Proceedings of the 38th Annual Aquatic Toxicity Workshop: October 2 to 5, 2011, Winnipeg, Manitoba

Comptes rendus du 38e atelier annuel sur la toxicologie aquatique: du 2 au 5 octobre 2011, Winnipeg, Manitoba

Editors/Éditeurs

K. Mathers, D. Heubert, M. Hanson, C. Wong, and /et L.E. Burridge

Fisheries and Oceans Canada,
Science Branch,
Maritimes Region
St. Andrews Biological Station,
531 Brandy Cove Road,
St. Andrews, New Brunswick, Canada
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Fisheries and Aquatic Sciences

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Les numéros 1 à 456 de cette série ont été publiés à titre de rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de rapports techniques de la Direction générale de la recherche et du développe-ment, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

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Editors/Éditeurs

K. Mathers[1], D. Heubert[2], M. Hanson[3], C. Wong[4], and /et L.E. Burridge[5]

[1]Stantec Consultants Ltd, Winnipeg, MB, R3C 3R6
[2]Stantec Consultants Ltd, Burnaby, BC, V5G 4L7
[3]University of Manitoba, Winnipeg, MB, R3T 2N2
[4]University of Winnipeg, Winnipeg, MB, R3B 2E9
[5]Fisheries and Oceans Canada, St. Andrews, NB, E5B 2L9

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Preface/Preface

The 38th Annual Aquatic Toxicity Workshop was held at the Fort Garry Hotel in Winnipeg, Manitoba from October 2-5, 2011. The Workshop included two plenary presentations, 58 platform and 45 poster papers. Total attendance was 182.

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 industry, 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.

L' 38ième atelier annuel sur la toxicité a eu lieu au Fort Garry Hotel au Winnipeg, Manitoba, Octobre 2-5, 2011. L'atelier a donné lieu à 2 communications lors de séances plénières, 58 exposés d'invités d'honneur et 46 communications par affichage. 182 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 organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations et des universités que le domaine intéresse. Ces derniers 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 critère 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 publies l'aide du ministre des Pêches et Océans.

Editors' comments/Remarques des editeurs

This volume contains 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, Ms. Rosalie Allen Jarvis and Ms. Monica Lyons for their assistance in the preparation of these proceedings.

Ces comptes rendus sont publiés en deux volumes, en raison de leur longueur, ils renferment le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs est aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La longueur et la forme des communications varient parce que ces dernières sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur les travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elles 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 d'emploi.

Les rédacteurs en chef voudraient remercier Dr. Jill Graham,Mme Rosalie Allen Jarvis et Mme Monica Lyons dans la préparation de ces comptes rendus.

38th Aquatic Toxicity Workshop Organizing

Committee/Comité d'organisation

Workshop Co-Chairs:

Karen Mathers	Stantec Consultants Ltd.
David Heubert	Stantec Consultants Ltd.

Committee Members:

Julie Anderson	University of Saskatchewan
Bozena Glowacka	ALS Environmental
Mark Hanson	University of Manitoba
Jocelyn Heibert	Stantec Consultants Ltd.
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MSc Swimming performance and energy homeostasis in juvenile laboratory raised fathead minnow *(Pimephales promelas)* exposed to uranium mill effluent

GOERTZEN, M. [1]***, DRIESSNACK, M.*** [2]***, JANZ, D.*** [2] ***and WEBER, L.*** [2]

[1]*Lorax Environmental Services,* [2]*University of Saskatchewan*

Research at the Key Lake uranium mill (Saskatchewan, Canada) suggests effluent discharged from the mill affects energy stores of resident fish, but the mechanisms by which energy homeostasis is affected and the subsequent effects on swimming performance are unknown. In the present study larvae were collected from laboratory raised adult fathead minnow *(Pimephales promelas)* exposed to 5% diluted uranium mill effluent or control (dechlorinated municipal) water, and reared in the same treatments to 60 days post hatch (dph). Critical swimming speed (U_{crit}) was significantly lower in effluent exposed 60 dph fish compared to control fish. Fish used in tests were considered fatigued and compared to fish without swim testing (non-fatigued). There were no differences in whole body glycogen or triglyceride concentrations between effluent exposed versus control fish. However, fatigued fish from both treatments had significantly lower triglycerides, but not glycogen, compared to non-fatigued fish from the same treatment. Whole body β-hydroxyacyl coenzyme A dehydrogenase activity was similar in fish from both treatments, but citrate synthase activity was significantly lower in effluent exposed fish. Our results suggest uranium mill effluent exposure in the laboratory affects aerobic energy metabolism and swimming performance in juvenile fathead minnow, which could affect wild fish survivability.

