests is as good as that of the QC tests based on lab cultures.

The collected results of intra-laboratory sensitivity comparison studies performed during the last 15 years by different laboratories on pure chemicals and on natural samples, with both laboratory cultured organisms and Daphnias hatched from dormant eggs, also revealed a similar sensitivity of the two types of test organisms.

Data on proficiency ringtests which have been organised in different countries since 2002 on the acute *D. magna* assay with either reference chemicals or with natural samples, and with either *Daphnia* microbiotests and/or with lab cultured Daphnias, also indicated that the sensitivity of the two types of neonates is similar. In most cases the precision of the *Daphnia* microbiotest was even found to be superior to that of the assays based on lab cultures.

Last but not least, it could also be deduced from the scientific literature that that there were no sensitivity differences between Daphnias hatched from dormant eggs produced by different *D. magna* strains.

Because of its technical as well as cost advantages, the *Daphnia* microbiotest is now in current use in several countries for toxicity testing in a regulatory framework, with about 10,000 assays performed annually with neonates hatched from dormant eggs.

On the basis of the extensive scientific evidence provided in the review and the fact that other ISO methods now already allow use of test organisms hatched from dormant eggs, the authors of the review recommend that this possibility should also be incorporated in national and international standards on the acute *D.magna* assay, as an alternative to the use of Daphnias taken from laboratory cultures.

The 29-page review has recently been published in the "free access" periodical "Knowledge and Management of Aquatic Ecosystems" (Persoone et al., 2009).

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A comparison of two automated systems used in invertebrate behavioural quantification.

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As part of a project looking at developing an early-warning biomonitoring system for drinking water, two automated systems used in invertebrate behavioural quantification are compared. The first is the MFB Multispecies Freshwater Biomonitor® (LimCo Int.), a non-optical system, which uses electrodes to measure changes in the current caused by movements of the organism. It is a non-optical BEWS that is based on quadrupole impedance conversion techniques. The system is flexible, modular and uses 24 channels, each containing an individual animal. *Daphnia magna, Hyalella azteca*, *Chironomus tentans*, and *Lumbriculus variegatus* were chosen as test organisms with this system. The second system, the ECOTOX®, is one of many image analysis systems in existence today. It is an automatic real-time behaviour analysis system that tracks several movement parameters of the organisms. *Euglena gracilis*, *Daphnia magna*, and *Hyalella azteca* were chosen as test organisms with this system. Endpoints measured are swimming velocity, motility, erratic swimming, and position in water column. The contaminants used for the comparison of the two systems are organometal (Tributyltin), a pesticide (Atrazine), a pharmaceutical (Ciprofloxacin), and a brominated flame-retardant (BDE47).

Methods for the assessment of feeding parameters in common laboratory assay organisms.

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[1]University of Saskatchewan.

Organisms such as *Hyalella azteca* and *Chironomus tentans* are routinely used in the assessment of sediment toxicity. Given their widespread use in freshwater toxicity testing, it is important to understand the feeding mechanisms and digestive processes by which these animals process sediments. In particular, for studies of contaminant uptake it is important to understand the time and conditions required for organisms to clear their gut contents. We used fluorescent particles to investigate the uptake and clearance of sediments by *H. azteca* and *C. tentans*. *Hyalella azteca* accumulated both 1 and 10 micron fluorescent particles when exposed to particle-spiked pureed fish food on clean sand, although a significant time-lag in uptake was observed. However, *H. azteca* avoided fluorescent particle ingestion when exposed to particle-spiked sediment, even after 48 h. Conversely, *C. tentans* rapidly accumulated both 1 and 10 micron particles when exposed to particle-spiked sediment. After 4 h of exposure most chironomids showed accumulation of particles throughout the length of the gut. After 12 h of depuration (on a clean sand substrate with pureed fish food) most chironomids had cleared all fluorescent particles out of the gut. Using these relatively simple techniques the feeding behaviours and physiology of test organisms can be evaluated to permit better control and refinement of bioassay procedures.

Biological assessment of zinc using spiked sediments and the echinoid embryo/larval sediment-contact test.

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[1]Environment Canada.

Method development research using an echinoid embryo/larval sediment-contact test has been conducted over the past several years by Environment Canada's Pacific and Atlantic Laboratories for Environmental Testing. Echinoid embryo/larval sediment-contact tests were conducted with four test echinoid species: *Lytechinus pictus*, *Arbacia punctulata*, *Stronglyocentrotus purpuratus* and *Dendraster excentricus*, on four field-collected sediments of varying grain size and organic carbon composition spiked with zinc. Spiked Sediment Toxicity Tests (SSTTs) were conducted to provide additional toxicological and chemical data on zinc to the Canadian Council of Ministers of the Environment (CCME) to aid in deriving Canadian Sediment Quality Guidelines (SeQGs). The target concentrations were spiked at levels based on the Canadian interim sediment quality guidelines level (ISQG) and probable effects levels (PEL) for zinc in marine sediment (CCME, 1999). The concentrations spiked were 9 times the PEL (2439 $\mu g \cdot g^{-1}$ Zn), 3 times the PEL (813 $\mu g \cdot g^{-1}$ Zn), at the PEL (271 $\mu g \cdot g^{-1}$ Zn), at the ISQG (124 $\mu g \cdot g^{-1}$ Zn) and the natural zinc content of the test sediments (no spike or control). The zinc concentration in the test sediment and overlying water was confirmed by chemical analysis. A summary and discussion of the test method and results will be presented.

A test suite for the assessment of soil contaminant toxicity using indigenous boreal forest soil microorganisms.

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Currently, ecological risk assessments do not include measures of soil microbial health because of a lack of available standardized test methods. As soil microorganisms play a significant role in terrestrial ecosystem nutrient cycling and organic matter decomposition, it is imperative that methods be developed to assess the toxic effects of soil contaminants on indigenous soil microbial communities. The development of these methods directly supports Canadian provincial and federal regulatory efforts to deal with contaminated land issues. Endemic soil microbial communities vary from site to site; therefore a set of tests with different assessment endpoints is needed. Microbial health tests are currently being employed to assess microbial activity, biomass, as well as diversity and community structure. The measurement of soil microbial activity will be pursued using bait lamina, substrate induced respiration (SIR), microbial soil respiration, nitrification, and organic matter decomposition methods. The fumigation/extraction (FE) method will be used to evaluate soil microbial biomass. Soil microbial diversity and community structure will be examined using denaturing gradient gel electrophoresis (DGGE), community level physiological profiling (CLPP), and enzyme assays. Preliminary results for petroleum hydrocarbon contaminated soils analyzed using the CLPP, DGGE, bait lamina, FE, and nitrification tests will be discussed.

Ontario's new laboratory bioaccumulation methods: the journey through development, evaluation, and validation.

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As toxicity tests and biological assessments have and are increasingly being used in industrial and environmental monitoring and regulatory programs, there has been pressure to standardize test methods. Numerous standard methods have been published for assessing the toxicity of effluents and sediment; however there is still a need to standardize methods which measure the bioaccumulation of sediment-associated contaminants. Despite the regulation of discharges, historical releases have lead to the contamination of sediment with toxic and persistent chemicals, which can move into the food chain through the process of bioaccumulation, posing a threat to wildlife and human health. As part of its commitment to the Canada - Ontario Agreement, the Ontario Ministry of the Environment (OMOE; in conjunction with the University of Guelph) has undertaken work on the development and standardization of sediment bioaccumulation methods. This presentation will outline the OMOE's new laboratory bioaccumulation method and summarize the various stages of method development, from a critical review of methods in the literature, to the selection and standardization of test species and exposure techniques, an evaluation of the bioaccumulation potential between species, and validation of laboratory methods with *in situ* field data. The goal of this research was to

develop a robust and effective method that could easily be applied to environmental monitoring programs provincially, nationally and internationally, and considered for use with new and emerging contaminants of concern.

Acute and chronic toxicity of high use pesticides on the sand shrimp (*Crangon septemspinosa*) in salt water.

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Pesticide runoff from potato fields has been of concern to the public and regulators in the Atlantic Region because of environmental effects such as fish kills. Pesticides which wash off agricultural fields are often quickly transported to estuarine or marine environments. Invertebrates in coastal marine systems are often sensitive to pesticides and there is a need to determine the sensitivities of locally important marine invertebrates. Our objectives were to measure acute toxicities, and to develop a chronic sublethal toxicity test using sand shrimp (*Crangon septemspinosa*) in order to study pesticide toxicity at environmentally realistic concentrations. Wild sand shrimp, a marine decapod crustacean, were collected from shallow lagoon beaches in Kouchiboguac National Park. 96-h acute toxicity tests were conducted with ten in-use pesticides (atrazine, azinphos-methyl, chlorothalonil, chlorpyrifos, diflubenzuron, endosulfan, glyphosate, hexazinone, methamidiphos, and methoprene) and results were used as range-finding tests for subsequent chronic exposures. Sand shrimp were also tested for chronic sensitivity to seven pesticides (atrazine, carbofuran, chlorothalonil, chlorpyrifos, deltamethrin, diflubenzuron and endosulfan) during 14-day exposures and three pesticides (carbofuran, diflubenzuron and atrazine) during 28-day exposures. The bioassays consisted of 20 replicate test containers per test concentration with each container holding one animal. Animals were fed and test solutions renewed three times per week. Chronic testing found no noticeable change in lethal concentrations or growth effects when the test exposure period was 14 days compared with 28 days. A standardized test procedure was developed using a 14-day exposure period.

Mécanismes de toxicité
Toxicity Mechanisms

Effect of injection time on the toxicity of 2,3,7,8-tetrachlordibenzo-p-dioxin to newly fertilized Japanese medaka (*Oryzias Latipes*) embryos.

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Japanese medaka (*Oryzias latipes*) are an ideal surrogate species to use when studying embryotoxicity of freshwater fish. Most toxicity testing on medaka embryos involve waterborne chemical treatments; however the actual dose transported into the developing embryo is difficult to estimate using this method. By injecting the chemical directly into the yolk sac, a known dose is delivered to the embryo. However, the

injection of multiple treatments and hundreds of eggs requires several hours during which time the embryos begin to develop. For this reason, it is important to understand the effect of the time from fertilization on the toxicity of injected chemical. In the current study, Japanese medaka embryos were exposed to 7.5 $pg \cdot nL^{-1}$ and 0 $pg \cdot nL^{-1}$ of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in a filtered triolein carrier via micro-pipette injection. These doses were injected at a volume of 1 nL/egg or about 0.1% of the egg volume. Eggs were typically fertilized by 8 am each day and injections were performed at 9 am, 11 am, 1 pm, and 3 pm. Embryos were allowed to develop for 11 days at which point they were scored for signs of blue sac disease (BSD) caused by TCDD including edema, hemorrhaging, craniofacial malformations, spinal deformities and presence or absence of blood flow. The 0 pg/nL (triolien control) concentration established the effect of injection time without toxicity being a factor. The 7.5 $pg \cdot nL^{-1}$ concentration was used to determine any differences in sensitivity to dioxin, associated with rapidly changing events during the first day of development. The results indicate no systematic time of onset of mortality, delay in hatching, or signs of toxicity in either the 0 $pg \cdot nL^{-1}$ or 7.5 pg/nl treatments except at 9 am, when the embryos appeared to be more sensitive.Therefore, injections of newly fertilized medaka embryos may take place throughout the course of day zero as long as the treatments to be injected are randomized. This will reduce the bias for a time effect.

The mode of toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is related to neural arrest resulting from an uncoupling of oxidative phosphorylation in the mitochondria of larval Sea Lampreys and Rainbow Trout.

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The pesticide TFM is used to control populations of parasitic sea lampreys (*Petromyzon marinus*) in the Great Lakes, where these jawless fishes parasitize/prey on economically important sport fishes. TFM specifically targets larval lampreys, which live burrowed in the substrate of streams. Despite its success in controlling lamprey populations for over 50 years, the mode(s) of TFM toxicity is incompletely understood. We hypothesized that TFM interfered with mitochondrial ATP production via inhibition of oxidative phosphorylation. As a result, we predicted that lampreys and non-target fishes would be forced to increase reliance on anaerobic glycolysis to meet ATP demands, and that death would ensue when reserves of glycogen were depleted. To test this prediction, larval lamprey and rainbow trout *(Oncorhynchus mykiss)* were exposed to TFM (12-h LC50) and sampled at different intervals for later measurement of changes in tissue glycogen and ATP. In lamprey, 9-12 h of TFM exposure caused an ~ 80-85% decrease in liver and brain glycogen, respectively, and complete depletion of liver ATP. Less pronounced, 50% reductions in brain glycogen were noted in trout. Exposure to TFM was also associated with an increase in the basal respiration rates (absence of ADP - State IV respiration) of mitochondria isolated from lamprey and trout. These effects were similar to those of 2,4-dinitrophenol, a known uncoupler of oxidative phosphorylation. We conclude that the mechanism of toxicity of TFM in both lamprey and rainbow trout is

impaired ATP production due to an uncoupling of oxidative phosphorylation in the mitochondria, which results in a failure of ATP supply to match ATP demand, and the eventual depletion of glycogen and ATP in brain. We suggest that tolerance to TFM and other uncouplers of oxidative phosphorylation are therefore related to the size of the glycogen pool in the brain and, likely, the liver.

Defining Phase III cellular detoxification: Three families of ABC transporters confer multixenobiotic resistance in aquatic organisms.

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ATP-binding cassette (ABC) transporter proteins have been well described as conferring multidrug resistance to structurally and functionally diverse chemotherapeutics in cancer cell lines, human tumours and pathogens. The role that transmembrane ABC transporters play in conferring multixenobiotic resistance through the cellular export of a wide variety of environmental toxicants has been underappreciated. Three sub-families of ABC transporters (B,C, and G) have been found to play the most important toxicological role in xenobiotic disposition, bioavailability, and fate. ABC xenobiotic transporters are expressed in organs and tissues involved in excretion (liver, kidney) or providing a barrier function (gut, blood-brain barrier, etc.). These ABC transporters have been described as a Phase III cellular detoxification system by exporting the metabolites of the well characterized Phase I (e.g. cytochromes P450) and Phase II drug metabolizing enzymes (e.g. glutathione-S- transferase). Unmodified amphipathic xenobiotics and phase I (predominantly cytochrome P450) metabolites are exported by ABCB1. Organic anions and their phase II conjugates are exported with some overlapping substrate specificity but varying affinity by ABCC family (predominantly glutathione conjugates, glucoronate and sulphate conjugates), and ABCG2 (predominantly sulphate and glutamylate conjugates). These ABC transporters have been shown to confer multixenobiotic resistance in aquatic organisms exposed to environmental contaminants. We will present data showing the role of ABC transporters B1, C2 and G2 in detoxification in concert with Phase I and II enzymes in multixenobiotic resistance populations of fish from PCB contaminated New Bedford Harbour, to PAH contaminated Sydney Tar Ponds, as well as Antarctic fish multiply exposed to cadmium and other contaminants.

Toxic effects of various pollutants in 11B7501 lymphoma B cell line from harbour seal (*Phoca vitulina*).

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Heavy metals and polycyclic aromatic hydrocarbons (PAHs) have often been measured in marine mammals. However, no information exists on their effects on the health of the harbour seal (*Phoca vitulina*). This species is found in a variety of habitats, including contaminated coastal areas, and can therefore accumulate relatively high

concentrations of pollutants. In this study, we assessed the immunotoxic and genotoxic effects of several heavy metals (arsenic, vanadium, selenium, iron, zinc, silver and chromium) and one PAH (Benzo[a]Pyrene or B[a]P) on a lymphoma B cell line from harbour seal. We evaluated cell viability, lymphocyte proliferation, phagocytosis, induction of micronuclei and the integrity of the cell cycle. A significant reduction in lymphocyte proliferation was registered following an exposure to 0.05 µM of B[a]P, 5 µM of arsenic or selenium, 50 µM of vanadium, 100 µM of silver and 200 µM of iron. Zinc increased the lymphoproliferative response at 200 µM. Phagocytosis decreased with 20 µM of arsenic, 50 µM of B[a]P or selenium, 200 µM of zinc and 500 µM of vanadium. Induction of micronuclei occurred with 0.2 µM of B[a]P, 100 µM of vanadium and 200 µM of arsenic or selenium. Exposure to 50 µM of arsenic decreased G2/M phase of the cell cycle. Chromium did not induce any effects. Concentrations of heavy metals (except silver) and B[a]P inducing a toxic effect are within the environmental ranges reported in the blood of pinnipeds. These results support the hypothesis that exposure to these contaminants could impair the health of harbour seals.

Secteurs préoccupants
Areas of Concern

Assessment of the health status of wild fish from Wheatley Area of Concern - 1. Biological effects.

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[1]Environment Canada.

Environment Canada has initiated studies readdressing fish health issues in the Canadian Areas of Concern, using various measures of endocrine function and overall fish health in brown bullhead (*Ictalurus nebulosus*) and goldfish (*Carassius auratus*) from the Wheatley Harbour Area of Concern. Fish health assessments were conducted following the National Environmental Effects Monitoring Programs (EEM) protocol using estimates of age structure, energy storage and energy utilization. Endocrine assessments included plasma steroid concentrations, *in vitro* steroid synthesis in female fish, plasma concentrations of vitellogenin, and thyroid status. Liver EROD activity was also measured. The gonads were collected and inspected for presence of deformities; liver samples were collected and analyzed for the presence of tumors. Exposure to endocrine disruptors was also investigated by deployment of semi-permeable membrane devices (SPMDs), which were extracted and analyzed using sex steroid binding protein and androgen receptor assays, also used to test pooled liver extracts for the presence of endocrine disrupting chemicals. Significant differences between exposed and reference site were detected for several variables, the details of which will be discussed.

Assessment of the health status of wild fish from Wheatley Area of Concern - 2. PCB body burden and hydroxylated metabolites in fish plasma.

DUSSAULT, E. [1], MUIR, D. [1], SHERRY, J. [1], MCMASTER, M. [1] and BROWN, S. [1]
[1]Environment Canada.

Since the 1970s, Wheatley Harbour, located on the northwest shore of Lake Erie, has suffered from poor water quality due to industrial discharges, notably from fish and vegetable processing plants. Historical sources of PCBs in harbour sediments present a significant environmental concern, and thus have been the focus of several investigations. In addition to the risk posed by the presence of PCBs, studies have suggested that hydroxylated PCBs (OH-PCBs), by-products of microbial and biological degradation, could also interact with fish thyroid function. However, few studies to date have investigated the environmental exposure and effects of these metabolites in wild fish populations. The present study investigated the environmental occurrence of PCBs and hydroxylated metabolites in brown bullhead (*Ictalurus nebulosus*) from Wheatley Harbour Area of Concern. PCB congener body burdens were determined and plasma OH-PCBs concentrations were quantified using GC-high resolution mass spectrometry. PCBs and OH-PCB concentrations were greater in fish from Wheatley Harbour than in those from Hillman Marsh, a reference site, and from all other Canadian Areas of Concern investigated. These results will be compared and contrasted with other sites in the Great lakes, as well as measurements of fish health, in the context of potential environmental health effects.

Assessment of the health status of wild fish from Wheatley Area of Concern – 3. Multivariate analysis.

DUSSAULT, E. [1], MCMASTER, M. [1], PARROTT, J. [1], SHERRY, J. [1] and BROWN, S. [1]
[1]Environment Canada.