BSc Immune response of rainbow trout *(Oncorhynchus mykiss)* co-exposed to benzo[a]pyrene and *Aeromonas salmonicida*

PHALEN, L. [1]

[1]*University of Prince Edward Island*

This study aimed to determine whether the use of four rainbow trout leukocyte-specific antibodies coupled with fluorescence assisted cell sorting analysis (FACS) could detect changes caused by benzo[a]pyrene to the immune response elicited by rainbow trout (*Oncorhynchus mykiss*) in response to inactivated *Aeromonas salmonicida*. Fish were exposed intraperitoneally to 2.5 or 25mg B[a]P kg^{-1} BW simultaneously with formalin inactivated A.s. for 1, 3 or 7 days in two replicate trials. Absolute erythrocyte

and leukocyte counts were measured in blood using a DiOC6 staining technique coupled with FACS. Leukocytes were isolated from spleen, peritoneum, head kidney and blood, stained using fluorescently labelled antibodies to determine the proportions of B-cells, T-cells, thrombocytes and myeloid cells using FACS. An approximate 25% decrease of total leukocytes in whole blood was observed at high dose on day 7 in Trial 1. A decrease in B-cell proportions was found at high dose on day 3 and 7 in spleen (~10%) and at low and high dose on day 7 in peritoneum (~16% and 21%). A decrease in percentages of viable cells was found at high dose on days 3 and 7 in peritoneum (~13% and ~40%) and head kidney (~4% and ~6%). An approximate 26% increase in the number of myeloid cells was seen at high dose on day 7 in peritoneum. These results imply that leukocytes die at the site of injection in the peritoneum and that movement of B-cells from spleen populations could be resulting in splenic B-cell depletion.

PRESENTATIONS

Toxicity of Metals

Dietary and waterborne mercury accumulation by yellow perch: A field experiment

HRENCHUK, L. [1], BLANCHFIELD, P. [1], PATERSON, M. [1] and HINTELMANN, H. [2]

[1]Fisheries and Oceans Canada, [2]Trent University

It is well accepted that the majority of monomethylmercury (MMHg) in fish originates in their food; however, the additional contribution of water as a source to fish MMHg levels remains unclear. We used isotope enriched Hg in a controlled field experiment to quantify uptake of mercury from ingested and aqueous sources by young-of-year yellow perch *(Perca flavescens)*. Water and zooplankton from a lake that received ^{202}Hg-enriched additions (spike Hg) for seven years during a whole-ecosystem loading study (METAALICUS) provided natural, low-level Hg exposure. Exposure pathways were separated by housing perch in one of four treatments: clean water + clean food; clean water + Hg spiked food; Hg spiked water + clean food; Hg spiked water + Hg spiked food. Fish accumulated MMHg directly from water, and this source accounted for up to 21% of MMHg in fish. Accumulation of spike Hg from water and food was additive, with food providing the majority of spike MMHg taken in.

Predictions from a bioenergetics model that excludes water as a source underestimated Hg in perch by 11%. This study illustrates the importance of acknowledging food and water as sources of Hg to fish and suggests that aqueous Hg should be included as a source of contamination in bioaccumulation models.

Rapid olfactory recovery of wild yellow perch *(Perca flavescens)* from metal contaminated lakes after short-term acclimation to clean water

AZIZISHIRAZI, A. [1]***, DEW, W.*** [1] ***and PYLE, G.***[1]

[1]*Aquatic Biotechnology and Ecotoxicology Laboratory, Lakehead University*

Fish depend on their sense of smell, or olfaction, for a wide range of vital life processes including finding food, avoiding predators, and reproduction. Various contaminants, including metals, can disrupt recognition of chemical information in fish, typically at lower concentrations needed to cause other adverse effects. Numerous studies have investigated the effects of metals on the olfactory system of fish in a laboratory setting; however, few have measured olfactory acuity using wild caught fish in lake water. In this study, we used electroolfactography to measure the olfactory acuity of wild yellow perch *(Perca flavescens)* from a clean lake (Geneva Lake) and two metal contaminated lakes (Ramsey and Hannah lakes) in Sudbury, ON. The results showed that Geneva lake fish have a greater olfactory acuity than either the Hannah or Ramsey lake fish in their own lake water. After fish from Geneva Lake were held in water from Ramsey or Hannah lakes for 24 hours, and vice versa, the results showed that the olfactory acuity of the Geneva Lake fish were reduced. However, fish from Ramsey or Hannah lake held for 24 hours in Geneva lake water resulted in a substantial increase in olfactory response. These results show that fish from metal-contaminated lakes have impaired olfaction that can rapidly recover within 24 hours of being held in clean water. On the other hand, fish from a clean lake with intact olfaction demonstrated an impaired olfactory response after being held for 24 hours in metal-contaminated water.

Functional and structural responses to metal-enriched leaf packs in small streams in British Columbia

WALLING, H. [1] ***and BENDELL, L.*** [1]

[1]*Simon Fraser University*

Metal pollution and contamination are persistent global issues. Depositions of metals from vehicle exhaust and industrial activities are of significant concern in urban watersheds. The impacts on stream community ecology are only beginning to be fully understood. How does metal-enrichment of basal food resources, such as plant leaves, impact the structure and function of stream invertebrate communities? We grew red alder *(Alnus rubra)* saplings under two metal-enriched treatments, corresponding to British Columbia's interim sediment quality guidelines (ISQG) and probable effect level (PEL) for Cd, Zn and Cu. Leaf tissue uptake was significantly different between treatments. Five grams of leaves were deployed in 10-millimeter mesh leaf packs in six Vancouver- area streams in October, 2010. After four weeks, we sampled mass remaining and invertebrate density and diversity that had recruited to those leaves. Preliminary investigation shows no differences in invertebrate density or numbers of orders present on leaf packs. There was greater mass loss from leaves grown at the PEL metal treatment, as opposed to those grown at or below BC's interim sediment quality guidelines. This may be a result of lower C:N in leaves grown under the PEL metal treatment. This could have implications to organic matter cycling in contaminated freshwater ecosystems and highly impacted urban watersheds.

Metal removal in engineered anaerobic bioreactors and wetland cells installed at Pb-Zn Smelter

DUNCAN, W. [1]***, MATTES, A.*** [2]***, VAN DER FLIER-KELLER, E.*** [3] ***and GOODARZI, F.*** [4]

[1]*Golder Associates Ltd.,* [2]*Nature Works Remediation Corporation,* [3]*University of Victoria,* [4]*Forbes Energy Group*

Historical disposal practices at the Trail Smelter have left legacy issues to be dealt with. An engineered collection system was constructed in 1997 to collect the contaminated seepages. The seepage can be pumped to the biological treatment system. Source control measures implemented by Teck Metals Ltd. have altered the seepage characteristics and volume over time.

The large-scale pilot biological treatment system was built in stages between 1997 and 2002. Since 2002, the system has been operated year-round and consists of 7 treatment stages all in series. Seepage is pumped to two upflow, anaerobic bioreactors

containing 60% pulp mill biosolids, 35% sand and 5% cow manure over a layer of dolomitic limestone. This is followed by three self-contained horizontal sub-surface flow gravel wetland cells. The flow is then treated by a slow sand filter before the final holding pond which irrigates a tree nursery.

The system treats seepage with zinc up to 3800 mg L^{-1} (average ~ 260 mg L^{-1}), arsenic to 3600 mg L^{-1} (average ~ 150 mg L^{-1}) and Cd to 83 mg L^{-1} (average ~ 4.7 mg L^{-1}) which are reduced to <0.5 mg L^{-1} (<0.02 mg L^{-1} for Cd). Flow rates are generally 15,000 to 21,000 L d^{-1} during the summer (May through September) and 7,000 to 15,000 L d^{-1} during the fall-winter (October through April). This large-scale pilot system demonstrates that passive biological treatment systems can effectively treat high concentrations of metals and reduce them to acceptable irrigation standards. The overall system and the treatment rates from 2002 to 2007 (including a spike event) are presented.

Multi-generational effects of seleno-methionine on adult female rainbow trout, brook trout, and cutthroat trout

PILGRIM, N. [1], RASMUSSEN, J. [1], PALACE, V. [2] and HONTELA, A. [1]

[1]University of Lethbridge, [2]Department of Fisheries and Oceans

This project investigates the effects of maternally transferred seleno-methionine (Se-met) on reproductive and physiological endpoints in rainbow trout, brook trout, and cutthroat trout. To test the hypothesis that species-specific sensitivities to Se exist and to investigate the mechanisms underlying these differences, trout were fed diets of 0, 15, and 40 µg Se g^{-1} for approximately 5 months prior to spawning. Tissue and blood samples were taken from adult fish at spawning. Eggs were fertilized and placed in a vertical incubator or under 10-15 cm of gravel in experimental flumes, to assess larval deformities and swim-up success. Plasma thyroid hormone T3:T4 ratio increased significantly in brook trout from both Se-met treatments, but not in the other two species. A dose-dependent decrease in plasma cortisol was only detected in rainbow trout. Liver LPO only increased in rainbow Se-met treatments, while total GSH increased in both rainbow and cutthroat trout Se-met groups. Swim-up success was lower in the Se-met treatments for rainbow and cutthroat trout but not in brook trout. Analysis of the terotogenic effects of Se in the larvae of the three salmonid species are in progress. This study will provide comparative data for the reproductive effects of selenium in three salmonid species for use in species-specific risk assessment of selenium. (Funded by MITHE-SN and ACA)

Chronic sublethal dietary selenomethionine exposure alters swim performance, metabolic rate and energy metabolism in adult zebrafish *(Danio rerio)*