Despite the development over the past decades of several types of multivariate statistical analyses which could be applied to the interpretation of biological data, considerable resistance remains toward their regular application. In the present study, the applicability of Principal Component Analysis (PCA) for the analysis of fish health data from Wheatley Harbour was investigated, to determine whether the resulting principal components could successfully summarize and explain differences between exposed and reference sites. Health status data for brown bullhead (*Ictalurus nebulosus*) and goldfish (*Carassius auratus*) were analyzed in a series of PCAs, the first three components of which were retained for further analyses. General physiological characteristics were represented on the first principal component, while the second principal component was usually characterized by plasma measurements of steroid hormones and vitellogenin. Differences between sites were evaluated by multivariate analysis of variance, using the first three principal components as independent variables. The present study demonstrated that the use of PCA summarized complex data into a smaller number of variables, which explained relatively well the variability found within the dataset, and for which significant site differences were detected.

Risk Assessment for Lake-Based Drinking Water Supplies under Ontario's Clean Water Act.

GOODYEAR, D. [1], DUCKETT, F. [2] and LONGSTAFF, B. [1]
[1]Lake Simcoe Region Conservation Authority, [2]Baird and Associates.

In 2006 the province of Ontario passed the Clean Water Act, which is intended to prevent the overuse and contamination of Municipal Drinking Water Supplies. The program employs a 'semi-quantitative' risk management approach, where vulnerable areas and potential quantity and quality threats are assessed to rank existing and potential risks to drinking water supplies. An 'Intake Protection Zone' is the vulnerable area with respect to lake-based water supplies, which has been delineated using a three dimensional hydrodynamic model for the seven municipal water supplies drawing from Lake Simcoe. Assessment of the chemical and pathogenic threats that various land use activities pose has been drafted. This ranking of significant, moderate and low existing and potential risks will be the foundation of Source Water Protection Plans that include policies and strategies to mitigate existing risks and prevent new risks from being established. The presentation will provide an overview of the Source Water Protection program, using the Lake Simcoe water supplies as case studies to exemplify the 'semi-quantitative' risk management approach that Ontario has adopted. (Interactive Workshop)

Présentations par affiches / Poster Presentations

Toxicologie aquatique générale
General aquatic toxicology

Increased accumulation of sulfur in lake sediments of the High Arctic.

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We report increases since c. 1980 in sedimentary accumulation of reduced inorganic sulfur in lakes of the Canadian Arctic archipelago and Svalbard. Sediment incubations and detailed analyses of sediment profiles from two of the Canadian lakes indicate that increases in sulfur accumulation may be due ultimately to a changing climate. Warming-induced lengthening of the ice-free season is resulting in well-documented increases in algal production and sedimentation of the resulting detrital matter. Algal detritus, as a rich source of labile carbon, is a potent stimulator of sulfate reduction and in these sediments increases the production of sulfide. According to stable isotope analyses, the sulfide is then oxidized to elemental sulfur and stored in sediment. A ramping up of the sulfur cycle in Arctic lakes could have profound effects on important biogeochemical processes (e.g., C flux, P storage, Hg methylation).

Biological and chemical contaminants in whitefish (*Coregonus clupeaformis*) from Northern Saskatchewan Lakes.

MYSIV, O. [1] and PIETROCK, M. [1]

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Whitefish is an important food source for Indigenous people in Northern Saskatchewan. Over the last years an increase in whitefish parasitism has been noted and concerns were expressed that this might be related to accelerating industrial activities in the North and water pollution. Therefore, a study has been initiated to investigate parasites and chemical contaminants in whitefish from Montreal and Reindeer Lakes to determine the risk related to whitefish consumption. In both lakes the parasite community of *C. clupeaformis* consists of cestodes, trematodes, nematodes, and acanthocephalans. A total of 11 species have been found so far. All fish were infected by at least one parasite. The most prevalent species is *Cotylurus erraticus*, which occurs in cysts around the heart. Contamination of fish with metals (as determined by ICP-MS) and organic compounds (analyzed on an Agilent 7890 Gas Chromatograph interfaced to an Agilent 5975 Mass Spectrometer) is low. Mercury concentrations in the fillet were 0.124 $mg \cdot kg^{-1}$ d.w. (Reindeer Lake) and 0.047 $mg \cdot kg^{-1}$ d.w. (Montreal Lake), respectively. No PCB congeners were identified and 4,4'-DDE was the only pesticide detected at concentrations above the method detection limit. Overall, the investigated whitefish harbour parasites which are typical for this host. The impact of environmental contaminants on fish parasitism is negligible in the sampled lakes. Further investigation is necessary to uncover the reasons for the perceived increase in parasitism in whitefish.

Development of a structure versus toxicity model for switchable surfactants.

ARTHUR, T. [1]

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For both environmental protection and industrial efficiency, switchable surfactants have been developed that are active surfactants above a critical pH, but not below. Hence the surfactant activity can be turned off by pH adjustment with CO_2. While offering environmental protection by restricting the conditions under which the surfactant works, the actual toxicity of these compounds relative to other surfactants is still unknown, and may vary with molecular structure. We conducted a series of experiments to test the acute toxicity of a switchable surfactant to 1-3g Rainbow Trout (*Oncorhyncus mykiss*). The surfactant has been produced in several variations which differ by the composition of their hydrophobic tail group. The primary goal of the study is to determine the least toxic model of the surfactant and to assess whether chain length causes a systematic change in toxicity. The preliminary results show that compounds with longer chain lengths are more toxic, indicating a linkage between hydrophobicity, bioavailability and chain length which could explain the toxicity of each version. This relationship can be modeled from the Kow (Octanol-Water Coefficient) of each compound.

Interactions between humic substances and marine microalgae: preliminary results.

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Abstract

The aim of this study was to characterize the sorption of humic substances onto the marine microalgae *Isochrysis galbana, Pavlova lutheri and Nannochloropsis sp.* Preliminary results shown the sorption of humic substances on marine microalgal cells. The amount of HS sorbed can reach 32.1 $mg \cdot m^{-2}$ depending on the nature of algae, time of contact, pH, and salinity.

Introduction

Humic substances (HS) are complex and ill-defined compounds with hydrophilic and hydrophobic moieties that interact strongly with toxic metals or lipophilic contaminants. These interactions modulate at the global scale, the transport and release of most contaminants in the sediments, soils, rivers and oceans. HS are also ubiquitously dissolved in the environment. These compounds form as high as 80% of the dissolved organic matter present in freshwaters and around 10-20% in the marine waters (Thurman 1985, Tremblay and Gagné 2009). Thus, every organism living in natural waters swims in a humic soup where it can meet humic molecules and interact with them. Recently, some interactions of HS with living surfaces have been detected.

In vitro experiments with freshwater phytoplankton have shown sorption of HS on living surfaces (Campbell et al., 1997; Slaveykova et al. et al., 2003; Knauer and Buffle, 2001) and changes in the membrane permeability (Vigneault et al., 2000; Nardi et al., 2002). In marine environments, studies have shown an increase in growth rates of phytoplankton in the presence of HS (Prakash and Rashid 1968; Prakash et al 1973). Moreover, the export of HS in coastal environments, such as the St Lawrence estuary, is often correlated with the occurrence of toxic blooms (Weise et al., 2002; Gagnon et al., 2005). The mechanism that promotes the development of marine algae in presence of HS is poorly understood. For freshwater algae, HS may affect phytoplankton cells indirectly (Matsunaga et al., 1998) by controlling the availability of metals or nutrients or directly by adhering to cells (Slaveykova et al., 2003; Knauer and Buffle, 2001). In marine environments, studies have shown indirect effects (Carlsson et al., 1993; Doblin et al., 1999). We do not know if direct adhesion of HS occurs on marine phytoplankton cells at natural pH.

The molecular mechanism of interaction of HS with living cells is rather poorly understood and is still an open question. Three explanations are suggested thus far: first of all, the size of HS molecules is considered too important to allow compounds to approach close enough to the cellular surface to provide an interaction or a reaction. Thus, these compounds do not sorb on cells. Second, the work of Campbell et al. (1997) highlights the importance of pH and suggests that HS adsorb to biomembranes via a hydrogen bonding. Third, the work by Elayan et al. (2008) proposes a two steps adsorption/absorption mechanism. In the first phase, the HS adsorb on cell membrane by hydrogen bridging and electrostatic interactions. In the second phase, the hydrophobic

moieties of the HS allow intrusion/absorption of HS into the cell membrane, causing some disturbances in its functioning.

The aim of this study was to investigate and characterize the sorption of humic substances onto the marine microalgae *Isochrysis galbana*, *Pavlova lutheri* or *Nannochloropsis sp* by using experimental conditions close to those found in estuaries or marine environments.

Materials and methods

The interactions between the microalgae and HS were studied using batch sorption experiments. In these assays the algae and HS were confined in a reactor vial for a specific time period to allow interactions to occur. Three algae species were studied: *Isochrysis affinis galbana*, *Pavlova lutheri* and *Nanochloropsis sp*. They were cultured at 20 °C using a light: dark photoperiod of 16:8 hours in a F/2 medium without SiO_2. The humic substances used were commercial Aldrich humic acid (HAAld) or humic acid extracted from the Aux-Outardes River, Québec, Canada (HAOut). The sorption measurements were carried out at 20 °C, under two salinity levels (12 or 24 PSU), and two pH (pH 8 for HAAld and a pH of 6 for HAOut). During the sorption experiments, the microalgal cells were exposed to HA concentrations ranging from 0 to 25 $mg \cdot L^{-1}$ for time periods of 0, 30, 60, 120 and 240 minutes. After these exposition times, the microalgae/HS solutions were filtered and the HS contents of the filtrates were determined by UV-VIS spectroscopy at 280 nm. The difference between initial HS concentration and the HS content at a specific time allows us to calculate the amount of HS sorbed by the microalgae. Calibration curves for humic acids analysis were made at each pH value. In addition, the size and cell counts were performed and the surface area was calculated with corresponding mathematical equations to evaluate the amount of HS sorbed by unit surface ($mg \cdot m^{-2}$).

Results and discussion

Previous studies on the sorption of HS onto freshwater microorganisms showed a strong influence of the pH on the sorption. The sorption was high at low pH and decreased almost to zero at neutral pH (Campbell et al 1997, Knauer and Buffle 2001). These data suggested that the sorption of HS onto microalgal surfaces would be very weak, if present, under estuarine or seawater conditions at pH around 8. We performed an exploratory work to determine if the sorption of HS to marine algal surfaces occurred under normal estuarine conditions. In these tests, microalgae were in contact with HAAld at pH 8 and salinity 12. Figure 1 shows the temporal changes in the absorbance at 280 nm of filtered microalgal broths. Clearly, the decrease of absorbance shows a sorption of HS under marine conditions for the three marine microalgae studied. As shown in figure 2, the amounts sorbed changed according to the phytoplankton species, varying from 0.9 $mg \cdot m^{-2}$ for *Pavlova lutheri*, to 1.6 $mg \cdot m^{-2}$ for *Isochrysis galbana*, and to 3.6 $mg \cdot m^{-2}$ for *Nannochlroropsis sp*.

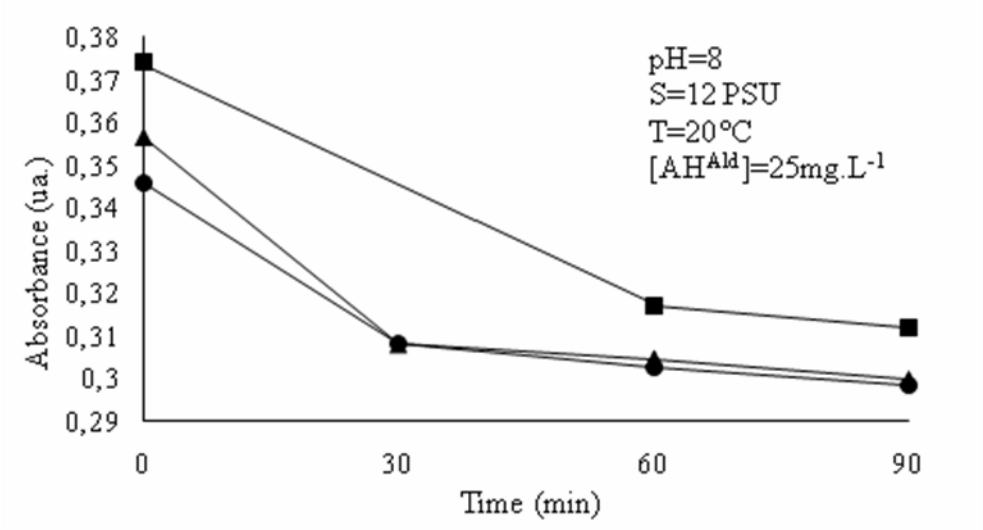

Figure 1. Variation in the absorbance of the broth cultures as a function of time for the microalgae *Isochrysis galbana* ▲, *Pavlova lutheri* ●, *and Nannochloropsis sp* ■

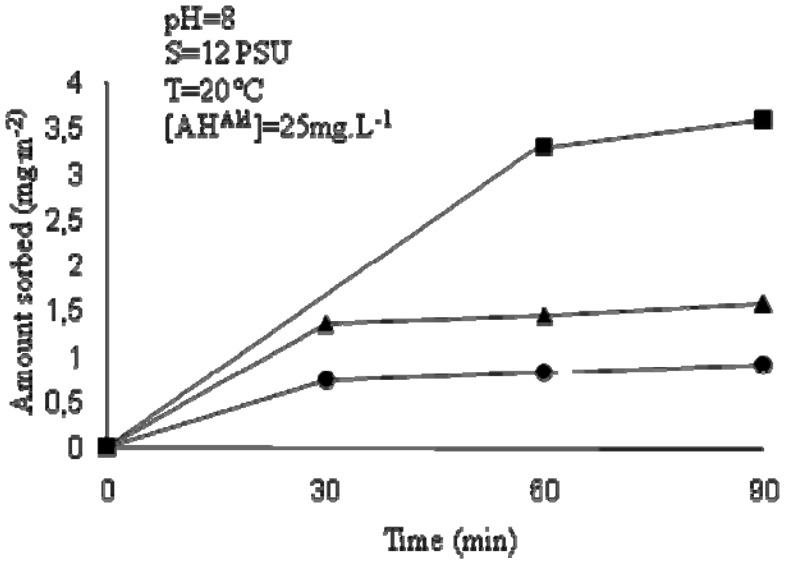

Figure 2. Variation in the amount of Aldric humic acids sorbed as a function of time for the microalgae *Isochrysis galbana* ▲, *Pavlova lutheri* ●, and *Nannochloropsis sp* ■

It is well known that commercial Aldrich or Suwanee humic substances are not representative of natural organic matter. To avoid the use of these compounds, we extracted humic acid from Aux-Outardes River (HA^{Out}) and tested the response of microalgae in contact with this natural organic matter. Figure 3 shows the amounts of HA^{Out} sorbed by *Isochrysis* at three concentrations of HA^{Out} under estuarine conditions. This microalga reacts quickly in the presence of HA^{Out} to reach a plateau in the sorption

in about 30 min. Even when concentrations in HA^{Out} change from 2.5 $mg \cdot L^{-1}$ to 10 $mg \cdot L^{-1}$, this plateau is reached. In four hours, Isochrysis sorbs between 12.6 and 32.1 $mg \cdot m^{-2}$ depending on the initial concentration of HA^{Out}.

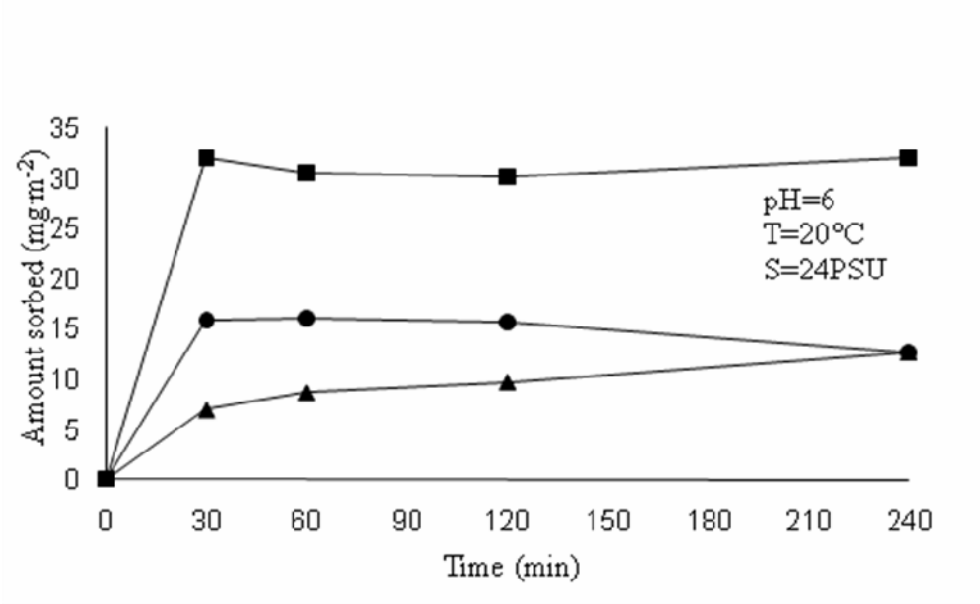

Figure 3. Variation in the amount of Aux-Outardes humic acids (HA^{Out}) sorbed by *Isochrysis galbana* as a function of time for three concentrations : 2.5 $mg \cdot L^{-1}$ ▲; 5 $mg \cdot L^{-1}$ ●; and 10 $mg \cdot L^{-1}$ ■

Conclusion

Marine microalgae exposed to humic substances sorb these compounds quickly after their contact under physico-chemical estuarine or marine conditions. The sorption depends on the nature of the phytoplanktonic species, time of contact, pH, and salinity.

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Using image analyis to investigate chemical stress-induced changes in the behaviour of *Daphnia magna*, *Euglena gracilis*, and *Hyalella azteca*.

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Behavioural changes are an important endpoint when considering early warning monitoring of water quality, as they are capable of providing much faster responses than the standard 24 and 48h bioassays. The Ecotox and Daphniatox image analysis systems use sequential digital image frames to measure a wide variety of behavioural parameters which change rapidly in response to contaminants. The behavioural changes of *Daphnia magna* (cladoceran), *Hyalella azteca* (benthic amphipod), and *Euglena gracilis* (chlorophyll-containing flagellate) were measured in response to the addition of Tributyltin, Atrazine, cadmium, Cipro, and PBDE. The chosen contaminants represent a variety of categories of pollutants, namely organometal, herbicide, heavy metal, pharmaceutical antibiotic, and organobromine respectively. The categories of behaviour observed with the Ecotox and Daphniatox system were motility; velocity; form factor in *E. gracilis* (given by circumference to area ratio), which changes from elongated to round under stress conditions; and finally gravitaxis (*E. gracilis*) and phototaxis *(D. magna)*, given by percent upwardly mobile cells, precision of orientation (r- value), and angle of main distribution of swimming. Five ecologically relevant concentrations were used for each contaminant in relation to a control, and measurements were taken every 10 minutes for 2 hours. 0.1% DMSO was used as a carrier for hydrophilic contaminants. This study is currently in progress and results are to be determined.

Development of an animal model methodological unit at the Aquarium du Québec.

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At present and long term, the Aquarium du Québec (AQ) seeks to establish different cultures of organisms (vertebrates and invertebrates) that are required in ecotoxicology research. The expertise and experiences of the AQ in this field will allow all members of various research institutions across Canada to have access to several organisms necessary to conduct a battery of tests more consistent with their needs. The private laboratories, institutional and industrial, that are required to perform these tests can achieve considerable savings by accessing these resources and organisms when necessary, while continuing to meet the highest standards of quality. Rearing and maintening live specimens and cultured organisms is generally a very laborious task that limits the use of this most appropriate testing method in the ecotoxicological studies. On these bases, the AQ could develop a market currently avalaible within the industry of Quéebec and Canada. Among the species that can be implanted in our laboratory phytoplankton species (*P. subcapitata*, *C. reinhardtii*, *Isochrysis sp*. etc.), some aquatic plants (*L. minor*), zooplanktonic species (*D. magna*, *A. salina*, etc.), some benthic species (*H. azteca*, *C. riparius*, *E. complanata*, etc.) as well as fish species (*F. heteroclitus*, *P. promelas*, *G. aculeatus*, etc.). A gradual plan for the introduction of live specimens and cultures will be organised in order to meet the greatest needs.

Contaminants anciens : Général
Legacy contaminants: General

Persistent organic pollutants in muscle tissue vs. whole fish in American eel (*Anguilla rostrata*).

LEBEUF, M. [1], BYER, J. [2], KEIR, M. [3], ALAEE, M. [3], BACKUS, S. [3], BROWN, S. [2] and HODSON, P. [2]
[1]Fisheries and Oceans Canada, [2]Queen's University, [3]Environment Canada.

This study examines whether persistent organic pollutants are accumulating in the muscle tissue and whole fish of American eel (*Anguilla rostrata*) at similar lipid weight concentrations and patterns. This question was raised in the context of preparing extracts of eel tissues to be used in embryo toxicity tests. Ten silver eels were captured in the fall 2007 at the mouth of Rivière Ouelle in the St. Lawrence Estuary. Average length of the eels was 1.11 (1.02-1.21) meters. A section of tissue ranging from 5 to 7 cm in length was removed posterior to the body cavity of each eel from which the muscle tissue was collected. The rest of each eel, representing between 90 and 92 % of the eel total weight, was used to prepare the individual whole fish homogenates. The muscle tissue and the whole fish homogenate of each eel were analysed for several persistent organic pollutants including various organochlorinated pesticides (e.g. DDE, DDT, Mirex, HCB), polychlorinated biphenyls (PCBs) as well as dioxin like PCBs, polybrominated diphenyl ethers (PBDEs) and polycholorinated dibenzo-p-dioxins and furans (PCDD/Fs). Comparison of sum of PCB congeners (□PCBs) and □PBDEs, reported on lipid weight basis, between the muscle tissue and the whole fish homogenate of each eel were within the analytical error of the methods (< 20%). No differences in relative patterns between muscle and whole fish were apparent for these classes of chemicals. Additional comparative data will be presented including the calculated 2,3,7,8-TCDD toxic equivalent concentrations.

Spatial occurrence of PCDD/Fs and dlPCBs determined in American eel.

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[1]Queen's University, [2]Environment Canada, [3]Fisheries and Oceans Canada.

There has been a drastic decline in juvenile American eel (*Anguilla rostrata*) recruitment to Lake Ontario since the early 1980s. This research was conducted to assess whether dioxin-like contaminants may be responsible for the decline of eels. Current levels of polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs), and dioxin-like polychlorinated biphenyls (dl-PCBs) were determined in whole fish homogenates for eels captured from seven locations in eastern Canada. Samples were analyzed by gas chromatography- high resolution mass spectrometry to determine the concentrations of 17 PCDD/F and four dlPCB congeners. Reference eels captured from rivers in eastern Canada that were tributaries to the Gulf of St. Lawrence, ranged from 61 to 80 cm in length, and from 439 to 1260 g in weight. Eels collected from suspected contaminated areas in the St. Lawrence and Lake Ontario ranged from 68 to 127 cm in length, and 518 to 3474 g in weight. Toxicity results were expressed as 2,3,7,8-tetrachlorodibenzo-p-

dioxin toxic equivalents (TEQs), calculated using the World Health Organization's (1998) fish-specific toxic equivalency factors. Total TEQ values ranged from 0.07 - 1.6 $pg \cdot g^{-1}$ ww and 0.64 - 4.0 $pg \cdot g^{-1}$ ww for reference and contaminated sites, respectively. Spatial trends in dioxin concentration and toxicity will be investigated.

The use of a model fish, *Fundulus heteroclitus*, to assess the role of persistent halogenated hydrocarbons in American eel (*Anguilla rostrata*) decline.

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[1]UQAR / ISMER, [2]Fisheries and Oceans Canada,, [3]Queens University.

Since the early 1980's, the St. Lawrence River American eel (*Anguilla rostrata*) population has been undergoing a dramatic decline associated with recruitment failure, possibly related to the accumulation of persistent halogenated hydrocarbons (PHHs) in eel tissues. These compounds are transferred to the eggs and could affect growth, behaviour and survival of early life stages. Our objective is to evaluate if there were temporal changes in the embryotoxicity of tissue extracts prepared from eels captured in Lake Ontario between 1988 and 2008. The impact of individual PHHs and of complex eel extracts will be assessed in the embryos of a model marine fish species, *Fundulus heteroclitus*. Survival, growth, malformations, hatching success and larval behaviour will be evaluated. Cytochrome P4501A activity, cerebral apoptosis and serotonin concentrations will also be measured. Preliminary experiments were conducted to test the use of a nano-injection method in our model species. The toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was assessed using two routes of administration: nano-injection in the egg yolk and topical applications on the eggshell. The two routes gave similar responses indicating that the nano-injection technique was adequate. *F. heteroclitus* was relatively resistant to TCDD. On the other hand, the relative toxicity of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) compared to TCDD was much greater in *F. heteroclitus* than in salmonids. Further experiments involve the nano-injection technique to evaluate the embryotoxicity of other individuals PHHs (non-coplanar PCBs, brominated compounds) and of eel extracts of known PHH composition and concentration. The ability to predict the embryotoxicity of eel extracts from chemical data will be assessed.

Germline mutations and reproductive effects in fathead minnows exposed to contaminated sediments from Randle Reef, Hamilton Harbour.

MILLER, J. [1], PARROTT, J. [1], SHERRY, J. [1] and QUINN, J. [2]
[1]Environment Canada, [2]McMaster University.

Sediments at Randle Reef, Hamilton Harbour, are highly contaminated with polycyclic aromatic hydrocarbons (PAH's) and metals, as well as various other contaminants. Studies there have demonstrated adverse effects in fish, including elevated mortalities and increased incidences of tumors. However, reproductive effects and heritable genetic alterations have not been evaluated. We exposed sub-adult fathead minnow (*Pimephales promelas*) to contaminated water and sediments at Randle Reef,

Hamilton Harbour, using two approaches. In the first, fish were caged at Randle Reef for six weeks, and in the second fish were exposed in-lab to whole sediments for three weeks. Fish from both studies were transferred to clean aquaria and evaluated for reproductive impairment immediately following exposures. We also determined germline mutation rates in fish exposed in-lab, using microsatellite DNA markers. Overall, we found no evidence to support our initial expectation that fish exposed to the highly contaminated sediments at Randle Reef would experience elevated germline mutations and reproductive impairment. This finding was unexpected, given the large number of studies that have reported effects following exposure to PAH's and PAH-contaminated sediments, including sediments contaminated with coal tar. A number of factors that may have contributed to results obtained are discussed.

Bioaccumulation assessment of pyrene using trout liver S9 and a predictive model.

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Bioaccumulation assessments are important for chemical substances with a log Kow value >3. REACH legislation will require bioaccumulation evaluations for chemicals imported into Europe in quantities >100 Tonnes. Currently, bioaccumulation potential is assessed using models such as BCFBAF (part of EPISuite) or the *in vivo* OECD 305 test. The ILSI HESI Bioaccumulation Group has developed a combined approach for estimating the biotransformation rates of potentially bioaccumulative chemicals using an *in vitro* fish S9 metabolism assay and a predictive model (Cowan-Ellsberry et al., 2008). We measured clearance of pyrene *in vitro* using S9 fraction from rainbow trout strains incubated with 5.0 μM, 1.0 μM, 0.5 μM, 0.3 μM, and 0.1 μM pyrene. Clearance rates obtained from the *in vitro* assay were extrapolated to whole fish biotransformation rates (kmet) and used to refine BCF computer model predictions based on log Kow, molecular weight and structure alone.The approximated BCF values, 61 – 574, estimated from this approach are similar to the *in vivo* BCF values,53 – 457, found in literature for pyrene (Carlson et al. 1979, Ogata et al. 1984, Jonsson et al. 2004). This study shows that the combined *in vitro* and *in silico* approach can be effective for predicting BCF values. In addition, our research shows that there is variability in the metabolic capacity of different rainbow trout strains, which requires further investigation.
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Contaminants d'intérêt émergent
Emerging contaminants

Analytical method for the monitoring of cyanotoxins in surface water and fish tissues by LC-MS/MS.

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Cyanobacterias blooms have increased significantly in Québec province Canada. These blooms appear from the beginning of June, and depending on different environmental parameters, they persist until end of October. They are caused by blue-green algae, amongst others, called cyanobacterias. These cyanobacterias release toxins in water, such as microcystins, anatoxin-A and others. The presence of microcystin-LR, -RR, -YR and anatoxin-A was determined with a liquid chromatograph coupled to a tandem mass spectrometer (LC-MS/MS). Surface water was monitored for toxins concentrations during the summer and fall seasons since 2000. A total cyanotoxins method was developed to quantify intracellular toxins and free toxins in water simultaneously. Drinking water treatment plants were also monitored for toxins in raw water and finished product. No results for microcystin-LR (toxic equivalent) in drinking water were over the Canadian provisional maximum acceptable level of 1,5 $\mu g \cdot L^{-1}$. The measured concentrations in surface water ranged from < 0.02 to 5700 $\mu g \cdot L^{-1}$ for total microcystins (LR+RR+YR). In recent years we were preoccupied by the fact that microcystins could be present in fish tissues and which recommendation could be acceptable for fish consumption. An extraction method for fish tissues was developed in 2007 and fish were sampled in lakes during the 2008 season, where blooms were significant. Results from this campaign will be present on this poster.

Automated solid phase extraction of carbamate and related pesticides in fortified water and natural water samples using LC-ESI/MS/MS.

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The identification and determination of carbamate pesticides have presented a challenge, both in specificity and sensitivity, when using conventional analytical techniques. Recent advances in solid phase extraction (SPE) technology combined with liquid chromatography tanden mass spectrometry (LC/MS/MS) have greatly improved this process. Hence, we developed a sensitive and robust analytical technique with supporting method detection limits (MDLs) using fortified Type 1 water. The applicability of the analytical method was then investigated in 2008 on approximately 100 natural water samples from rural and urban watersheds, including the Niagara River in Ontario. Six carbamate insecticides and one fungicide in water were simultaneously extracted by an automated Autotrace SPE Workstation. The 500 ml fortified or natural water samples were loaded onto an Supelco Envicarb cartridge at a flow rate of 4ml/min., dried with nitrogen for 1 minute, eluted with 12ml of 80/20 DCM in methanol and concentrated to 1ml for analysis by LC-ESI/MS/MS. Recoveries in fortified Type 1 water

samples were 87 % or higher for all compounds using a sample set of n=12. Instrument and method detection limits ranged from 0.09 pg/uL (Methomyl) to 0.27 pg/uL (Metalaxyl) and 0.16 ng·L^{-1} (Carbaryl) to 0.70 ng·L^{-1} (Aldicarb), respectively. Maximum observed concentrations in natural water samples from Ontario were 949 ng·L^{-1} for Carbaryl, 899 ng·L^{-1} for Methomyl and 292 ng·L^{-1} for Oxamyl and 286 ng·L^{-1} for Carbofuran. Most detections occurred in streams from fruit-growing areas followed by streams from urban and row crop areas.

Occurrence of glyphosate and AMPA in open waters and tributaries of Lake Erie.

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Application of the herbicide glyphosate (GLY-P) has escalated in recent years with the widespread increase in corn and soybean production in the Great Lakes Basin. Previous environmental concerns with this herbicide focussed on ecotoxicological issues, but compelling evidence now suggests that GLY-P may have other, more pervasive effects, contributing to the selective development of harmful algal blooms (HABs) in receiving waters. Research shows that the GLY-P breakdown product aminomethylphosphonic acid (AMPA) can be exclusively assimilated by some cyanobacteria as an alternative phosphorous (P) source, providing a selective enhancement and competitive edge in algal communities. It has been proposed that AMPA may contribute to the recent increase of toxic/noxious cyanobacterial blooms in the Lower Lakes - yet levels of this P-ester in these systems are largely unmeasured. To address this issue GLY-P and AMPA were measured in the Erie pelagic zone and major US tributaries in June 2008 following spring application of the herbicide in surface (1m) samples collected from 32 lake, river mouth and upstream stations. GLY-P and AMPA were primarily detected in the tributaries and upstream stations and not at any pelagic stations unless these were close to tributary inputs. Maximum GLY-P and AMPA concentrations were 1.72 ug·L^{-1} and 0.732 ug·L^{-1} respectively, well below the Canadian Water Quality Guideline (65 ug·L^{-1}) considered protective of aquatic life. Levels of AMPA were also significantly below those supporting *in vitro* cyanobacterial growth (~10 uM or 1 mg·L^{-1}).

Occurrence of pharmaceuticals compounds and steroids in drinking water, surface water and wastewater: From 2003 to 2008.

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Since 2003, CEAEQ laboratory monitored water and wastewater for Pharmaceutical residues, steroids and others contaminants. These compounds originating in sewage effluent have been detected in surface and ground water in other provinces and countries. Detected drugs include analgesics/anti-inflammatory, lipid (cholesterol) regulators, non-steroids anti-inflammatory, antiseptic, antibiotics, steroids and hormones,

surfactants and others chemicals for a total of 47 compounds. Three methods has been applied to more than 300 samples, representing a variety of matrices, including municipal drinking water facilities from across the Québec province, wastewaters, and surface waters. One method used liquid-liquid extraction, derivatization and GC-MS analysis to detect pharmaceutical compounds. The second method used solid-liquid extraction, derivatization and GC-MS analysis to detect mainly steroids, hormones and surfactants. The last one used solid-liquid extraction and LC-MS/MS analysis to detect pharmaceuticals compounds mainly antibiotics residues. Limit of detection varied from 1 to 100 $ng \cdot L^{-1}$ in surface water, from1 to 100 $ng \cdot L^{-1}$ in drinking water and 5 to 400 $ng \cdot L^{-1}$ in wastewater. Of the 47 compounds analyzed, 27 were found in surface waters, 12 in drinking waters and 33 in wastewaters. This presentation will discuss the method performance and summarize general findings on occurrence in different matrices from samples taking in different regions of the province.

Consortium for research on pollutants in the Lake Ontario – Gulf of St. Lawrence corridor.

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This poster presents a new initiate among federal government organisations mandated to examine and /or monitor the presence of chemicals pollutants in the Lake Ontario – Gulf of St. Lawrence corridor. In early 2009, a group of scientists from the departments of Environment Canada and Fisheries and Oceans Canada created a Consortium for research on pollutants from the Lake Ontario – Gulf of St. Lawrence corridor. The Consortium is first focusing on chemicals that are persistent and accumulating in sediments, and for which the dynamic of suspended particles is important. These chemical pollutants comprise mainly metals and organohalogenated compounds. The aims of such a consortium are diverse and include: 1) sharing experience and development on technical aspect of collecting and measuring contaminants in sediments, 2) exchanging and integrating data on levels, trends of legacy chemicals as well as on presence of emerging chemicals found in the different areas of the Lake Ontario – Gulf of St. Lawrence corridor, and 3) collaborating nationally on new projects, production of scientific documents and delivery of scientific advices. The poster provides additional information on the aims of the Consortium and reports a specific example of integrated data on polybromimated diphenyl ethers (PBDEs) in sediments and suspended particles along the lake Ontario - Gulf of St. Lawrence corridor.

Polybrominated diphenyl ethers in air as part of the Chemicals Management Plan.

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Polybrominated Diphenyl Ethers (PBDEs) were identified as a high priority in the Chemicals Management Plan (CMP) in December 2006. Environment Canada's Air Quality Research Division, through its Integrated Atmospheric Deposition Network (IADN) provides regular updates of the atmospheric deposition of toxic chemicals to the Great Lakes and the comparison of those inputs to other sources. The atmosphere has been identified as a potential pathway for the distribution of PBDEs. This study reports on the determination of PBDEs in ambient air samples at Canadian IADN sites in the Great Lakes Basin and in urban and remote sites. The spatial and temporal behaviour and gas-particle partitioning will be shown as well as preliminary results of modelling the global sources of penta-BDE to the Canadian environment.

The effects of brominated flame retardants on reproductive behaviour and physiology of American kestrels: conservation implications.

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Brominated flame retardants (BFRs) are persistent organic pollutants that have reached global distribution in the environment. BFRs are lipophillic and bioaccumulative, and consequently have been recorded in wildlife, with some of the highest ever levels seen in some raptor species. Other organic pollutants (e.g. DDT/DDE) have had devastating effects on avian populations in the past, thus it is important that we gain an understanding of the effects of BFRs on reproduction in these birds. The aim of our work was to determine the effects of exposure to two BFRs, PBDEs (DE- 71) and HBCD, on the reproductive behaviour and physiology of captive American kestrels, at levels modeled after concentrations recorded in wild birds. This work has been conducted at McGill University during the breeding seasons of 2005 – 2008. Our findings have shown that both of these types of BFRs affect reproduction and growth of kestrels. Exposure to the technical PBDE mixture, DE- 71, decreased reproductive success, lowered incubation temperatures, caused eggshell thinning, and caused males to perform fewer courtship behaviours. Pairs exposed to HBCD laid eggs earlier and had larger clutches, but the frequency of courtship and brood-rearing behaviours was reduced. Chicks exposed during development to PBDEs tended to be large and grow more rapidly, whereas those exposed to HBCD grew more slowly, relative to control chicks. In males, testes weights were increased with both PBDE and HBCD exposure, and in females, egg mass was decreased. These changes have the potential to affect reproductive output with possible repercussions for wild raptor species.

Chronic toxicity of phthalates, bisphenol A and a Canadian bottled water stored under different light regimes using the cnidarian *Hydra viridissima*.

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There is general concern whether human exposure to selected Canadian bottled waters and the chemicals which may leach from them could potentially cause deleterious effects. This research was designed to determine whether exposures to bottled water and plastic leachates caused toxicity to the freshwater Cnidarian *Hydra viridissima* (green hydra). Three chemicals used in the production of polycarbonate and polyethylene plastics, bisphenol A (BPA) and two phthalate esters: dibutyl phthalate (DBP) and bis (2-ethylhexyl) phthalate (DEHP) along with one type of commercial bottled water were investigated. One brand of bottled water was analyzed over four months (stored in light and dark conditions) along with lab water similarly stored in glass, polycarbonate and polyethylene bottles. Following 2, 4, 8 and 16 weeks in each of the two treatments, hydra bioassays were conducted and hydra were affected reproductively and morphologically. Levels of DBP, DEHP and BPA were detected by GC-MS and may correspond with the effects that were observed. Chronic toxicity tests were also conducted on the two phthalates and BPA. Concentrations that were used included 0.1, 1.0, 10, and 100 $ug \cdot L^{-1}$. The chronic toxicity tests showed that BPA caused effects on hydra morphology and population at low doses and DBP and DEHP both showed signs of hormesis.