THOMAS, J. [1] ***and JANZ, D.*** [1]

[1]*University of Saskatchewan*

This study investigated effects of chronic sublethal dietary selenomethionine (SeMet) exposure on repeat swimming performance, oxygen consumption (MO_2), cost of transport (COT) and energy metabolism in adult zebrafish. Adult fish were fed 1, 3, 10 and 30 µg selenium (Se) g^{-1}, dry weight in the form of SeMet for 90 days. After the exposure period, fish from each treatment group were divided into three subgroups: a) no swim, b) swim, and c) repeat swim. Fish from the 'no swim' group were euthanized at 90 days and whole body triglycerides and glycogen were determined. Individual fish from 'swim' and 'repeat swim' groups were introduced in a swim tunnel respirometer, and critical swimming speed (U_{crit}) and MO_2 were determined. Fish from 'swim' group swam only once in the swim tunnel respirometer where as fish from 'repeat swim' group swam twice (i.e., two U_{crit} tests) in the tunnel with a 60 min. recovery period between tests, and then fish were euthanized to measure energy stores. Impaired swim performance was observed in fish fed greater than 3 µg Se g^{-1} in the form of SeMet, however no differences were observed in repeat U_{crit} tests within each treatment group. MO_2 and COT were significantly greater in fish fed elevated dietary SeMet. Whole body triglycerides and glycogen increased with increasing dietary SeMet exposure. The results of this study suggest that chronic SeMet exposure can alter swim performance, metabolic rate and energy metabolism in adult fish, which could threaten fitness and survivability of adult fish in natural environments.

Understanding the role of copper, nickel, and selenium in metal mine effluent toxicity: A single metal versus mixed metals approach

OUELLET, J. [1]***, DUBÉ, M.*** [1] ***and NIYOGI, S.*** [1]

[1]*University of Saskatchewan*

Aquatic discharge of metal mine effluents (MMEs) is a potential cause of metal toxicity to benthic and fish communities. These MMEs are a source of increased metal concentrations below discharge points. Past studies within a northern Ontario metal mine have found reproductive impairment in fathead minnows [FHM *(Pimephales promelas)*] along with increased tissue metal accumulation resulting from exposures to a particular MME. Several metals, specifically copper, nickel, and selenium have been

identified as metals of concern, due to their accumulation in the biofilm, benthic invertebrates, and fish tissues in artificial stream studies. The purpose of this research was to determine whether reproductive effects observed in FHMs during exposure to a particular MME with elevated copper, nickel, and selenium would also be observed from exposures to each of the 3 metals or the 3 metals in combination. Cumulative mean total egg production was observed to decrease over a 21-day period in exposures to the MMEs (Two-sample Kolmogorov-Smirnov; p0.05). In addition, tissue metal accumulation in each of the single and mixed metal exposures closely mimicked the metal accumulations from the MME. These results suggest that copper, nickel, and selenium may not be responsible for reproductive impairment in fathead minnows exposed to MMEs. Further work will be done to determine whether other metals cause the reproductive effects we have found, and whether or not changes to water chemistry can mitigate these effects.

Chronic exposure to dietary selenomethionine increases gonadal steroidogenesis in female rainbow trout

HECKER, M. [1]***, WISEMAN, S.*** [1]***, THOMAS, J.*** [1]***, HIGLEY, E.*** [1]***, HURSKY, O.*** [1]***, PIETROCK, M.*** [1]***, RAINE, J.*** [1]***, GIESY, J.*** [1] ***and JANZ, D.*** [1]

[1]*University of Saskatchewan*

Selenomethionine (Se-Met) is the major dietary form of selenium (Se). Detrimental effects have been associated with exposure to elevated dietary selenium. Previous studies have demonstrated effects of Se on the endocrine system, in particular effects on cortisol and thyroid hormones. However, no information is available regarding effects of Se on sex steroid hormones. In the present study, effects of dietary exposure to an environmentally relevant concentration (4.54 mg kg^{-1} wet weight (ww)) of Se-Met for 126 days on concentrations of sex steroid hormones in blood plasma of female rainbow trout were determined. Furthermore, the molecular basis for effects of Se-Met on plasma sex steroid hormone concentrations was investigated. Concentrations of androstenedione (A), estrone (E1), and estradiol (E2) were 39.5-, 3.8-, and 12.7- fold greater in plasma of treated females than the untreated controls, respectively. Testosterone (T) was detected only in plasma of treated females. The greater E2 concentration stimulated greater transcript abundance of vitellogenin (vtg) and zona-radiata protein (zrp). Female rainbow trout exposed to Se-Met had greater transcript abundance of key steroidogenic proteins and enzymes, including peripheral benzodiazepine receptor (pbr), cytochrome P450 side-chain cleavage (P450scc), and 3-hydroxysteroid dehydrogenase (3-hsd). Exposure to Se-Met did not affect transcript

abundance of luteinizing hormone (lh) or follicle stimulating hormone (fsh). Similarly, there was no change in transcript abundance of luteinizing hormone receptor (lhr) or follicle stimulating hormone receptor (fshr). Long-term exposure to dietary Se-Met has the potential to stimulate vitellogenesis in female rainbow trout by directly stimulating ovarian tissue steroidogenesis. This is the first study to report effects of Se on sex steroid hormone production in fish.