Nanotoxicologie
Nanotoxicology

Toxicological effects of a new nanoscale drug delivery system to rainbow trout hepatocytes (*Oncorhynchus mykiss*).

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In recent years, nanotechnology has gained interest in drug delivery strategies. The use of nanocarriers in drug development was shown to increase bioavailability and reduce side effects of drugs mainly by site-selective delivery. With its highly branched and nanoscaled globular structure, PAMAM Dendrimer has been highlighted as one of the most potent candidates for future medical advances. Promising results of this nanocarrier have been shown for cancer-targeting chemotherapies and treatment of neurological disorders. Increasing interest for research and development in nanomedicine will inevitably lead to the release of various drug nanocarrier in the environment. However, the safety of these nanoscale delivery systems to the aquatic organisms remains to be investigated. Concerns about their potential interactions with pharmaceuticals found in municipal effluents are justified. The objective of the present work was to address the toxicity of PAMAM dendrimers of low generations (G2, G4 and G5) and their pharmaceutical vectorization properties on primary cultures of rainbow trout hepatocytes.

Cytotoxic effects and vectorization properties of dendrimers were assessed by cell viability assays combined to nano and drug-related biomarkers.

Effect of core shell copper oxide nanoparticles on cell culture morphology and photosynthesis in *Chlamydomonas reinhardtii*.

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The effect of core-shell copper oxide nanoparticles on algal cellular systems is still not well understood. Documenting these effects is urgent since core-shell copper oxide nanoparticles are currently a component of boats' antifouling paint. This paint may induce alteration effects in aquatic ecosystems. Here, the toxic effect of core-shell copper oxide nanoparticles on green alga *Chlamydomonas reinhardtii* was investigated with regards to the change of algal cellular population structure, primary photochemistry of photosystem II and formation of reactive oxygen species when algal cultures have been exposed 6 h to 0.004, 0.01 and 0.02 $g \cdot L^{-1}$ of core-shell copper oxide nanoparticles. We found that core shell copper oxide nanoparticles induce cellular aggregation processes and a deteriorating effect on photosynthesis. The inhibition effect of photosynthetic electron transport induced a strong energy dissipation process via non-photochemical pathway. Such a deterioration effect was found to be induced by reactive oxygen species formation formed by core shell copper oxide nanoparticles.

Biomarqueurs
Biomarkers

Identification of a novel biomarker for evaluating the disruption of thyroid function.

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Thyroid hormone (TH) is a key component of the vertebrate endocrine system and is required for normal growth, development and metabolic functions. However, the permissive effect of TH on numerous physiological processes and tight regulation of vertebrate TH levels makes evaluation of perturbations to the thyroid system difficult to assess. Despite the importance of TH, there is little known about specific functions of this hormone, and few biological endpoints available as markers of TH action. The potential for environmental contaminants to disrupt thyroid function in vertebrates is a concern, as the chemical structures of some of these substances exhibit similarities to TH. Furthermore, there is evidence that exposure to these toxicants can disrupt thyroid function directly, and indirectly through disruption of other inter-related endocrine pathways. In this study, a unique biomarker of thyroid hormone action was identified in

rainbow trout. Fish in the salmon and trout family undergo a dramatic developmental transformation that involves changes in behaviour, physiology and morphology. During this developmental period, ultraviolet-sensitive (UVS) cone cells in the retina responsible for ultraviolet and polarized light detection naturally degenerate. This process was shown to be induced with exogenous TH treatment and mitigated with exposure to an anti-thyroid drug. The presence of UVS cones detected with immunohistochemistry was correlated with retinal changes in gene expression assessed using quantitative real-time RT-PCR: up-regulation of the nuclear TH receptor (TR), TRB, and down-regulation of the TH-regulating enzyme deiodinase 2 and the UVS cone visual pigment SWS1 opsin. These results demonstrate that UVS cone loss in rainbow trout is a TH-dependent process that can be used as a biomarker in endocrine disruption research.

To protect rainbow trout, dial 9-1-1: the use of Rad9-Rad1-Hus1 (the 9-1-1 complex) checkpoint genes and proteins as a genotoxicity biomarker.

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Cell-cycle checkpoint proteins help maintain genomic integrity by sensing damaged DNA and initiating a repair mechanism or apoptosis. Cells with DNA damage contain checkpoint proteins with altered expression levels and/or phosphorylation states. The detection of checkpoint proteins with these altered levels provides a means to determine whether DNA in a cell is damaged or not. Steinmoeller et al. (2009) showed checkpoint proteins are suitable biomarkers for detecting genotoxins in rainbow trout (*Oncorhynchus mykiss*) and other fish species. In current research, three evolutionary conserved checkpoint proteins, Rad9, Rad1, Hus1 (the 9-1-1 complex) have been cloned from rainbow trout, and antibodies against these proteins will be developed to determine their efficiency as genotoxicity biomarkers. Furthermore, Environment Canada has a list of 2,000 new chemical substances which need to have their environmental effects assessed. Ultimately, rainbow trout will be used as a tool to help monitor aquatic systems.

Études de suivi des effets environnementaux
Environmental effects monitoring

Assessing metal bioavailability in field sediments using both metal bioaccumulation and the SEM-AVS approach.

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Because metals released into the aquatic environment tend to accumulate in sediments, being able to predict their bioaccumulation and ecological impact is of great importance for assessing ecological risk in aquatic systems. Since not all metal in sediments is readily available for uptake by benthic animals, measurements of total metal concentrations in sediments can be misleading. Thus we compared two alternative approaches for evaluating metal bioavailability in sediments: metal measurements in

sediment-dwelling invertebrates and the SEM-AVS approach, which assumes that only metals (Ag, Cd, Cu, Ni, Pb, Zn) not bound to sulfides and organic carbon are bioavailable. We applied these approaches to several metals (As, Ba, Cd, Cr, Cu, Mn, Ni, Se, Zn) in sediments contaminated by half a century of mining activities in the Oujé-Bougoumou region of northern Québec, where sediments downstream of mining operations tend to have much higher metal concentrations than those upstream. Overall, our results suggest that high trace element concentrations in sediments downstream from mining activities do not necessarily mean that these contaminants are in a form that can be taken up by animals living in these sediments. Considering the fact that metals bound to acid volatile sulfides (AVS) are not bioavailable does not improve predictions of metal bioaccumulation using sediment metal concentrations. We suggest that measurements of trace elements in sediment-dwelling animals provide a more direct means of assessing the bioavailability of these contaminants. However, optimal use of benthic animals as biomonitors requires further research into how they accumulate metals.

Linking spatiotemporal variations of diuron contamination to biofilm-induced tolerance in a river.

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Biofilms represent useful potential early warning indicators for monitoring of rivers. When assessing the effects of toxicants on natural microbial communities, special attention must be given to the distinction between these effects and those resulting from other environmental parameters. Pollution-induced community tolerance (PICT) approaches offer possibilities to partially isolate effects of individual toxicants within a complex ecosystem by studying shifts in community sensitivity. To validate the pertinence of PICT methodology for risk assessment, the aim of our study was to investigate if diuron tolerance levels induced in photoautrophic biofilm communities were proportional to their previous *in situ* exposure level to this herbicide. A field survey was conducted for 9 months at two sites located in a river chronically contaminated by diuron. Spatiotemporal variations of diuron tolerance capacities within photoautotrophic communities were estimated monthly from short-term photosynthesis bioassays. Even if we observed a possible influence of three co-varying environmental variables (nitrates, conductivity and temperature) in diuron tolerance induction processes, statistical analysis clearly demonstrated that the main factor explaining variation in diuron sensitivity was the diuron exposure level during biofilm colonization periods. A remarkable exponential correlation between EC50 values and *in situ* diuron concentrations was thus recorded, confirming that PICT can serve as a relevant tool for environmental monitoring of rivers in complement to other existing bioindicator methods.

Impact of phosphorus on the tolerance of riverine biofilms to copper and diuron.

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Induced tolerance of periphytic communities can be attributed not only to the presence of pesticides in rivers: various environmental factors, such as phosphorus, could also play an important role in the acquisition of tolerance. A study was undertaken to highlight the potential impact of a phosphorus gradient on the sensitivity of periphytic microbial communities to copper and diuron. Biofilms were grown in indoor microcosms that were clean or exposed to realistic environmental toxic contamination, with a phosphorus gradient. Periphyton sensitivity to copper and diuron was assessed by short-term inhibition tests based on photosynthetic activity, extra cellular enzyme activities (b-glucosidase and Leucine-Aminopeptidase) and substrate-induced respiration activity. The impact was also evaluated by measuring pesticide concentrations in biofilms, biomass parameters (chl a, AFDW) and community structure (18S and 16S rDNA gene analysis by DGGE, and HPLC or PhytoPAM pigment analysis). Generally, chronically exposed biofilms were more tolerant to copper and diuron than control biofilms, whatever the concentration of phosphorus and the physiological parameter. The phosphorus gradient stimulates the acquisition of tolerance of biofilm to copper, but not to diuron. AFDW and chl a are correlated with the increase of phosphorus concentrations. Green algae were detected in chronically exposed biofilms but not in control biofilms.

The Ostracodtoxkit microbiotest as an alternative screening solid phase assay to the conventional sediment contact test with *Hyalella azteca*.

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Sediments are the centre of intense biological activity which co-determine the good ecological functioning of all aquatic ecosystems, and which are affected by pollutants that accumulate in the sediments. The toxicological impact of sediment contaminants in freshwater bodies is to date also determined regularly with “solid phase tests” based on selected benthic species such as the amphipod crustacean *Hyalella azteca*. In analogy to other “culture/maintenance free” Toxkit microbiotests which are now used extensively for toxicity testing of contaminated waters, a “direct contact” microbiotest has recently been developed for sediment toxicity testing with the ostracod crustacean *Heterocypris incongruens*. Over the last 8 years river sediments of the hydrographic basin in Flanders, Belgium, have been monitored annually for their toxicity with both the *H.azteca* assay and the alternative Ostracodtoxkit microbiotest. Comparison of more than 700 data pairs from a network of several hundred sampling sites revealed that the Ostracodtoxkit is a good alternative to the conventional contact test with *H.azteca* for routine toxicity monitoring of river sediments in Flanders. The converted response of *Heterocypis* in an ecotoxicological class - used in the triad approach to rank the ecological status of sediments in Flanders - is indeed identical to the class calculated with the conventional test for half of the samples or only differs by one class (i.e. either a higher or a lower toxic signal) for most other samples. The good correspondence in

sensitivity and the practicality and cost-efficiency of the Ostracodtoxkit microbiotest is certainly worth further consideration as an alternative screening solid phase assay for routine toxicity monitoring of contaminated sediments.

Using 48-h acute test without food as a surrogate for 7-d sub-lethal test with food for metal mining effluents.

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The Metal Mining Effluent Regulations require effluent quality monitoring using sub-lethal toxicity tests with feeding. Reference site water would ideally be used for dilution for these tests but this is currently not routinely done because of the large volumes required. Research to develop predictive model for metal toxicity have highlighted that the food required to conduct longer term sub-lethal test can greatly reduce metal toxicity, especially for metal with a binding affinity for organic matter such as copper. The current project tested whether shorter term acute test without feeding would in fact be more sensitive to mine effluents than longer test with feeding. Acute 48-h without food and 7-d sublethal tests with food were conducted on *Ceriodaphnia dubia*. Results indicated that *C. dubia* was more sensitive to copper, zinc and nickel in the 48-h acute tests without food than in the 7-d sublethal test. However the reverse was obtained for cadmium. Nevertheless, 48-h tests with two metal mine effluents were also more sensitive than the 7-d sub-lethal test for *C. dubia,* which suggests that the added food in fact reduces the mine effluent toxicity. Our data suggest that for *C. dubia*, 48-h acute test could be considered to monitor effluent quality more efficiently, using site reference water for dilution.

Age and growth of darters, sticklebacks, and minnows: an area in need of further study.

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Small-bodied fish play significant ecological and economic roles in all aquatic ecosystems. Despite this significance, comparatively little study has been directed toward age and growth analysis of darters (*Percidae*), sticklebacks (*Gasterosteidae*), and minnows (*Cyprinidae*). These studies have focused primarily on southern populations, such as those in the Mississippi, Missouri, and Tennessee River watersheds. Such studies frequently used scales and length frequency distributions to interpret age and growth. However, recent studies suggest these interpretations may be inaccurate or imprecise due to the challenge of interpreting small scales and narrow length distributions. One recommendation to address this challenge is to use otoliths or opercular bones to estimate age. This recommendation reflects the observation of higher age of maturity and maximum age from otoliths and opercular bones compared with the other methods. Consideration of available studies indicates the disparity in estimated growth rates and maximum age between the methods is greater in northern compared with southern

populations. Such disparities may represent errors in age estimates and could complicate or compromise management efforts, which is of particular concern for species of high conservation importance particularly in northern locales. For this presentation, we review select studies of age and growth for northern and southern populations of minnows, darters, and sticklebacks. We then recommend the use of otoliths or opercular bones for estimates of age, with a caveat that the use of these bones for the majority of species considered has not been formally validated. These views are of particular relevance to the environmental effects monitoring studies that use small-bodied fish in Canada.

Multiple reference areas – pooling data collected over time instead of space – an alternative approach in EEM programs when you just cannot get the fish numbers you need?

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As part of a federally-regulated EEM program, fish populations are monitored at exposure and reference areas according to several endpoints in order to determine whether or not there is an effect of the effluent. Although a minimum of one reference location is required, federal guidance recommends the use of multiple reference areas to achieve greater statistical power and provide an indication of variability amongst reference areas. In this case, differences found in the exposure area that are outside the range of values seen at a number of reference areas are more likely to be ecologically relevant. As part of an on-going EEM program, mummichog (*Fundulus heteroclitus*) population endpoints have been monitored through several cycles at the same exposure and reference areas. In the latest cycle, the minimum number of fish could not be collected at the reference site. Given this deficiency, we considered potential strategies to determine whether or not there is an effect of the effluent on fish. We investigated the possibility of pooling data collected from the reference area across the cycles to assess variability within the reference fish population and for comparison with the exposure population from the latest cycle. The potential usefulness of this approach for this particular site and for EEM programs at other locations will be discussed.

Assessment of fish health around the Terra Nova Oil Development Site on the Grand Banks.

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Bioindicators or health effect indicators have the potential to identify adverse health conditions in fish in advance of effects on populations. American plaice (*Hippoglossoides platessoides*) was an important commercial flatfish on the Grand Banks and was initially chosen by the oil industry in consultation with Fisheries and Oceans Canada as an indicator species for Environmental Effects Monitoring (EEM) programs in

the area. We report here on fish health studies carried out at the Terra Nova oil development site before and after release of produced waters, which began in 2003. These studies constitute one component of the overall Terra Nova EEM program. Fish were collected in the near vicinity of the Terra Nova development site as well at a reference site located approximately 20 km southeast of the development. Approximately 500 fishes in total were studied over 5 survey years from 2000 to 2006. The health effect indicators studied included fish condition, visible skin and organ lesions, levels of mixed-function-oxygenase (MFO) enzymes, haematology (differential cell counts) and a variety of histopathological indices in liver (e.g. nuclear pleomorphism, megalocytic hepatosis, foci of cellular alteration, macrophage aggregation, neoplasms) and gill (e.g. epithelial lifting, oedema, fusion and aneurysms). These indicators have been extensively used in laboratory and field investigations with various fish species. Although a slight elevation of MFO enzyme activity was observed in fish from the development site in 2002, before release of produced water, and in 2006, a suite of other health bioindicators were found to be generally absent or similar between the development and reference sites. Overall, on the basis of the various indicators studied, the results support the hypothesis of no significant project effects on the health of American plaice.

Réhabilitation des milieux aquatiques dégradés
Remediation of degraded aquatic environments

Effects of nitrate addition on phosphorus retention in an euthophic reservoir: laboratory experiments.

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Ibirité reservoir (Southeast Brazil) receives treated effluents from an oil refinery and domestic untreated sewage from cities in its surroundings, which are the main source of nutrients and cause of eutrophication. The application of calcium nitrate was used as a sediment remediation technology to reduce the availability of phosphorus. The experiments were carried out in microcosms incubated for different periods until 135 days. The organisms *Ceriodaphnia silvestrii* and *Vibrio fischeri* were used for the acute toxicity assessment of water column and pore water of sediments and the organism *Chironomus xanthus* for the assessment of bulk sediment. The chemical results showed that the high values of acid volatile sulfide (6063 mg kg^{-1}) present in sediments decreased 99% after 135 days of incubation. Moreover, almost 50% of the soluble reactive phosphorus was removed from the water column. Nitrate concentration reached levels above 1,200 mg $N\text{-}NO_3\text{-}$ L^{-1} in the samples of pore water of sediments, which is the most probable cause of toxicity to the tested organisms. For *Chironomus xanthus* the sediments samples were deleterious to the exposed organisms. Thus, for the experimental conditions

of this work, the application of calcium nitrate as a sediment remediation technology is not adequate, from the ecotoxicological point of view.

Le canal de Lachine: Un habitat pour le poisson.

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Un inventaire ichtyologique et benthique a été réalisé récemment dans le canal de Lachine afin d'évaluer les effets potentiels de la contamination des sédiments sur les organismes vivants (risques écotoxicologiques). Au total, 19 espèces de poissons, distribués sur l'ensemble du canal, ont été recensées à l'automne 2008. Les plus abondants sont le Crapet-Soleil, le Méné Émeraude, la Perchaude, le Crayon d'Argent, et le Crapet de roche. Des espèces comme le Doré noir, le Méné à nageoires rouges et le Lépisosté osseux ont été observées pour la première fois. L'analyse de la distribution des tailles des poissons les plus abondants permet de constater que le milieu serait une bonne aire d'alevinage pour certaines espèces en plus d'être une aire d'alimentation. Par contre, la majorité des espèces recensées (85 %) possède une tolérance intermédiaire à la pollution et seulement 15 % sont tolérantes. L'absence complète d'espèce intolérante peut être un signe d'une dégradation de l'habitat du poisson dans le canal. Au niveau de la faune benthique, les nématodes, les gastéropodes, les bivalves, les oligochètes et les insectes sont les plus abondants. Le nombre de taxons variait peu d'une station à l'autre (de 11 à 20). Par contre, le nombre d'individus par m^2 pouvait varier énormément, passant de 300 à plus de 2600 ind./m^2. La présence de sédiments contaminés a probablement des effets sur la diversité et l'abondance de la faune ichtyologique et benthique et pourrait expliquer, en partie, les résultats. Par contre, il ne faut pas négliger d'autres facteurs environnementaux, comme la granulométrie, la présence de berges abruptes en béton sur une grande partie du canal, les variations du niveau d'eau, voire l'assèchement du canal par moment.

Sables bitumineux
Oil sands

Salinity and solvent effects on the characterization of naphthenic acids from Athabasca oil sands using electrospray ionization.

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Mass spectra of naphthenic acids, obtained using a variant of electrospray ionization coupled with a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer, are shown to vary greatly, reflecting their dependence on the combined effects of salinity and solubility of the oil sand acids in organic solvents. Whereas monocarboxylic compounds ($C_nH_{2n+z}O_2$) in the z= -4, -6, and -12 (2, 3 and 6-ring naphthenic acids respectively) family in the carbon number range of 13 to 19 were prevalent in the dichloromethane/acetonitrile cosolvent systems, salting out effects resulted in (i) a general reduction of these species; (ii) the complete elimination of

dicarboxylic acids and (iii) 80% reduction in O_3 species with similar carbon number range and z values. The dicarboxylic acids are generally less toxic than their monocarboxylic counterparts. Salt interactions of naphthenic acids are expected to occur in oil sands processed waters, and thus the surrogate method is intended to serve as a guide in the identification of principal toxic components; which in turn supports efforts to remediate oil sands contaminated soils and groundwater.

Modeling the ecotoxicity of naphthenic acids.

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[1]HydroQual, [2]ExxonMobil Biomedical Sciences, Inc, [3]University of Waterloo, [4]University of Delaware.

Oil sand development has recently gained renewed interested due to the strong global demand for petroleum products. Oil sand-derived process water effluents are mixtures of thousands of compounds, all of which contribute in varying degrees to observed ecotoxicity. Recent modeling advances show promise for application in estimating oil sand ecotoxicity due to naphthenic acids (NA). The ecotoxicity of these complex mixtures has been described successfully using an extension of the hydrocarbon block method that relies on a database of physicochemical properties for individual hydrocarbons, including naphthenic acids, in the PETROTOX oil-toxicity model. The toxicity of ionizable compounds appears pH-dependent and can be described using chemical speciation and biota partitioning models. Analysis of the model predictions suggests that the high MW (e.g., >350) compounds from the higher Z-families (e.g., -8, -10 and -12) are contributing most to the observed toxicity. The model, however, overpredicts the toxicity of the highest MW, e.g., residual, fractions. This suggests that the bioavailability of the highest MW compounds is limited and further model refinement may be required. Future efforts should focus on evaluation of model NA compounds across a wide range of MW and Z-families.

Identification of causes of oil sands coke leachate toxicity.

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A series of chronic (7-day) toxicity tests showed that oil sands coke leachates (CL) are acutely toxic to *Ceriodaphnia dubia*. However, the cause of coke leachate toxicity was not known. Therefore, the purpose of this study was to generate CLs in the laboratory to evaluate the toxicity response of *C. dubia* to CLs and to perform toxicity identification evaluation (TIE) tests to identify the cause(s) of CL toxicity. Coke was subjected to a 15-day batch leaching process at two pH values (5.5 and 9.5). Leachates were filtered on day 15 and used for chemical and toxicological characterization. The 7-day LC50 estimates for *C. dubia* survival were 6.3 and 28.7 % for pH 5.5 and 9.5 CLs, respectively. Addition of EDTA to CLs significantly ($p \leq 0.05$) improved survival and reproduction in pH 5.5 CL, but not in pH 9.5 CL. A cationic resin treatment removed toxicity of pH 5.5 CL only. Conversely, the toxicity of pH 9.5 CL was completely removed with an anion resin treatment, suggesting that the pH 9.5 CL contained metals

that form oxyanions. Trace element characterization of the CLs showed Ni and V levels to be well above their 7-day LC50 values for *C. dubia*. Further, toxicity reappeared when Ni and V were added back to resin-treated CLs at their original levels. The TIE results combined with the trace element chemistry of the CLs suggest that both Ni and V could be acting as toxicants in the pH 5.5 CL, whereas V appears to be the primary cause of toxicity in pH 9.5 CL.

Gas production, oxygen demand and microbial activity in sediments of wetlands constructed with oil sands mine tailings.

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We determined change in Sediment Oxygen Demand (SOD) in 2 reference and 9 oil sands process material (OSPM; sediments and water) affected wetlands since their construction in 1992. SOD was measured by tracking the rate of O_2 depletion in *in situ* test chambers resting on the sediment surface within the test pond areas. SOD measurements taken in 2008/09 show a lower rate of oxygen consumption than those collected in 1993, suggesting that sediment-associated reducing compounds (primarily ammonia) had been depleted. CO_2 is dominantly respired by methanogens, which use the carbon as a terminal electron acceptor in conjunction with hydrogen to produce CH_4. Analysis of the gases eluted *in situ* from wetland sediments provided an independent measure of likely biological vs. chemical oxygen demand. Higher CH_4 concentrations suggest that OSPM affected sediments promote the increase of methanogenic bacteria. To test this hypothesis, samples of evolved gas, pore water, and intact sediment cores were collected at each pond. The biological signatures of samples from each pond will allow us to determine whether significant differences in biogeochemical composition have developed over time. The maximum likely number of surficial autotrophic and sediment-dwelling heterotrophic bacteria will be compared. The relationship between microbes and sediments of reclaimed wetlands is imperative in assessing current wetland reclamation designs' effectiveness at restoring productivity to that observed during premining conditions.

Seasonal and spatial trends in production and stable isotope signatures of primary producers in Alberta oil sands reclamation wetlands.

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[1]University of Waterloo.

Bitumen is recovered from the Athabasca oil sands through a mining process that produces large amounts of wastewater containing naturally occurring compounds such as polycyclic aromatic hydrocarbons (PAHs) and naphthenic acids (NAs). Aquatic organisms may be exposed to varying levels of PAHs and NAs in addition to ammonia as a function of the reclamation strategies. The objective of this study is to determine the carbon and nitrogen dynamics in primary producers using stable isotopes in process-affected and reference wetlands. Planktonic and periphytic samples from artificial

substrates were collected for community biomass (chlorophyll-*a* and biomass) as well as carbon and nitrogen isotope analysis. Periphyton was collected in 14-20 day intervals for 3 time periods in 2007 and 2 in 2008; seasonal trends in isotopic composition could then be analyzed. Periphyton data showed δ15N enriched values (12.81-18.76 ‰) for some consolidated tailings (CT; Mature fine tailings (MFT) and gypsum ($CaSO_4$)) sites in 2008. However, other sites with only MFT (1.80-3.50 ‰), as well as non-MFT sites (0.01-7.19 ‰) did not have enriched δ15N values. The CT data suggest that there are differences in the quality of CT (ie. ammonia levels) between oil sands operators. Differences in the quality of CT result in differences in the δ15N values of periphyton dominated by algae (this study), as well as periphyton dominated by microbes. Microcosm studies will also examine the effects of DOC from various reclamation substrates (peat, CT, and MFT) on carbon and nitrogen isotopic composition of primary producers.

The effects of nutrient enrichment on oil sands reclaimed wetlands: A field microcosm study.

CHEN, H. [1], FARWELL, A. [1] and DIXON, G. [1]

[1]University of Waterloo.

The extraction of bitumen from oil sands in Alberta, Canada, generates a large amount of processed material that must be reclaimed. Using processed material to create wetlands and shallow lakes is a challenge, as this material has the potential to have a strong impact on aquatic faunal and floral colonization. In this case, the quality of the substrate may be poor due to physical (particle size, organic content) and/or chemical characteristics. Present in the processed materials are naphthenic acids, polycyclic aromatic hydrocarbons, and increased salinity, which pose toxic threats to the environment. One approach to improving the quality of the substrate used in reclamation is the addition of nutrients. Nutrient enrichment promotes primary productivity which settles out creating a biological layer to confine toxic processed material and provide a more favourable substrate for colonization. The objective of this study was to examine the influence of nutrient addition on primary production by means of chlorophyll *a* and total biomass analyses. Nutrients were added to microcosms to create differing levels of fertility (oligotrophic to hyper-eutrophic conditions) for each of three water types that vary in naphthenic acid concentration and for each of three different potential reclamation substrates (sand; mature fine tailings (50%) + sand (50%); peat + mature fine tailings/sand)). Results showed different levels of growth depending on both the water and substrate type. Generally, combined planktonic and periphytic growth was highest in water with high levels of dissolved organic and inorganic carbon.

Inferring energy sources in constructed wetlands through stable isotope analysis of microbial biofilms.

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[1]University of Windsor, [2]Suncor Energy.

Stable isotope ratios are promising means of discriminating among sources of nutrition, speculating energy flow, and tracing trophic linkages within food webs in constructed wetland ecosystems. This study presents a unique means of sequestering microbial biofilm by using artificial substrates which were fixed within 8 wetlands, differing in age and construction materials, for 2 y in the oil sands lease areas of northeastern Alberta. Autotrophic and heterotrophic biofilm samples were collected from subsurface and epibenthic zones of the outer and innermost pipe surfaces, respectively, of each submerged substrate assembly. A mixing model of the 3 isotopic signatures, δ13C, δ15N, and δ34S, will be used to infer the contribution of 4 prospective nutrient sources to the biofilm. Dominant living and senescent emergent as well as submergent macrophytes, particulate organic matter, sediment (i.e. detritus), algae (i.e. metaphyton), hydrocarbons (bitumen), dissolved organic carbon, and invertebrates were collected for comparison to signatures of biofilm in each wetland. Results will clarify the role of primary production in reference vs OSPM-affected wetlands, relative to heterotrophic processes based on assimilation of oil sands-derived hydrocarbons and autochthonous detrital pools.

Emergence, growth, and dispersal of *Chironomidae* in reclaimed wetlands in the Athabasca oil sands region of Alberta.

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[1]University of Windsor.

In recent years there has been growing concern over the environmental repercussions of oil sands extraction in the boreal region of northeastern Alberta. The objective of this study was to determine whether emergence, growth, and dispersal of *Chironomidae* differ in reclaimed wetlands created using oil sands process materials (OSPM) when compared to reference wetlands. Emergence was quantified using five floating 30-cm diameter 'halo' traps per wetland deployed for 24 h. Exuviae trapped in the surface water film were collected, identified, and counted. Cultures of chironomids were lab-reared from egg masses collected from two OSPM- affected wetlands and two reference wetlands paired according to geographic proximity under controlled conditions for one generation. Chironomid larval growth will be quantified *in situ* in complementary reference/OSPM wetland pairs, by reciprocally transferring second instar, second generation culture larva. Dispersal was quantified by tabulating the number of adults caught on suites of five sticky insect traps situated along 3 radially-arranged transects extending distances of 0-150 m from each wetland. OSPM-affected wetlands are expected to support chironomid communities exhibiting less emergence, reduced richness, and slower growth than reference wetlands. Preliminary analysis suggests that fewer chironomids emerged from OSPM-affected wetlands but that there were no differences in dispersal distance between OSPM-affected and reference wetlands.

Effects of oil sands sediments in fish.

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[1]Environment Canada, [2]University of Saskatchewan.

A collaborative project under the Panel of Energy Research and Development (PERD) began in 2009 with researchers and environmental scientists from Environment Canada and University of Saskatchewan Toxicology Centre. The group is collaborating on a 4-year study to assess the potential toxicity to laboratory fish of several oil sands sediments and river waters, and of reclamation pond waters and sediments. As the first step in this process, three sediments from rivers were assessed for potential to cause effects in fathead minnow eggs/larvae, to set the "background" of effects in the oil sands area. Fathead minnow eggs and larvae were exposed to sediments for 18 days. Hatching, larval survival, development and growth were monitored. Naphthenic acids, metals, and polycyclic aromatic hydrocarbons (PAHs) and C1-C4-substituted PAHs were measured in sediments and in SPMDs deployed in the field, to determine whether these compounds can be correlated with the toxicity observed. The research will continue and expand over the next few years, assessing walleye eggs exposed to sediments, and *in situ* fish exposures. Fractionation of extracts of the sediments will begin, and Toxicity Identification and Evaluation (TIE) will attempt to isolate fractions that contain compounds that affect fish growth and development.

Contaminants anciens - Métaux
Legacy contaminants –Metals

Analyse des métaux traces dissous par ICP-MS en conditions propres.

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L'analyse des métaux traces dissous requiert bon nombre de précautions et nous astreint à plusieurs contraintes d'ordre logistique. Par exemple, le délai entre le prélèvement de l'échantillon et sa réception au laboratoire pour permettre la filtration de celui-ci en un temps opportun, est un obstacle de taille lorsque l'échantillon est prélevé en région éloignée. L'utilisation de matériel exempt de contamination est une étape critique pour l'obtention de résultat fiable. La filtration sur le terrain, jusqu'à présent, requiert un appareillage pas toujours facile à transporter et nécessite des étapes de décontamination fastidieuse. Pour les besoins d'une étude comparative menée par la DSÉE, le CEAEQ a adapté et validé des méthodes d'analyse de métaux traces dissous, dosage par ICP-MS en salle propre. Les échantillons ont été filtrés à leur arrivée au laboratoire et ensuite acidifiés ou encore ceux-ci ont été filtrés sur le terrain à l'aide d'une seringue préalablement décontaminée. Les métaux dosés par cette méthode sont les

suivants: argent, aluminium, arsenic, bore, baryum, béryllium, cadmium, cobalt, chrome, cuivre, fer, manganèse, molybdène, nickel, plomb, antimoine, sélénium, strontium, uranium, vanadium et zinc. Les limites de détection varient de 0,0008 $\mu g \cdot L^{-1}$ pour l'uranium à 0,7 $\mu g \cdot L^{-1}$ pour le zinc. Suite aux conclusions tirées de l'étude, la DSÉE a effectué, durant l'été 2009, le suivi des concentrations de métaux traces dissous dans plusieurs rivières du Québec, en utilisant la filtration sur seringue.

Temporal changes in metal concentrations in the insect biomonitor *Hexagenia* from Rouyn-Noranda area lakes.

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Emissions from the metal smelter in Rouyn-Noranda, Québec, have decreased considerably over the last few decades. We set out to determine if this reduction in inputs to nearby lakes has resulted in comparable reductions in bioavailable metals by measuring metal concentrations in larvae of the mayfly *Hexagenia limbata*. This burrowing insect was chosen as a biomonitor because it is present in 90% of the lakes in the area, is of large size (up to 2 cm in length), readily accumulates trace metals, and is an important prey for fish. To achieve our goal, we collected this mayfly, lakewater and sediment from 11 lakes situated along a metals gradient near Rouyn-Noranda. In lakewater, we measured major ions, organic and inorganic carbon and metals (Cd, Cu, Zn, etc.) so that we could calculate free metal ion concentrations using a chemical speciation model (WHAM). Mayfly larvae were dissected into 4 parts: gills, gut, sediment in the gut, and remaining parts. We compare metal concentrations in each insect part to those in water and sediment to determine if metal concentrations in this insect are correlated with those in its surroundings. We then compare mayfly metal concentrations measured in 2009 to those measured 20 years earlier to determine if bioavailable metal concentrations have declined over time in Rouyn-Noranda area lakes.

Patterns of methylmercury bioaccumulation by the phantom midge *Chaoborus sp.*: involvement of diel vertical migration and feeding habits.

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Methyl mercury (MeHg) is a well known contaminant that can easily be accumulated and magnified through aquatic food webs. For instance, pelagic invertebrate predators are trophic pathways of MeHg between zooplankton and fishes. However, several studies reported that the widely distributed *Chaoborus* larvae (Insecta, Diptera) don't follow this classical pattern of bioamplification. Their ability to bioaccumulate MeHg seems to be lower than those of their zooplankton prey. The aim of this study was to investigate the patterns of MeHg bioaccumulation of different instars of *Chaoborus*, and relate them to *Chaoborus'* diel migratory behaviour and feeding habits. Larvae at different stages of development and their potential zooplankton prey were collected in two lakes, Croche and Geai (QC, Canada). On the one hand, the presence of fish in Lake

Croche induces migration of both *C. flavicans* and *C. punctipennis*. On the other hand, the absence of fish in Lake Geai allows the coexistence of migratory (mature *C. trivittatus*) and non-migratory larvae (*C. americanus* and immature instars of *C. trivittatus*). In addition, crop contents of *Chaoborus* larvae were analyzed and MeHg concentrations were measured in both predators and their presumed prey (size fractions and sorted zooplancton species). Preliminary results show that MeHg concentrations in *C. flavicans* and *C. punctipennis* of Lake Croche are lower than those in *C. americanus* and *C. trivitattus* of Lake Geai. Furthermore, in both lakes, migratory instars seem to be less contaminated than non-migratory larvae. According to their crop contents, it appears that larvae feed mainly on the less contaminated zooplankton fractions and types of prey (rotifers, copepods and juvenile *Chaoborus*).

Toxicokinetic and food web models to quantify the effects of *Hemimysis anomola* on Lake Ontario food webs.

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[1] Queen's University, [2] Ministry of Natural Resources.

Hemimysis anomala (bloody red shrimp), an invasive invertebrate of Great Lakes, prefer areas with rocky substrate, cavities, or hard surfaces in nearshore of lakes. Their rapid propagation and maturity (individuals can reach adulthood after approximately 45 days, and will produce between 2-4 broods) and opportunistic omnivore feeding habits (zooplankton, phytoplankton, insect larvae, etc) probably will significantly influence the food web dynamics of today's Lake Ontario. We sampled nearshore habitats along the Canadian side of Lake Ontario for fish, macroinvertebrates and other key species. We also designed and used bottle traps and bucket traps to collect *Hemimysis.* All food web components will be analysed for mercury, stable nitrogen and carbon isotopes. We will discuss our findings in light of the role of *Hemimysis* in the food webs of today's Lake Ontario in nearshore habitats, clarifying which trophic positions the *Hemimysis* occupies in the webs and how they influence mercury transfer patterns in Lake Ontario.

Heavy metal concentrations in commercial fish sold in the Baie des Chaleurs, New Brunswick.

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Fish and seafood bioaccumulate heavy metals. Heavy metal can have deleterious effect on human wellbeing and health. Fish and seafood consumed by the public are bought mainly in groceries and fish markets. Nonetheless, few studies have examined the heavy metal concentrations in commercial halieutic products. The objectives of this study are to establish the origin of fish and seafood sold in the region and to determine their heavy metal levels. In 2008, interviews were conducted in four fish markets and four grocery stores. We then bought for analysis species that were the most purchased. Results show that 36% of seafood species sold come from the Baie des Chaleurs. Lobsters, shrimps, scallops and oysters are the most consumed species. In lobster, cadmium and

copper concentrations are higher in the hepatopancreas than in its other parts. Aluminium, iron and manganese concentrations in canned mussels are six times higher than in fresh mussels. No metal levels in the samples collected exceed the World Health Organization tolerable daily intake for an adult who consumes the recommended two portions of seafood per week. However, choice of species, portion size and seafood consumption frequency remain important to consider. Furthermore, at-risk groups such as pregnant women and young children need to be careful in selecting their seafood.

Cyanobactéries
Cyanobacteria

Validation of a phycocyanin probe for the monitoring of cyanobacteria.