Energetic and endocrine modulation in fish exposed to selenium

JANZ, D. [1]

[1]*University of Saskatchewan*

Our field research in several fish species collected from areas in Canada receiving complex mining effluents elevated in selenium (Se) revealed consistently greater energy stores (triglycerides and glycogen) and an impaired physiological stress response compared to fish collected from ecologically similar reference sites. Although whole-body Se concentrations in these fish were high (ranging 21 - 43 ug Se g^{-1}dry wt), clear cause-effect relationships could not be demonstrated. In subsequent laboratory experiments, adult zebrafish exposed for 90 days to elevated dietary selenomethionine (SeMet, the major form of Se in natural diets) exhibited similar elevations in energy stores at whole-body Se concentrations exceeding 10 µg g^{-1} dry wt. Despite having greater energy availability, the exposed zebrafish had similar condition factor compared to fish fed a basal Se diet. In addition, zebrafish exhibited impaired swim performance and greater oxygen consumption in swim tunnel challenges, suggesting altered energy mobilization (lipolysis and/or glycogenolysis). Juvenile rainbow trout exposed for 120 days to a diet elevated in SeMet exhibited similar elevations in energy stores, greater basal cortisol levels in blood plasma, and an attenuated acute physiological stress response. Interestingly, female trout exposed to SeMet also exhibited greatly increased ovarian steroidogenesis. Overall, these studies in 8 species of freshwater fish indicate that dietary Se exposure causes consistent alterations in a variety of physiological functions including lipid and carbohydrate homeostasis, steroid hormone synthesis, and stress responsiveness.

Relationships between metal concentrations and zooplankton species richness in 24 northern Saskatchewan lakes and pit-lakes

PRESTIE, C. [1], VANDERGUCHT, D. [1], HELPS, D. [1], SEREDA, J. [1], BOGARD, M. [1] and HUDSON, J. [1]

[1]University of Saskatchewan

Saskatchewan is a major producer of Uranium. Uranium mining in Northern Saskatchewan has exposed certain watersheds to elevated concentrations of metals (e.g., Cu, Ni, Zn, U, etc.). A difference in the tolerance of zooplankton to metals has the potential to alter plankton diversity in impacted systems. Therefore, we assessed if elevated metal concentrations were associated with a reduction in zooplankton species richness. We sampled 24 water bodies encompassing pristine lakes to heavily contaminated pit-lakes between 2003 and 2005. Zooplankton samples were collected with a Van Dorn sampler from a central location in each system and the number of species present (richness) was determined. Concentrations of 24 different contaminants were obtained from Areva Resources Canada Inc. and Cameco Corporation for each water body. Zooplankton species richness had a range of zero to nine (mean = 4.7). Metal concentrations ranged from undetectable to 2 mg L-1. Nickel and uranium were frequently found in the greatest concentrations. Zooplankton species richness was significantly and negatively correlated with arsenic, copper, molybdenum, nickel, uranium, zinc and selenium. A relationship between zooplankton richness and other contaminants was not observed. Pit-lakes typically had the greatest metal concentrations and lowest species richness.

The effect of chronic nickel exposure on swim performance and bioaccumulation in rainbow trout *(Oncorhynchus mykiss)*

ELLIS, J. [1] and MCGEER, J. [1]

[1]Wilfrid Laurier University

Previous research has shown nickel to act as a respiratory rather than an ionoregulatory toxicant in both acute and chronic exposures. Rainbow trout (*Oncorhynchus mykiss*) have been shown to accumulate nickel within various tissues and exhibit a significantly reduced swim performance during chronic exposures. The goal of this research is to contribute towards the understanding of the effects of chronic Ni exposure on the accumulation of metal and how tissue concentrations relate to effects in rainbow trout. Juvenile rainbow trout were exposed to 0, 0.75 and 2 mg Ni L^{-1} for 23 days in a preliminary study and for 35 days in a subsequent chronic study and both

exposures were in moderately hard water (80 mg L^{-1} $CaCO_3$, pH 7.2, T=12-15°C). On d 3, 7, 14, 23, 28 and 35 sustained swim performance (U_{crit}) was assessed and liver, kidney, gill, muscle and plasma samples were collected before and after each swim trial. Tissue Ni burden, plasma ions (Ca^{2+}, Na^{+}, Mg^{2+}) and white muscle ATP levels were measured. Nickel accumulated in the gill and kidney in a time and exposure concentration dependent manner, but did not accumulate significantly in the liver or muscle. Swim performance was decreased as a result of chronic exposure, with reductions in U_{crit} observed after 14 d of exposure. This research aims to integrate Ni accumulation at the cellular level with whole organism effects. Supported through the NSERC Discovery Program.