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In support of the cyanobacteria issue in the province of Québec, laboratory analyses are performed on a regular basis for cyanobacteria identification and enumeration and for cyanotoxins quantification. As a potential complementary tool, a phycocyanin probe (YSI BGA-PC 6131) that reports results in cell ml^{-1} was tested and validated in laboratory. We determined the background level, the detection limit, the linearity, the repeatability and the fidelity. Different modes of calibration were used and compared. We also performed measurements with real samples during summer 2008 and compared the results of the YSI probe with those of microscopic enumeration and biomass (biovolume). The detection limit was estimated at 1221 cell ml^{-1}. To improve its performance, the YSI probe must be calibrated in cell ml^{-1} with a laboratory culture (*Microcystis aeruginosa*). This procedure is more complicated than using the manufacturer's default calibration but produce more reliable results. In the range of 5 000 to 100 000 cell ml^{-1} with *Microcystis* culture and calibration in cell ml^{-1}, the difference with the true value varies generally between 10 to 30% with sometimes large differences of up to 100%. The comparison with microscopic results of lake samples showed that YSI is more correlated with biomass than with cell ml^{-1}; the latter may be strongly biased by the cell biovolume and showed differences of up to one order of magnitude in comparison with microscopic analysis.

Pesticides
Pesticides

The influence of detection limits on the interpretation of pesticide data in surface waters, particularly in regards to monitoring programs.

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Method detection limits (MDLs) are often treated as a tangential issue in studies involving pesticides in surface waters. Naïve methods of handling observations below MDL have even been construed as "data fabrication (e.g. Helsel 2006), where inappropriate values are substituted for values below MDL. The nature of MDLs may have a dramatic influence on the interpretation of data obtained by monitoring programs. Our objective was to determine the influence of MDLs on the interpretation of pesticide residues in surface waters. We used the "native" MDLs that were obtained for 37 pesticides in surface waters from southern Ontario (n = 650) analyzed by the National Laboratory for Environmental Testing (NLET) and by Axys Analytical, as well as modeling increasingly high MDLs from 50 to 1700 $ng \cdot L^{-1}$. All fractions were analyzed by gas chromatography/ mass spectrometry (GC-MS). MDLs for the pesticides examined ranged from 0.37 to 79.5 $ng \cdot L^{-1}$, with an overall average of 16.04 $ng \cdot L^{-1}$. The different measures used—means, medians, frequency of detections, and number of simultaneously detected pesticides--were affected by the MDLs. Depending on the method used for replacement of observations below detection limits, means or medians were either overestimated (½ MDL) or underestimated (0 in lieu of MDL). Although the mean or median concentrations of atrazine did not change much when the MDL was shifted from the native (5.76 $ng \cdot L^{-1}$) MDL to 25 to 50, subsequent increases in MDL increasingly biased both means and medians; medians were no longer viable at an MDL of 80, as the median was the ½ the MDL, or 0. However, even small increases in the MDL affected the estimate of the number of pesticides detected in surface waters; an MDL of 50 $ng \cdot L^{-1}$ reduced the number of detected pesticides by half. Analytical costs can be a major constraint on monitoring studies that seek to quantify pesticide concentrations in the environment. There is substantial incentive to minimize these costs to allow for extensive sampling, such as for screening or looking for exceedances of guidelines. However, the high MDLs may restrict the types of inferences one may make about pesticide residuals in surface waters, particularly claims about the absence of pesticides. MDLs can, ultimately, determine the objectives that any given monitoring program is capable, or incapable, of achieving.

Metolachlor in the Great Lakes and Ontario small lakes.

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Concentrations and stereoisomer proportions of the chiral herbicide metolachlor were determined in the Great Lakes and Ontario small lakes between 2005-2007. Median metolachlor concentrations ($ng \cdot L^{-1}$) followed the order: Erie (18) > Ontario (13) = Michigan (13) > Huron (4) > Superior (0.3). Lower median concentrations, ranging from 0.2-5 $ng \cdot L^{-1}$, were found in small lakes of southern Ontario which lacked tributary contributions, while the median for Ontario streams was 94 $ng \cdot L^{-1}$. The four stereoisomers of metolachlor were determined by LC-MS/MS on a chiral-phase column; results were expressed as the stereoisomer fraction SF = herbicidally active isomers/total isomers. SFs fell between 0.89 for currently used S-metolachlor, which is enriched in the active stereoisomers, and 0.50 for older racemic metolachlor. Median SFs in the Great Lakes were: Erie (0.84) > Huron (0.74) ~ Michigan (0.72) > Ontario (0.68) > Superior (0.53), Median SFs in small lakes ranged from 0.75-0.89 and tended to be higher in the southern lakes close to agricultural sources. The median SF in Ontario streams was 0.84. Deviation in lakes from the SF of currently used S-metolachlor may be related to hold-up of older racemic metolachlor within the lakes or stereoselective degradation.

Air-water gas exchange of currently used pesticides in the Canadian Arctic.

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[1]Environment Canada, [2]Fisheries and Oceans Canada.

Currently used pesticides (CUPs) were determined in air and water samples collected on board the CGSS Amundsen during the summers of 2007-2008 on an excursion from Québec City, through the St. Lawrence Seaway, along the northern shore of Labrador, through Hudson Strait and into Hudson Bay. Additional samples were taken in 2008 off Banks Island on the eastern edge of the Beaufort Sea. CUPs identified were dacthal, chlorothalonil, endosulfan, chlorpyrifos and trifluralin. Highest concentrations in air were found in the St. Lawrence Seaway except for trifluralin, which was highest off Banks Island. For water, a gradient was seen for dacthal (3.7-42 $pg \cdot L^{-1}$), trifluralin (<2-21 $pg \cdot L^{-1}$) and endosulfan-I (0.8-3.7 $pg \cdot L^{-1}$) with higher concentrations in the east than the west. Chlorpyrifos (2.3-28 $pg \cdot L^{-1}$) showed the opposite trend with higher concentrations in the west. The water/air fugacity ratio (FR) was calculated from concentration data, the Henry's law constant at the water temperature, and the air temperature. FRs were <1 for all chemicals except trifluralin, indicating net gas-phase deposition.

Apport en pesticides vers le lac Saint-Pierre.

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Situé sur le parcours du Saint-Laurent, le lac Saint-Pierre est le dernier bassin d'eau douce avant l'estuaire. Ce tronçon du Saint-Laurent est vulnérable à la contamination puisqu'il reçoit les eaux de plusieurs rivières dont le bassin versant est à forte vocation agricole. C'est le cas des rivières Bayonne, Maskinongé et du Loup sur la rive nord et des rivières Yamaska, Saint-François et Nicolet sur la rive sud. L'échantillonnage effectué à l'embouchure de ces rivières en 2004 et 2006, montre que l'eau de ces rivières transporte plusieurs pesticides. Selon les rivières et les années, on y détecte 9 à 15 pesticides. Les produits décelés sont surtout des herbicides (atrazine, métolachlore, dicamba, bentazone) mais des insecticides sont parfois aussi présents. Sur la rive nord quelques insecticides sont détectés dans les rivières Bayonne, Maskinongé et du Loup. Le chlorpyrifos, notamment, est présent sporadiquement en concentration qui dépasse les critères de qualité de l'eau pour la protection des espèces aquatiques. Sur la rive sud des herbicides sont régulièrement décelés, mais leurs concentrations ne dépassent pas les critères pour la protection des espèces aquatiques. Cependant elles atteignent souvent des valeurs qui dépassent les critères pour les eaux d'irrigation. Compte tenu du débit respectif des six rivières, la charge en pesticides vers le lac Saint-Pierre générée par les tributaires de la rive sud est plus importante que celle générée par les tributaires de la rive nord, ce qui implique que la zone affectée par la présence de pesticides est probablement plus importante sur la rive sud du lac.

Chemical mixtures in the environment: effects of glyphosate-herbicides and fertilizers on the emergence of adult aquatic insects from wetlands.

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Glyphosate-based herbicides are some of the most commonly used herbicides in the world, in domestic, forestry, and agricultural applications. Although generally considered to have a low direct toxicological risk to animals, few studies have attempted to investigate the ecosystem-level effects of formulated glyphosate products in conjunction with other likely co-occurring environmental contaminants. Small wetlands are ubiquitous in agricultural landscapes and have considerable potential for contamination by both herbicides and fertilizers. Wetlands support a diverse array of vertebrates, including amphibians and birds that depend on emerging insects. Twenty-four wetlands, each split with an impermeable plastic barrier to allow for direct comparison between treated and untreated halves, were used to examine the effects of Roundup WeatherMax® with simulated fertilizer inputs. Barriers were installed in wetlands located on Canadian Forces Base Gagetown, near Oromocto, NB, in summer 2008; treatments were applied in spring 2009. One half of each pond was over-sprayed with the herbicide in two separate treatments to achieve either an environmentally realistic or worst case maximal aqueous concentration of the glyphosate-based herbicide

RoundUp WeatherMAX® with the other half left untreated. Six ponds of each treatment also received several applications of inorganic fertilizer nutrients (phosphoric acid and ammonium nitrate) to maintain two-fold higher aqueous nutrient concentrations. Five preservative-free emergence traps were deployed on each side of each wetland, once before and three times post-treatment, to measure impacts on emergence of aquatic insects. Differences in taxonomic richness, abundance and biomass of insects collected from treated and untreated sides of each wetland will be compared.

Growth, survivability, and reproductive effects of pulse-dosed endosulfan on Florida flagfish *(Jordanella floridae)* over one life-cycle.

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Endosulfan is a commonly used organochlorine pesticide in Durham Region, Ontario, which has known toxic effects on non-target organisms including fish. This research investigated the effects of endosulfan on American flagfish (*Jordanella floridae*), using both continuous and pulse-exposure. The 96-hour continuous exposure LC50 in larval flagfish was 4.35 $\mu g \cdot L^{-1}$; sub-lethal observations included hyperactivity, convulsions, and some axis malformation. The effects of a 4-h endosulfan pulse-exposure on 7-8 d-old larval growth, reproduction, and survivability were investigated over one full life-cycle. The 4-h pulse-exposure LC50 value for larval flagfish was 49.7 $\mu g \cdot L^{-1}$; there were no growth or reproductive effects of endosulfan pulse-exposure up to the highest exposure concentration of 10 $\mu g \cdot L^{-1}$. Thus, the life-cycle 4-h pulse-exposure NOEC and LOEC were 3.2 and 10 $\mu g \cdot L^{-1}$ endosulfan, respectively, due to significantly higher mortality.

Chronic effects of single intra-peritoneal endosulfan injection on rainbow trout (*Oncorhynchus mykiss*) and field observation of caged fish in Oshawa Creek.

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The organochlorine pesticide endosulfan has been shown to be highly toxic to fish and there is some evidence to support that it may act as an endocrine disrupting chemical. Juvenile rainbow trout (*Oncorhynchus mykiss*) were caged at 4 sites in Oshawa Creek during the fall and spring of 2008 and 2009 while another group was intra-peritoneal injected in the laboratory with varying concentrations ($mg \cdot Kg^{-1}$) of endosulfan. Plasma vitellogenin (VTG) levels, liver ethoxyresorufin-O-deethylase (EROD), citrate synthase (CS), lactate dehydrogenase (LDH), and brain acetylcholine esterase (AChE) (caged fish only) enzymatic activities were measured. Trout injected with endosulfan experienced an increase of the anaerobic (LDH activity) and a decrease of the aerobic (CS activity) metabolic pathways, while male VTG levels increased. Since it was a singular injection, VTG results have to be confirmed. Fall caged trout showed increased EROD activity and inhibited AChE activity while those caged in the spring experienced an unexpected exposure to the lampricide 3-Trifluoro- Methyl-4-Nitro-Phenol (TFM) which disrupted

metabolic parameters (inhibited CS and increased LDH activity). Both fall and spring caged trout experienced no induction of VTG activity. Further research is needed since the spring exposure was altered due to the unplanned TFM treatment and thus did not represent a valid temporal replicate.

Toxicologie des amphibiens et toxicologie faunique I
Amphibians and Wildlife Toxicology I

Influence of agricultural contamination on the reproductive system of male bullfrog (*Rana catesbeiana*).

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The surrounding land of the Yamaska River drainage basin is heavily utilized for agriculture, mainly for corn and soybeans, leading to pesticide levels in surface waters that have surpassed the Canadian Water Quality Guideline up to 20-fold. Frog populations of the Yamaska River have been decreasing in the past years. The goal of the study was to characterise the influence of agricultural run-off on the reproductive system of adult American bullfrogs, an aquatic sentinel species. Adult males were collected at 6 strategic sites with low, medium and high agricultural intensity in the Yamaska River drainage basin during the summers of 2007-2008. Morphometric measures were taken, and testes and nuptial pads were weighed, measured and processed for histological analyses. There were no differences in testicular size or volume or lobule diameter among sample sites. There were significantly fewer spermatogonial cell nests along the lobule wall in animals from highly contaminated sites. Throat color and nuptial pad thickness (secondary sexual characteristics) demonstrated slight differences between two contaminated sites and reference sites. These results suggest alterations in androgens and spermatogenesis, which could impair reproductive capacity, possibly due to agricultural run-off. These results contribute to characterising the effects of agricultural pollution on the reproductive system of a ubiquitous sentinel species that can be used for environmental monitoring.

Development of an early life stage toxicity test using the Pacific Tree Frog (*Pseudacris regilla*): case study using selenium and sulphate.

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Environmental toxicity testing is a useful environmental monitoring and risk assessment tool. Despite their importance to ecosystems, amphibians are under-represented in toxicity testing programs, and standardized amphibian testing species and protocols do not exist in Canada. In this study, we have explored the development of an early life stage toxicity test using the Pacific tree frog (*Pseudacris regilla*), a species

common to North America. In the first test, *P. regilla* tadpoles were exposed to a range of concentrations of sodium selenate until 70% of controls had reached metamorphosis (112 days). The endpoints examined were mortality, developmental stage, wet weight and snout-to-vent length. The second test exposed tadpoles for 21 days to sulphate in two different water types, moderately hard water and soft dechlorinated tap water. In addition to the aforementioned endpoints, hind limb length and body length were also measured. The resulting LC50s (and 95% confidence limits) were 4.6 (3.3 - 5.3) $mg \cdot L^{-1}$ selenium, 1495 (817 - 2047) $mg \cdot L^{-1}$ sulphate using moderately hard water and 2233 (1494 - 8505) $mg \cdot L^{-1}$ sulphate using soft dechlorinated municipal tap water. Information was obtained from these tests with respect to appropriate exposure duration, test chamber size, temperature, food type and light intensity for early life stage tests using this species.

Évaluation des risques environnementaux
Environmental risk assessment

Comparative evaluation of the impact of different disinfection procedures on the toxicity of the wastewater of the city of Montreal.

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Urban wastewaters are one of the main sources of surface water pollution in Canadian bodies of water (in volume). The Saint-Laurent (St. Lawrence) River receives the effluent from the most important water-treatment station in North America, that of Montreal. Some years ago, the City of Montreal set up a program to monitor the ecotoxicology of its wastewater. Its objective was to study the impact of the disinfection of effluent using three different methods: ultraviolet irradiation (UV), performic acid and ozone. Brook trout (*Salvelinus fontinalis*) were exposed in tanks with different dilutions, representing the respective sectors of the panache of distribution of the wastewater discharged to the island called Île aux Vaches. The panache distances were 300 m, 1 km, 4.2 km and 10 km as well as a control tank. After 90 days of exposure, we observed that the treatment of disinfection by ozone had no effect on the weights or lengths of the fish and did not prevent lymphocytes T and B from proliferating, thus does not seem to have increased the toxicity of effluents. It did not increase the phagocytosis and it had a weak effect at 300 m on cytotoxic cells (NCC). This effect disappeared at 1 km and 4.2 km.

DIESE : un projet pour l'évaluation des risques écotoxicologiques liés aux sédiments contaminés.

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Les enjeux écologiques, réglementaires et économiques de la gestion des sédiments contaminés imposent le développement des méthodologies robustes d'évaluation des risques environnementaux. Il importe que ces méthodologies soient organisées selon une approche graduée, qui permette de hiérarchiser et proportionner les efforts mis en œuvre pour l'évaluation selon les risques potentiels sur les écosystèmes. Sous la conduite de l'Université de Metz, le Cemagref, l'Université de Reims et EDF R&D ont lancé en 2008 le projet « Outils de Diagnostic de l'Ecotoxicité des Sédiments (DIESE) », financé par l'Agence Nationale de la Recherche (ANR). Le projet DIESE structure les travaux sur le développement d'une batterie d'outils de mesure et de modélisation pour l'évaluation du risque écologique spécifiquement applicable aux sédiments contaminés. Le projet est construit en trois axes: le premier vise au développement d'outils permettant de mieux identifier l'exposition potentielle ou réelle d'organismes du sédiment; le deuxième axe s'attache à développer des outils d'observation et d'interprétation des effets; le troisième axe vise à intégrer ces outils dans le cadre d'une approche graduée des risques, et à en démontrer leur faisabilité sur des études de cas concrètes. Les résultats présentés s'insèrent dans le deuxième axe du projet et concernent les microbiotests en tant qu'outils opérationnels de screening des effets toxiques et la batterie des tests écotoxicologiques représentant plusieurs niveaux d'organisation taxonomique. Cette batterie joue un rôle central dans l'évaluation de la qualité des sédiments et intègre la biodisponibilité des substances dans un contexte de multi pollution.

The use of monospecific and multispecific bioassays to assess the ecocompatibility of alum application in eutrophicated lake restoration.

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Saint-Augustin Lake (Québec, Canada) is strongly eutrophicated (120 $\mu g \cdot L^{-1}$ Ptot, Chlorophyl-*a* (max) 62 $\mu g \cdot L^{-1}$, Secchi: 1.5 m). The main sources of phosphorus in this system are the superficial sediments (300 $mg \cdot kg^{-1}$ P extractable) and the groundwaters ($P>40$ $\mu g \cdot L^{-1}$). Metals and metalloïds are also presents in sediments at high concentrations. The aim of this study is to assess the ecological compatibility of a restoration method for this eutrophicated lake that consists of precipitate/flocculate P in the water column with alum (Al_2SO_4). The present work focuses on the impact of an application of 20 $mg \cdot L^{-1}$ of alum. Organisms were introduced in media 24 hrs after alum application; first, using monospecific bioassays *P. subcapitata* (growth), *L. minor* (growth), *D. magna* (survival and reproduction), *H. azteca* (survival and reproduction), *C. riparius* (survival, growth and emergences), *O. mykiss* (survival and growth), *P. promelas* (survival and growth), and then, using 2 litre microcosms that group the 5 first

above-cited organisms. The bioassays were performed with formulated water and sediments as laboratory control, and then with water and sediments from the Saint-Augustin Lake. This presentation will emphasize the results obtained with water and sediments from Saint-Augustin Lake. *P. subcapitata* growth and *D. magna* reproduction were inhibited in monospecific bioassays while no significant effect was observed in microcosms.

Development of cadmium bioassays using the green alga *Chamydomonas reinhardtii.*

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Certain trace metals are major environmental pollutants due to their high toxicity at low concentrations, non-biodegradability, and concentration in the food chain. Effective means of their quantification in water and soils are required to monitor the risks posed to ecosystems and human health. Although analytical chemistry provides excellent methods for quantifying metals, complete environmental risk assessment requires knowing whether or not a metal is bioavailable, i.e. present in toxic or benign species. To achieve the difficult task of quantifying specifically bioavailable metal species, we are developing bioassays using the green alga *Chlamydomonas reinhardtii* to detect trace amounts of cadmium (Cd). The characteristics of such a bioassay are specificity for Cd at environmentally relevant concentrations, the capacity for *in situ* measurements, and the technical simplicity required for field work. Using global transcriptome analysis, we recently identified ten genes that are activated by trace concentrations (from 7.8 nM to 9.0 μM) of Cd^{2+} (Simon *et al*. 2008). We focused on one gene, AOT4, because it shows the highest level of induction (42 fold) at low [Cd^{2+}].

The toxicity of oil contaminated peat following biodegradation.

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Contaminated soil from crude oil pipeline spills must be remediated in accordance with environmental regulations. The current Canadian Council of Ministers of the Environment criteria indicates the maximum allowable levels of hydrocarbons resulting from an oil spill. However, it assumes that all detectable hydrocarbons are petroleum hydrocarbons (PHC), and does not account for naturally occurring biogenic hydrocarbons (BHC). This failure to account for the naturally occurring BHCs means that soils in question may be wrongly assessed as being PHC contaminated. False identification of contaminated soils could lead to unnecessary bioremediation, which is costly and potentially disruptive to functioning ecosystems. This study is part of a larger project to distinguish between natural and petroleum F3 hydrocarbons in pipeline oil-spill impacted muskeg material. The objective of the current study was to examine the toxicity of oil contaminated muskeg (peat) following biodegradation in laboratory microcosms. Preliminary toxicity tests, an earthworm (*Eisenia Andrei*) reproduction bioassay and a

Northern Wheatgrass (*Elymus lanceolatus*) growth bioassay were completed using locally purchased Sphagnum peat moss contaminated with Federated Crude oil (10,000 $\mu g \cdot g^{-1}$ Fraction 3; 22,000 $\mu g \cdot g^{-1}$ total petroleum hydrocarbons). The most sensitive endpoint was the number of juvenile earthworms. For Northern Wheatgrass, root and shoot growth stimulation was observed at low concentrations. Federated Crude oil contaminated peat aged under simulated conditions was also tested and compared with fresh oil contaminated peat. Based on the number of juvenile earthworms produced, 10,000 $\mu g \cdot g^{-1}$ Fraction 3 contaminated peat was more toxic than uncontaminated controls at day 0 and day 150, but by day 300 the toxicity was eliminated.

Métaux aqueux ou associés à la nourriture
Waterborne and diet-borne metals

Biodilution and biomagnification of silver in Lake Moreno, Patagonia, Argentina.

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Silver (Ag) concentration in sediments and biota from Lake Moreno, Patagonia, Argentina, have been found to be enriched in association with human population growth. Fish liver Ag concentations are among the highest ever reported globally. The goal of this study was to analyse the trophic transfer of Ag in Lake Moreno in Nahuel Huapi National Park, in light of the ecotoxicological risk. During 2001-2008 the biota of Lake Moreno was sampled, and stable N and C isotopes were used to characterize food web transfer of Ag. There was Ag biodilution between phytoplankton and zooplankton. In addition, no toxic effects of diet-borne Ag were found in relation to [Na] in zooplankton. Interestingly, brook trout (*Salvelinus fontinalis*) showed hepatic biomagnification, although no biomagnification trends were detected for muscle tissue samples. When whole biota was analysed, significant Ag biomagnification was observed for the whole food web. Both Ag biodilution and biomagnification operate simultaneously in Lake Moreno. Biodilution in the phytoplankton and zooplankton food web decreases the toxicological risk for organisms in planktonic food webs, whereas biomagnification in fish livers and whole biota increases the toxicological risk to terrestrial and avian predators such as gulls. However, human risk is very low due to the absence of fish liver consumption in the region.

Prise en charge et adsorption de l'aluminium et du fluorure par les algues vertes dans les eaux résiduaires d'alumineries.

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Ce projet, en collaboration avec l'industriel Rio Tinto Alcan, examine l'accumulation et l'adsorption de l'aluminium et du fluorure par les algues vertes dans des conditions similaires à celles que l'on retrouve dans les eaux résiduaires d'alumineries. L'accumulation désigne la portion des éléments qui pénètre à l'intérieur de la cellule alors que l'adsorption désigne la portion des éléments se liant à des sites fonctionnels présents sur la paroi et la membrane cellulaire. Nous tenons aussi compte de la spéciation de l'aluminium dans le milieu en considérant que dans les lacs acides, tels que ceux du Bouclier canadien, l'aluminium peut former des complexes stables avec le fluorure. Ceci nous permettra d'étudier le rôle des fluorures sur l'adsorption et l'accumulation de l'aluminium. Éventuellement, une comparaison de diverses espèces d'algues vertes et de leur capacité à accumuler et à adsorber l'aluminium et le fluorure sera effectuée. L'algue verte unicellulaire *Chlamydomonas reinhardtii* est exposée durant 4 jours à des milieux synthétiques contenant de l'aluminium et du fluorure. Au terme de la période d'exposition, la méthodologie permet de connaître la proportion de métaux qui a été adsorbée et absorbée. Ce projet permettra de faire avancer les connaissances sur le sujet tout en explorant le potentiel des algues à épurer les eaux résiduaires d'aluminerie.

The effect of Zn on photosynthetic energy dissipation processes of freshwater cyanobacteria and algae: acclimated versus non acclimated species.

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Zinc is one of the most common metals in polluted freshwater systems. Zn is an essential element for photosynthetic organisms, but it is toxic at high concentrations. Although Zn is known to affect photosynthetic processes, little is known of its effect on photosynthetic energy dissipation processes. Our main goal was to understand how different species may change their photosynthetic energy dissipation processes when acclimated to low Zn concentration. Furthermore, we investigated the toxic effect of Zn on Zn-acclimated and non-acclimated species. For the acclimation experiment *Phormidium sp.*, *Oedogonium sp.* and *Gomphonema angustatum* were grown in presence of 25 and 50 $\mu gZn \cdot L^{-1}$. After this acclimation period, Zn toxicity tests (100 and 300 $\mu gZn \cdot L^{-1}$ for 6 and 30h) were performed. For both experiments we measured cell growth, photosynthetic processes (PhytoPAM and PEA fluorometers), pigment content, oxygen evolution and PSI/PSII stoichiometry. Our results indicate that acclimation to low Zn concentration induced changes in energy dissipation processes and pigment content, which were different among species. These adjustments seem to induce higher tolerance to elevated Zn concentrations compared to non acclimated species. For the Zn toxicity tests, species not acclimated to Zn presented higher reduction of photochemistry (Φ_M, Φ'_M, qP, oxygen evolution) than Zn-acclimated species. The decrease in photochemical activity was counterbalanced by an increase in non-photochemical processes (NPQ). The

obtained results bring new information to explain at the photosynthetic level why some species are found when pulses of high Zn concentration occur or when low levels of Zn are present in aquatic ecosystems.

Bioaccumulation of Cd in binary metal mixutres by *Chlamydomonas reinhardtii*.

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Simple equilibrium models such as the Free Ion Activity Model (FIAM) or the Biotic Ligand Model (BLM) generally provide good prediction of the bioaccumulation or toxicity of trace metals. Nonetheless, these models have not been sufficiently tested under natural conditions or under conditions containing complex contaminant mixtures. The objective of this study was to quantify the effects of metals in complex mixtures by comparing stability constants for single metals and for solutions containing binary metal mixtures. Stability constants were evaluated from intracellular metal concentrations obtained following the exposure of *Chlamydomonas reinhardtii* to an increasing concentration of Cu and Pb, in presence and absence of 5x10-7 M Cd^{2+}, in a well controlled medium (10-2 M HEPES with 5x10-3 M Ca). The results show that the presence of Cd did not affect the internalisation of Pb for the exposure concentrations that were examined. On the other hand, initial results have shown that copper internalization increased in the presence of Cd at the lowest Cu exposure concentrations that were examined. At higher concentrations of both Cu and Pb, an inhibition of cadmium internalization was observed, as would be predicted by the BLM. Cadmium bioaccumulation decreased to a much greater extent in the presence of Pb as opposed to results observed in the presence of Cu. In a second set of experiments, the effects of multiple metals (Cu, Pb, Ni and Zn) on the short term bioaccumulation (≤ 30 mins.) of Cd by *C. reinhardtii* were examined in a complex Tris-Acetate-Phosphate (TAP) medium, for a short period of time. Under these conditions, the presence of five-fold excess in the concentration of a second metal, the intracellular Cd decreased. Results from both these experiments are discussed in the context of the BLM and its potential applications to complex media, including natural waters.

The complex relationship between metal-gill binding and toxicity in trout exposed to Pb plus Cd mixtures.

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The majority of metal toxicity research focuses on individual metals, rather than mixtures which are more likely in contaminated waters. Pb and Cd are two metals that may coexist in contaminated waters. These metals compete for the Ca channels on the gill, and are thus assumed to interact competitively. Our goal was to determine how Pb plus Cd toxicity was related to their interactions at fish gills. We predicted increased toxicity as Pb plus Cd exposure concentrations increased, and that this would be related

to additive metal-gill binding. Accordingly, rainbow trout (~1-2 g) were exposed to a matrix of Pb and Cd concentrations (6-24 nM and 600-2400 nM respectively) for 10 days in soft water (Ca^{2+}~100 μM; pH~6). Sub-samples of gills were collected at 3 h for metal-gill quantification to determine if metal-gill accumulation was predictive of toxicity (LT50). Increased concentrations of Pb reduced Cd-gill binding and toxicity when fish were exposed to 6 nM Cd, (96-h LC50 or 1 TU). When Cd concentrations were 12 nM (~1-2 TU), however, Pb was no longer protective as Cd-gill binding and toxicity increased. Unlike the addition of Pb, addition of Cd (6-24 nM) to the water resulted in greater Pb-gill accumulation in trout but paradoxical decreases in toxicity, suggesting possible competition for binding sites on the gill. We suggest that Pb protects against Cd toxicity at low Cd concentrations due to competitive interactions between metals. At higher Cd concentrations, however, the complexity of these interactions is greater, making it difficult to predict toxicity. Mathematical modeling of Pb-Cd-gill interactions may shed additional light on the nature of these unexpected findings.

« Omiques » en toxicologie aquatique
"Omics" in aquatic ecotoxicology

Transcriptional response of fathead minnows (*Pimephales promelas*) to chronic exposure of environmentally relevant dietary or waterborne copper.

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Although essential, copper can become toxic to fishes when environmental concentrations are elevated, typically from industrial activities. We used fathead minnows (*Pimephales promelas*) to investigate copper-induced gene expression changes in different tissues using a 15K gene microarray. Tissues included gill, gut and olfactory epithelia of fish chronically exposed (25 days) to environmentally relevant waterborne (10 $\mu g \cdot L^{-1}$) or dietary (~100 $\mu g \cdot g^{-1}$) Cu concentrations. One-way ANOVA asymptotic selection was used to identify genes where expression changed 2-fold or more in Cu-exposed fish relative to controls. Fewer genes were differentially expressed in waterborne exposures compared to diet-borne Cu exposure in both gill (20 and 28 genes, respectively) and gut tissues (28 and 30 genes, respectively). The majority of these genes were down-regulated. In olfactory epithelia, waterborne Cu resulted in 20 genes being differentially expressed. Among these, 7 genes were down-regulated and were associated with signal transduction, transcription, protein synthesis and structural proteins. Apoptotic genes were up-regulated. Differential expression of these genes may have caused impairment to the olfactory system, as fish exposed to waterborne copper showed impaired responses to olfactory stimuli in behavioural assays. These findings suggest that fathead minnows are useful models in identifying mechanisms of copper toxicity, and have a role to play in environmental monitoring and ecological risk assessment.
(NSERC-MITHE Strategic Network)

Indicateurs au niveau de l'organisme ou de la population
Organism or population level indicators

Evaluation of contamination history using deformed insects.

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Animals can persist in contaminated environments and yet show toxic effects. Such is the case for the sediment-dwelling insect *Chironomus*, which can survive in contaminated lakes and yet be physically deformed. The frequency of such abnormalities has been used to reveal the contamination state of the environment in which the larva lives. A case in point is Parry Sound harbour (Georgian Bay), which has suffered from inputs of sewage, metals and oil (a 1950 oil spill covered the harbour area). In the 1970's, ~80% of the *Chironomus* larvae collected in the harbour area were reported to have deformed mouth parts, whereas those outside of this area had none. We set out to determine if, 30 years later, the quality of the environment has improved. We did this by collecting *Chironomus* larvae and examining them for deformities, as well as by evaluating the structure of the benthic community, both at contaminated and uncontaminated sites in Parry Sound. Results indicate that the incidence of deformities has declined over the past 30 years, which suggests that contaminated sediments are being gradually buried such that larvae are less exposed to the contaminants they contain. We are also measuring organic and inorganic contaminants in *Chironomus* larvae from contaminated and uncontaminated sites so that we can determine the causative agent of the deformities.

Identifying the cause(s) of toxicity at sites in a stormwater management facility to the freshwater amphipod *Hyalella azteca*.

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[1]Environment Canada.

The Terraview-Willowfield stormwater management facility (Toronto, Ontario) receives inputs of multiple contaminants, including road salt, PAHs, and metals, via run-off from Highway 401 (a multi-lane, divided highway with controlled access) and the surrounding residential area. A previous study determined that salinity in the overlying water may influence the toxicity in deeper sections of the ponds. In order to investigate the effects of salinity in these ponds on benthic invertebrates, overlying water and sediment samples were collected from 4 sites in Fall 2007 (low salinity) and 6 sites in Spring 2008 (high salinity), and four-week sediment toxicity tests were conducted with *Hyalella azteca*. Survival and growth were not significantly affected in fall samples (200 mg $Cl^{-} \cdot L^{-1}$). However, significant effects on survival and growth in spring samples (1400-4000 mg $Cl^{-} \cdot L^{-1}$) were observed, and were not related to changes in PAH levels or bioavailable metal concentrations. Four-week toxicity tests conducted with NaCl confirmed that effects observed in stormwater pond tests were the result of chloride exposure: four-week EC25 (growth), LC25, and LC50 were 425, 957, and 1210 mg $Cl^{-} \cdot L^{-}$

[1], respectively, and complete mortality occurred above 1800 mg $Cl^{-} \cdot L^{-1}$. Although PAHs and metals are also of concern, our results indicate that chloride from road salt is the primary cause of toxicity in this laboratory study of the Terraview-Willowfield stormwater management facility.

Cancer regulating proteins in neoplastic *Mytillus edulis* and *M. trossulus* from contaminated sites in Greater Vancouver.

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Haemocytic leukemia affects bivalves and is fatal in most cases. Contaminant exposure, abnormal temperatures, or viral infections may play roles in the disease etiology. Disease incidences tend to be more prevalent in contaminated areas. A characteristic of the disease is overproduction of haemocytes, the equivalent to monocytes in vertebrates. p53 proteins are "guardians of the genome" and regulate the transcription of genes involved in DNA repair and tumour suppression. The p53 gene family is very highly conserved; thus regulation of gene expression by environmental factors may have direct implications for human health. Proteins from the p53 family are present in normal and leukemic individuals from various salt water mussel and clam species, including *Mytilus edulis*, *Mytilus trossulus*, and *Mya arenaria*, whereas p97 is down-regulated in leukemic cells. Our western blot analysis of *M. trossulus* and *M. edulis* that we caged at sites in the Greater Vancouver Region confirmed that an antibody recognizing a homogenous domain of p63/p73, recognized a 73 kDa band in leukemic cells that was not detectable in normal cells of both species. The homodomain is not p53 and the antibody does not appear to cross-react with p97 in normal cells. This up-regulated and unidentified 73 kDa protein may be an alternate form of p73, which has been modified in some way to become an oncogene. The 73 kDa protein has strong potential as a biomarker protein for the identification of moderately or incipiently leukemic individuals in environmental sites of interest.

Comparison of tumor-suppressor gene expression in two mussel species with different susceptibilities to haemic neoplasia, a cancer of the haemolymph.

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Mussels of the genus *Mytilus* are frequently used in environmental monitoring programs across the world. Despite the many morphological, genetic and physiological similarities between the individual species in this genus (especially between *M. edulis*, *M. trossulus*, *M. galloprovincialis*), the mussels also show a variety of dissimilarities, which have to be taken into account in environmental assessments. Local species distribution and the occurrence of hybridization zones may contribute to variations in results of parameters tested in environmental monitoring programs. These species-specific differences need to be taken into careful consideration. A comparison between *M. edulis* and *M. trossulus* was initiated by Metro Vancouver during a pilot receiving environment

effects monitoring program. A fatal neoplastic disease of the haemolymph of many bivalves, haemic neoplasia or leukemia, is thought to be affected by genetic, environmental and potentially viral factors. The occurrence of the disease and other physiological endpoints were monitored on caged *M. edulis* and *M. trossulus* over two years in Burrard Inlet, Vancouver, Canada. Despite identical environmental conditions and exposure periods, M. *edulis* showed a much lower disease level than *M. trossulus*. Previous work implicated the tumor suppressor family p53 in the disease. We now compare the expression levels of the p53 family and key regulatory genes in *M. edulis* and *M. trossulus* with the objective to identify potential differences in gene expression levels that may contribute to the different prevalence in disease levels we have observed.

Comparison of benthic macroinvertebrate community to sediment quality guidelines classification using various indices: The case of the St. Lawrence River.

DESROSIERS, M. [1], CORMIER, M. [2], MASSON, S. [3], ARCHAIMBAULT, V. [4] and PINEL-ALLOUL, B. [5]

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Many studies have showed that impact of chemical contamination on benthos can be hard to demonstrate in the presence of unmeasured factors. Although influence of some habitat characteristics has been identified in parts of the St. Lawrence River, the use of benthos to assess chemically degraded sites remains uncertain. The purpose of this study is to compare the potential of various benthic macroinvertebrates' community indices (diversity, richness, biotic) for assessing sediment quality in comparison with chemical sediment quality guidelines classification. In an attempt to identify possible key factors that could be weighing in on benthic macroinvertebrates' community structure, we will also regroup sites based on prospect habitat characteristics. This study is a part of a large research project aimed at developing an ERA tiered framework for sediment management, in the context of integrated management of contaminated sediment, site restoration and sustainable navigation. During falls 2004-2005, macroinvertebrates were collected at 59 sites in the St. Lawrence River, especially in its three fluvial lakes and in the harbour zone of Montreal. Organic (PCBs, PAHs, petroleum hydrocarbons), inorganic (As, Cd, Cu, Cr, Hg, Ni, Pb and Zn) contaminants and sediments characteristics (e.g. grain size, metal-binding phases, nutrients) were measured in whole sediment. The preliminary results and the implication for the development of an ERA tiered framework will be discussed.

Comparison between functional traits and taxonomy of benthic macroinvertebrates as tools for ecological risk assessment (ERA): the case of the St. Lawrence River.