Copper, calcium, and olfaction in fathead minnows *(Pimephales promelas)*: Considerations for a chemosensory based BLM

DEW, W. [1] ***and PYLE, G.*** [1]

[1]*Aquatic Biotechnology and Ecotoxicology Laboratory, Lakehead University*

The current gill based biotic ligand model (gbBLM) is an acute model that uses water quality measures to predict site-specific safe copper (Cu) concentrations. Recently there has been an effort to develop a chemosensory based BLM (cbBLM) based on the olfactory epithelium. To further this effort, the current study investigated the effects of varying Cu concentration and exposure duration on Cu-induced olfactory dysfunction, as well as determining if calcium (Ca) is protective of the effect of Cu. Fathead minnows *(Pimephales promelas)* were exposed to a range of Cu concentrations for 1 h to 96 h with and without added Ca. Electro-olfactography (EOG), a neurophysiological technique, was used to determine if there was a reduction in olfactory acuity induced by the various treatments. The results show that at the low, ecologically-relevant Cu concentrations tested there was a significant inhibition of EOG function. Interestingly, over time there was a concentration-dependent recovery of olfactory function during continuous exposures. In addition, Ca was not protective of Cu-induced olfactory dysfunction and it caused olfactory dysfunction itself. Furthermore, EOG and behavioural trials revealed that Ca acts as an odourant for fathead minnows. These results demonstrate that unlike Ca interactions at the gill where it is protective, Ca is not protective of Cu-induced olfactory dysfunction. Any future cbBLM should consider Ca, not as a competing cation, but a metal capable of inducing its own independent effects on fish olfaction.

Sediment-associated uranium bioavailability and toxicity to the freshwater midge *Chironomus dilutus* (poster)

CRAWFORD, S. [1]***, DOIG, L.*** [1] ***and LIBER, K.*** [1]

[1]*University of Saskatchewan*

The importance of metal bioavailability in aquatic systems has been well established; however, our understanding of the mechanisms controlling the bioavailability of many metals is incomplete. In particular, there is limited information regarding uranium (U) bioavailability and toxicity in freshwater sediments. The objective of our ongoing research is to investigate the effects of specific sediment characteristics on the bioavailability of U to a model benthic organism, *Chironomus dilutus*. The goal of this pilot study was to determine the bioavailability and toxicity of sediment-associated U in a wide range of U-spiked sand treatments. This was examined in a 10-d whole sediment toxicity test, exposing 8-d old *C. dilutus* larvae to different concentrations of U-spiked sand. Test endpoints included survival and growth, as well as U concentrations in organisms, whole-sediment and water samples (overlying and porewater). Results from this research will guide future studies with more complex sediment, while also informing risk assessments of U contaminated sandy sites and possibly the development of site-specific sediment quality guidelines.

Evaluating sublethal effects of dietary selenium exposure on juvenile fathead minnow (poster)

MCPHEE, D. [1] ***and JANZ, D.*** [1]

[1]*University of Saskatchewan*

Selenium (Se) is known to cause chronic toxicity in aquatic species. In particular, dietary exposure of fish to selenomethionine (SeMet), the primary form of Se in the diet, is of concern. Recent studies suggest that chronic dietary exposure to SeMet alters energy and endocrine homeostasis in adult fish. However, little is known about the direct effects of dietary SeMet exposure in juvenile fish. The objective of the present study was to investigate sublethal physiological effects of dietary SeMet exposure in juvenile fathead minnow *(Pimephales promelas)*. Twenty days-post-hatch fathead minnow were exposed for 60 days to different concentrations (1, 3, 10, 30 ug Se g^{-1}dry weight) of Se in food in the form of SeMet. After exposure, samples were collected for trace metal analysis and fish were subjected to a swimming performance challenge (critical swimming speed, U_{crit}). Tail beat amplitude and frequency, as well as oxygen consumption was determined during the swim performance test. A number of

important indicators of energy homeostasis were subsequently determined in both swam and un-swam fish. Energy storage capacity will be measured via whole-body glycogen and triglyceride concentrations, and the stress biomarker cortisol will also be determined. Two key enzymes involved in energy metabolism, citrate synthase and β-hydroxyacyl coenzyme A dehydrogenase (HOAD) will be determined in whole-body fish. The results will then be compared between exposure groups as well as between swam and un-swam fish to gain new insights into the sublethal effects of dietary Se exposure on a juvenile fish species.

Acute toxicological effect of copper sulfate and formaldehyde on *Artemia franciscana nauplii* (poster)

TRUJILLO-AGUIRRE, R. [1]***, BARTOLOMÉ, M.*** [1]***, RUEDA-JASSO, R.*** [1] ***and CORTÉS, R.*** [1]

[1]*Universidad Michoacana de San Nicolas de Hidalgo*

Formaldehyde and copper sulphate are commonly used in the industry as surface cleaning agents and algaecides in water treatment plants; after used they are discharges into water bodies. Their impacts have been studied thoroughly. Polluting agents, such as metals, are highly dependent on abiotic factors, which modify the chemical species that are present. The cupric ion (Cu^{+2}) found in water bodies at a 7.4 of pH is the most available toxic species and able to be added to food chains and unleashing bioconcentration, bioaccumulation and biomagnification processes. The objective of this study was to evaluate the water's acute toxicity (LC_{50}) of copper sulphate, formaldehyde and their binary mix (1:1 proportion) in *Artemia franciscana* nauplii I (24 hours age). The nauplii were exposed to a synthetic sea water solution with formaldehyde concentrations of 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1 and 2.4 mg L^{-1} for a period of 24 hours. The LC_{50} values for copper sulphate and formaldehyde were 1.4 mg L^{-1} and 1.38 µL L^{-1}, respectively. Compared to the acute toxicity classifications proposed by the Organization for Economic Cooperation and Development and the US Environmental Protection Agency, copper sulphate's LC50 corresponds to a moderately toxic substance while formaldehyde's LC50 corresponds to a highly toxic one. The acute toxicity results of the mix show 24-h LC50 values of 1.16 mg L^{-1} for copper sulphate and 0.71 µL L^{-1} for formaldehyde. The mixture's type of interaction is synergistic according to the Synergic Proportion and Concentration Addition models.

Water monitoring data and regulatory decisions for pesticides

VILLENEUVE, J. [1]***, LARIVIÈRE, É.*** [1]***, NWOBU-NNEBE, O.*** [1] ***and KIRBY, S.*** [1]

[1]*Health Canada*

The Pest Management Regulatory Agency (PMRA) considers water monitoring data to enhance regulatory decisions on the risk of pesticides to human health and the aquatic environment (PMRA policy document SPN2004-01). Water monitoring data are typically available from a variety of sources including, government departments at the federal, provincial and municipal level, registrants, public literature and various databases maintained in the United States. Ideally, abundant high quality monitoring data would be available to estimate exposure concentrations of pesticides in aquatic habitats and drinking water. However, in reality the quantity, variability and uncertainty associated with existing monitoring data can make it challenging to estimate nationally representative exposure concentrations. Challenges encountered include determining when the use of monitoring data to generate exposure estimates is appropriate, the best statistical approach to analyze data and other ways data can be used to enhance regulatory decisions. The utility of the monitoring data within a regulatory context would be strengthened with the inclusion of additional information on study design, as well as adequate temporal and spatial ancillary data relative to processes that influence pesticide movement in the environment The consideration of water monitoring data in risk assessments for pesticides is important for the regulatory process as such, continued collaboration with other jurisdictions within the federal, provincial, territorial and municipal levels of government as well as with registrants is essential.

Sampling strategies for monitoring and risk assessment in aquatic environments

PURDY, J. [1]

[1]*Abacus Consulting Services Ltd*

The number, duration and frequency of samples taken in aquatic products are critical components of field work projects the aquatic environment for monitoring and risk assessment. The strategy for sample collection is often a simple linear series of grab samples designed to minimize cost, without attention to the specific needs of a project. There are opportunities to use extended collection times, pooling and subsampling to

ensure that the, the compartments or locations to be sampled, the required replication are adequately covered for the duration and purpose of the study. Cost is a major design constraint in environmental sampling. In most cases the cost of collecting and analyzing a complete and properly designed set of samples is not greatly increased, and it may in some cases be decreased. Proven and accepted strategies and sampling designs for both the field phase and the analytical phase of aquatic monitoring and risk assessment studies will be presented.

Atrazine Ecological Exposure Monitoring Program: Study design and conduct

MILLER, P. [1], HARBOURT, C. [1], HENDLEY, P. [2], CARVER, L. [1], CHEN, S. [2], TRASK, J. [1] and SNYDER, N. [1]

[1]Waterborne Environmental, Inc, [2]Syngenta

An Atrazine Ecological Exposure Monitoring Program (AEMP) was initiated in 2003 and continues in 2011. Monitoring in 72 corn and sorghum agricultural watersheds in nine Midwestern states produced over 150 "site years" of land use, residue, total suspended solids, meteorological, and stream flow data. Sampling locations were identified on streams at the outlets of watersheds based on defined criteria for potential atrazine use and watershed scale. Watershed sampling locations were equipped with an integrated system of weather stations, automatic samplers, and stream stage measurement stations. Monitoring in each watershed was designed to collect four-day grab samples during the five month growing season. Runoff event based and daily automatic sample collection programs were used during the study. Land use was characterized by customized satellite image classification or USDA NASS Cropland Data Layer data. Results show that the AEMP study design adequately captured atrazine runoff events following chemical applications for each growing season.