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This study is a part of a large research project aimed at developing an ERA tiered framework for sediment management, in the context of integrated management of contaminated sediment, site restoration and sustainable navigation. The purpose of this study is to assess sediment quality by exploring the relationships between chemical contamination and benthic community structure using classical taxonomy and functional trait approaches. During falls 2004-2005, macroinvertebrates were collected in 59 sites in the St. Lawrence River, especially in its three fluvial lakes and in the harbour zone of Montreal. Organic (PCBs, PAHs, petroleum hydrocarbons), inorganic (As, Cd, Cu, Cr, Hg, Ni, Pb and Zn) contaminants and sediment characteristics (e.g. grain size, metal-binding phases, nutrients) were measured in whole sediment. Taxonomy and functional traits — i.e., biological traits such as size, morphology, life cycle or ecological traits (e.g. preferences for different habitat characteristics that can inform on ecosystem functioning — of benthic macroinvertebrates in the St. Lawrence River will be used to assess the relative impact of chemical contamination and sediment characteristics, applying multivariate analyses and variance partitioning. We will compare the potential of taxonomy and functional trait approaches for assessing sediment quality in relation to chemical sediment quality guidelines classification. The preliminary results and the implication for the development of an ERA tiered framework will be discussed.

Analysis of age in pearl dace used for environmental monitoring in Ontario: understanding the implications of past incorrect age estimates.

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Small-bodied fish can represent an integral component of an Environmental Effects Monitoring (EEM) program for aquatic habitats associated with mine sites across Canada. These fishes are of particular importance for headwaters of watersheds lacking large-bodied fish. For an ore extraction mine in northern Ontario, pearl dace (*Margariscus margarita*) were used to meet the EEM monitoring requirements. Studies conducted over the last decade at this mine have generated extensive information on the pearl dace, including age analysis, body condition, length-frequency distributions, and maturity schedules. These analyses indicate healthy populations and strong sexual dimorphism for adult fish. Past age analyses of the pearl dace involved different methods that led to variable estimates of age. Recent image-analysis of pearl dace otoliths identified spawning females as usually 10 or older whereas the males were always younger. Additional analyses of pearl dace age were completed with opercular bones and mass spectrometry of microelemental concentrations of otoliths to confirm age estimates

from image analysis of lapillus otoliths. These varied methods led to a confirmation of observations and estimates of length-at-age. This information suggests pearl dace achieve higher ages than reported in past studies, and indicates a longer generation time and lower productivity than inferred previously. These results suggest that small-bodied fish populations may be incorrectly assessed with simple age analyses involving scales or length distributions, and this could compromise long-term EEM monitoring efforts.

Tests de toxicité
Toxicity Tests

Investigation of cadmium toxicity in *Chlamydomonas reinhardtii* strains with and without cell wall by using chlorophyll fluorescence parameters.

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Photosynthetic apparatus shows high sensitivity to toxic effects induced by metals. Such effects may alter different biochemical and biophysical functions of photosynthetic apparatus and consequently cause inhibition at algae cellular physiological state. In this study, we examined specificity of cadmium toxicity in two strains of *Chlamydomonas reinhardtii* having different cell wall properties. Here, we used chlorophyll fluorescence parameters to evaluate influence of cell wall on cadmium toxicity. Different fluorescence parameters dependent on Photosystem II photochemistry were used as reliable and sensible biomarkers to evaluate cadmium toxicity. Those parameters have been analysed after 24 and 48h of exposure of *C. reinhardtii* to different concentrations of cadmium. The important effect of cadmium toxicity has been noticed on cellular division in both strains of *C. reinhardtii*. However, cell wall defficient strain was more sensitive to cadmium effect on cellular division. For this strain, chlorophyll fluorescence parameters revealed that Photosystem II photochemistry was more altered compared to wild type alga having cell wall. We demonstrated fluorescence parameters showing non-photochemical energy dissipation to be the most sensitive indicators of cadmium toxicity. Our results indicate that cell wall defficient strains of algae may be used as highly sensitive indicators of metal toxicity.

Comparative study of different herbicides' and pesticides' acute toxicity in *Chlamydomonas reinhardtii*.

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Alga *Chlamydomonas reinhardtii* is frequently used as a bioindicator for different pollutants' toxic effects. The most sensitive target of toxicity is known to be primary photochemical reactions in photosynthesis. In this study, toxicity was induced by aldicarb, cyanazin, malathion and picloram at low concentrations (0.1-50 $mg \cdot Kg^{-1}$) permitting us to evaluate possible protocols of rapid toxicity tests. We used rapid rise of

fluorescence, which is dependant on photosystem II primary photochemistry and plastoquinone reduction as electron carrier between photosystems II and I. Fluorescence yield at transitions I and P were used to determine sites of actions. The majority of those pollutants were acting as electron transport inhibitors, therefore energy dissipation via photosystem II was altered. This effect provided information on energy dissipation via non-photochemical pathways. Such a parameter was very important for use as a sensitive and reliable indicator. We found very high diversity in sensitivity of *Chlamydomonas reinhardtii* to investigated pollutants. Since the aquatic environment is frequently contaminated with such pollutants, rapid biotests are supposed to be necessary for evaluation of toxicity risks. Our results are important to define sites of pollutants' action and practical approaches to developing toxicity tests. Those toxicity indicators will be also useful for developing new fluorimetric methods for practical applications in the field.

Investigation of physiological stress in *Chlorella vulgaris* exposed to non-equilibrated composition of nitrogen.

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In this study, the investigation of functional and structural properties of *Chlorella vulgaris* has been done when algae were exposed to nitrogen deficiency and high concentrations of acetate-inducing inhibitory effects on biomass growth. Here, high cellular concentrations of *C. vulgaris* (15 x 106 cells mL^{-1}) grown in BG11 medium as algal biomass were used as initial biomass for exposure to physiological stress. Algal cells were exposed 18 days to physiological stress under continuous illumination. Gradually, algal chloroplasts were transformed in chromoplasts. Such transformation has been associated with the change of structural and functional properties of the photosynthetic apparatus. We found a strong decrease of chlorophyll, Photosystem II and I photochemistry and gradual increase of carotenoids content. Algal cultures have been continuously exposed to light but photoinhibition did not show altered effect. Formation of high concentrations of carotenoids induced protection effect. After 18 days of exposure and drastic transformation of chloroplast in chromoplast, cellular culture was able to recover if it was transferred again to BG11 medium. The important question posed was: is this transformation induced by nitrogen deficiency or acetate toxicity effect? We attempt to interpret the relationship between environmental physiological stress and structural and functional modification of the photosynthetic apparatus.

Confusing effect of phosphorus and nitrogen present in pulp and paper effluents on the results of the aquatic toxicity test with *Pseudokirchneriella subcapitata*.

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Effluents from pulp and paper industries having a secondary treatment plant are rich in nutrients such as phosphorus and nitrogen. This characteristic often leads to unusual results when this kind of effluent is analyzed for its environmental toxicity using a growth/inhibition algal test. Indeed, inhibition and/or stimulation are frequently observed and concentration-response curve is often atypical. This could hide the real toxicity of the effluent. The goal of the project was to determine if high concentrations of P and N added to every concentration of the test, including the control group, could eliminate the interference of this nutrient-rich effluent on the results of the algal toxicity test. Results indicate that enrichment with P can largely attenuate the interference of rich P effluent and lead to expression of toxicity response initially masked by P. The enrichment with P also permits reestablishment of a more typical concentration-response curve for the majority of cases studied. When an effluent is suspected to be rich in nutrient, it is advisable to perform both a standard algal test and a test with enrichment of P.

The toxicity of Harmony Landfill leachate to green Hydra.

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Harmony Landfill is a 9-acre former industrial waste disposal site which is located immediately adjacent to Harmony Creek in Oshawa, Ontario, Canada. Although environmental monitoring took place during the landfill's operational period, from 1957 to 1985, the current environmental impact of the landfill on the aquatic environment is unknown. Historical and recent analyses have revealed elevated levels of metals including aluminum, cadmium, chromium, copper, iron, lead, manganese and zinc in landfill leachate samples. Laboratory toxicity tests were conducted on the freshwater invertebrate *Hydra viridissima* (Green Hydra) using varying concentrations (0%, 3.2%, 10%, 32%, 100%) of leachate samples diluted with laboratory water. *Hydra viridissima* were pulse-exposed for 24 hours and population reproduction at 25 °C was recorded daily for 7 days. Reproductive toxicity was observed at the 100% leachate concentration. Current findings indicate that the leachate flowing from Harmony Landfill has low potential to negatively affect aquatic invertebrate populations. Future research will test leachate collected seasonally and assess toxicity under field conditions.

Behavioural and biochemical responses of *Chironomus tentans* and *Lumbriculus variegatus* to tributyltin.

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Chironomus tentans and *Lumbriculus variegatus* have been extensively used in the past as bioindicators in aquatic systems. However, previous experiments have been based around biochemical results such as bioaccumulation while observing for non-repeatable biological endpoints such as death. The behaviour of these two benthic organisms while exposed to sub-lethal concentrations of toxicants is one aspect that has been repeatedly overlooked. This study attempts to correlate observerable sub-acute behavioural responses with the biochemical response of respiration of *C. tentans* and *L. variegatus* during exposure to the biocide tributyltin (TBT). Three behavioural endpoints were observed for both invertebrates and were captured using live video feed. The larval insect *C. tentans* showed distinct behavioural responses of stress at all three endpoints, in correlation with a decrease in respiration rate at TBT concentrations of 1, 5, 10, and $20\mu g \cdot L^{-1}$. The aquatic worm *L. variegatus* showed an increase in respiration; however, no behavioural responses were observed at TBT concentrations of 0.02, 0.2, and $2.0\mu g \cdot L^{-1}$. The specific concentrations used for each species were determined by previous studies and environmental relevance. Future work will be done in order to discover what concentrations of TBT will elicit a behavioural response in *L. variegatus*. This study illustrates the usefulness of behavioural responses in the attempt to identify the presence of a toxicant as well as its utility in future live-feed bioassays used in monitoring freshwater systems.

Determination of effects of tributyltin on *Daphnia magna* and *Hyalella azteca* using the Multispecies Freshwater Biomonitor

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The analysis of organism behaviour represents a sensitive and ecologically relevant method to monitor water ecosystem quality. Behaviour demonstrates a whole-organism response to stimuli, including exposure to a toxicant. The specific effects of several pollutants on two aquatic organisms have been tested and quantified using the Multispecies Freshwater Biomonitor (MFB). The MFB is a non-optical, fully automatic, online, real-time biomonitor designed to record behavioural patterns of aquatic vertebrates and invertebrates using quadrupole impedance conversion techniques. *Daphnia magna* and *Hyalella azteca* were chosen as test organisms based on their sensitive responses to toxicants, their behavioural characteristics, and the position they inhabit in the aquatic system. By using these organisms, a holistic view of ecosystem health can be determined. This study is to determine the abilities and limitations of the MFB system in regards to using the above mentioned organisms both individually and collectively. The exact reaction of these organisms elicited by specific toxicants or toxicant families is also to be determined. This is a work in progress. Pollutant tested to this point is Tributyltin (TBT), a biocide used to clean the hulls of ships at concentrations

of 1, 10, and 100 $\mu g \cdot L^{-1}$. TBT was administered with the use of an organic carrier, DMSO, to ensure even dispersion through the test water and to prevent its adsorption to equipment surfaces. The effect of DMSO was examined for both the organisms. Use of 0.1% DMSO showed no adverse effects on the animals. Tests were conducted over 2 hours. Visual analysis of the organisms throughout all the tests was done to corroborate the data acquired by the MFB. Swimming behaviour, ventilation, and survival of the organisms were used as endpoints.

An assessment of thallium speciation and the relative toxicity of thallium species.

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Thallium was recently proposed as a recommended effluent quality parameter under the metal mining effluent regulation (MMER) environmental effects monitoring program (EEM). Thallium has been shown to significantly increase in both water and aquatic biota after exposure to metal mine effluent; however, there is a lack of knowledge as to its fate and effect in the aquatic environment. The objective of this project was to 1) establish speciation analysis to assess thallium speciation in test waters and selected mine effluents, and 2) conduct an effects assessment to assess the relative toxicity of thallium (I) and (III) on three aquatic species: the algae *Pseudokirchneriella subcapitata*, the invertebrate *Ceriodaphnia dubia* and the vertebrate *Pimephales promelas*. Speciation analysis proved difficult at environmentally relevant concentrations (</= 10 $\mu g \cdot L^{-1}$), however analysis with solutions >125 $\mu g \cdot L^{-1}$ revealed that over a 7-day period, the majority of thallium (III) was converted to thallium (I). It was only in solutions <24-h old where recovery of thallium (III) was greater than 80%. The lowest IC25s generated during our effects assessment for both thallium (I) and (III) were more than 10-fold greater than the highest concentration recorded in receiving environments (8 $\mu g \cdot L^{-1}$) and more than 100-fold greater than the current guideline (0.8 $\mu g \cdot L^{-1}$). Based on our speciation analysis, it would seem that the majority of thallium (III) in our test water was converted to thallium (I) over a 7-day period. This may hold true for receiving environments, but without an accurate, repeatable method to assess thallium speciation at low concentrations we cannot draw any firm conclusions as to whether this is the case. Development of a reliable speciation method is ongoing.

Application of TIE (Toxicity Identification Evaluation) in pluvial waters of the fertilizer industy in Brazil.

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A fertilizer industry in Brazil that produces approximately 1,443,668 tons of fertilizer per year is located in an important industrial region. Effluents generated by this industry are treated by an efficient physical-chemistry system and are completely reused in the industrial process. The pluvial water generated in this factory, however, presents a

high acute toxicity. Prior to choosing a treatment system for this pluvial water, the industry applied the TIE procedure to determine the components or compounds causing the acute toxicity. In this industry there are 2 tanks of the pluvial water storage, namely Station 1 and Station 2. Phase I of the TIE was applied to these 2 stations as per the methodology of the US-EPA 600/6-91/005F, 1992.TIE consists of the several steps where physical and chemistry manipulations, such as pH alterations and volatilization, are made in a sample to modify the toxicity. Using this concept as a base, it becomes easy to infer that the physical and chemical manipulations of the environmental sample can reduce the concentrations, or can remove different compounds, making them biologically unavailable. The changes of these compounds are measured in toxicity tests with manipulated and non-manipulated samples (Dorn & Compernolle, 1995). Comparison of these test results makes it possible to verify which manipulations were responsible for reduction or removal of the toxic compound(s) (Nascimento et al., 2002).In this study, 16 manipulations of each sample had been carried out, being: baseline, pH adjustment (acid and alkaline), pH initial + filtration, pH 7 + filtration, pH 9 + filtration, pH initial + aeration, pH 7 + aeration, pH 9 + aeration, filtration + post C18 Solid Phase Extraction Column, EDTA addition (2 concentrations) and Sodium Thiosulfate Addition (2 concentrations) tests. Toxicity tests making use of the *Daphnia similis* organism-test were employed, as in USEPA 712-C-96-114/1996. TIE results on Station 1 showed that the toxic compounds in this sample were: solids, oxidants, bivalent metals, ammonia and fluoride. On Station 2 the toxic compounds were: solids, bivalent metals, ammonia, fluoride and organic compounds.

Toxicity tests in marine sediments using amphypods *Tiburonella viscana*.

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In Brazil, most companies involved in sediment dredge operations must conduct several sediment analyses prior to beginning such activities. These analyses include: physical-chemistry analyses, benthonic survey and toxicity tests, as per federal law CONAMA N°344. Amongst the different organisms used in toxicity tests with sediments, amphipods are the most sensitive taxa, being the first species of the marine benthonic communities in contaminated places to disappear, and having an important function on benthonic ecosystems. In Brazil the species most used for sediment total toxicity testing is the digger amphipod *Tiburonella viscana* (Amphipod: Platyschnopidae). Recently (June 2009) 90 sediment samples around Santos Harbor, which receive domestic and industrial effluents were analyzed using the amphipod *Tiburonella viscana* following the methodology described by ABNT (Brazilian association of Norms Techniques) – NBR 15638:2008. The test organisms were collected in Ilhabela/SP (between 23°30'S e 25°02'S) through a benthonic catching during low tide. Six thousand organisms were collected and stored in several polyethylene pots with sediment and water from the place of collection, and were placed under aeration until arrival in the laboratory. On arrival the organisms were transferred to larger basins and maintained under aeration for a 4-day period of acclimatization. Once acclimatized, sediment tests with 90 samples were initiated. The control-sediment used was the local sediment where the amphipods were

collected. Three replicates of each sample were tested, each containing 20 organisms. The sensitivity of the organisms was measured through the use of ammonium chloride in the same 10-day time period as the sample analysis. Upon completion, test results were statistically analyzed using chi-square, Shapiro Wilk´s, F-test, Students' T-test and Wilcoxon´s Rank Sun. The bioequivalent test (R=0,69) was attempted, but the results were unsatisfactory as this test requires the use of more than 3 replicates of the sample.

Toxicity assessment of alkyl-polycyclic aromatic hydrocarbons and their metabolites to Japanese Medaka (*Oryzias latipes*).

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Polycyclic aromatic hydrocarbons (PAHs) are lipophilic environmental contaminants derived from petroleum and the incomplete combustion of organic compounds. Alkyl substituted PAHs predominate in crude oils and can also be found downstream of pulp and paper mills. The alkyl substitution of PAHs results in an increase in molecular weight and a higher octanol-water partition coefficient (KOW) and hydrophobicity. Studies suggest that some alkyl-derivatives such as retene (7-isopropyl-1-methylphenanthrene) are more toxic than their un-substituted parent PAH. Previous work in our lab points to a link between the metabolism of alkyl-PAHs such as alkyl phenanthrenes (APs) and the prevalence of toxicity in early life stages (ELS) of fish. The metabolism of alkyl-PAHs was found to generate ring (phenols) and chain hydroxylated (benzylic alcohols) derivatives, with a strong preference for chain hydroxylation in the case of APs compared to a preference for ring hydroxylation in alkyl anthracenes (AAs). Alkyl chain hydroxylation was implicated as the major metabolic pathway for APs and the proposed first step in the formation of highly reactive intermediates leading to enhanced toxicity. The higher toxicity of APs as compared to AAs leads us to predict that chain hydroxylated metabolites are more toxic than ring hydroxylated metabolites. The main objective of this research is to test this hypothesis by assessing the chronic toxicity of a series of ring and chain hydroxylated AP derivatives to the ELS of Japanese Medaka, comparing their effect with one another and their un-substituted parent compound.

Field versus laboratory comparison using passive samplers for detection of narcosis-based toxicity.

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Past studies have shown that passive samplers saturated with hydrophobic organic chemicals (HOCs) in static toxicity tests helped maintain the concentrations of HOCs throughout the exposure period. In the current study, sites that appear to be contaminated with HOCs and exhibiting impoverished benthos were identified using a sediment profile imaging (SPI) camera. Based on the SPI images, Tabbs and Aransas Bays, Texas, were selected as HOC-contaminated and reference sites, respectively. Hydrocarbon inclusions

were visible in SPI images of Tabbs Bay. Several stations were analyzed at a gradient from the most contaminated area. A transect across Aransas Bay was analyzed for selection of the reference station. Thin films of polyethylene (PE), used as passive samplers, were exposed to contaminated and reference sediments for 15 days both *in situ* and in the laboratory. Results of toxicity tests with sea urchin early life stages using these passive samplers as sources of HOCs in filtered seawater, as well as in pore water from the same sediments, will be presented and discussed relative to benthos conditions as visualized in SPI images. Data will be used to further evaluate the utility of passive samplers in sediment toxicity assessments and to compare the effectiveness of *in situ* deployment of PE relative to laboratory exposures.

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