Crayfish and mussels as monitors of POPs (poster)

CRAIG, G. [1] and MIDDELRAAD, I. [2]

[1]G.R. Craig & Associates, [2]D.G. Dixon & Associates

Indigenous crayfish *(Camarus robustus)* and mussels *(Ellipio complanata* and *Lasmigona costata)* have been used for over 10 years to monitor the release of a low concentration persistent organic pollutant from a point source to a southern Ontario river. The techniques used have included continuous and consecutive placement of mussel cages and the scheduled collection and caging of indigenous crayfish. The study demonstrates how the seasonal fluctuation of exposure concentrations can be determined, mechanisms of contaminant transport can be identified, and the

importance of determining clearance rates within monitoring organisms to characterize exposure dynamics. Long term monitoring has identified a consistent trend in response to remediation.

Emerging Contaminants and Chemicals of Concern

Examination of BDE and OH-BDE in vitro exposure on androgen production in male brown trout *(Salmo Trutta)* testes using LC/MS/MS.

PETERS, L. [1], DARLING, C. [2], BESTVATER, L. [1], GEMMILL, B. [2] and TOMY, G. [2]

[1]University of Manitoba, [2]Fisheries and Oceans Canada

Polybrominated diphenyl ether (PBDE) flame-retardants are lipophilic persistent organic compounds used in manufacturing of plastics, electronic equipment, polyurethane foam and textile materials. PBDEs have become an increasingly important environmental problem due to their ability to bioaccumulate and biomagnify. Increases of these compounds have been measured in wildlife and human adipose tissue, with 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) being one of the leading PBDE congeners found. The fully brominated, 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether (BDE-209), is the predominant congener of the DecaBDE commercial formulation. Recent studies have focused on the potential endocrine disrupting properties of PBDEs, which include estrogenic/antiestrogen activity and effects on thyroid hormones, but very little work has been done to explore possible effects on androgen biosysthesis and metabolism. To address this deficiency, the effects of environmentally relevant concentrations of BDE-209, BDE-47, as well as the 3OH-, 5OH- and 6OH- hydroxylated metabolites of BDE-47, on the *in vitro* steroidogenic capacity of brown trout (*Salmo trutta*) testicular tissue were examined. Preliminary results show that BDE-47 had no significant affects on the production of testosterone, testosterone glucuronide and 11-ketotestosterone by fish testicular tissue. Exposure to 6OH-BDE-47 resulted in increased production of all androgens measured, with significant affects observed in 11-ketotestosterone under gonadotropin stimulated conditions. Both 5OH- and 3OH-BDE-47 decreased hormone production of the testicular tissue, with 3OH- being the most potent antiandrogen. Possible mechanisms of action, as well as the results from the BDE-209 exposure will be discussed.

Understanding the relationship between contraceptive choices made by society and the resultant estrogenic load on the environment

KHAN, U. [1] ***and NICELL, J.*** [1]

[1]*McGill University*

More than a decade of evidence suggests that the estrogenic content of wastewaters being released to the environment feminizes fish and that such feminizing effects could potentially comprise the reproductive capacity of the effected fish. Environmentally-relevant estrogens can be broadly categorized in two classes; namely, those that are endogenously produced and those that are exogenously consumed. Of the two, the excretion and therefore the subsequent environmental presence of endogenous estrogens can be considered to be omnipresent since they are a fixed characteristic of our biology. When considering exogenously consumed estrogens used for human contraception, the most important by far in terms of amounts consumed and environmental relevance is the female birth control pill, containing the synthetic estrogen ethynylestradiol as its active ingredient. The pill was first approved 50-years ago as a form of contraception and is currently used by approximately 11 million women in the USA for this purpose. Since the use of ethynylestradiol, and hence the estrogenic content released as a consequence, is a contraceptive choice made by members of society, a logical question arises: When evaluated from an environmental perspective, should the use of ethynylestradiol be mitigated or even eliminated? And if so, do greener alternatives exist to this contraceptive? How does one go about making such an evaluation? Further, the mere suggestion that society's contraceptive choices be altered to mitigate environmental impact should be analyzed in a broader context. Are contraceptive choices that are greener also clinically and personally preferred? Would such a switch to more greener contraceptive choices also be financially feasible? This study will present a first attempt at answering these questions.

Decamethylcyclopentasiloxane (D5) spiked sediment: Bioaccumulation and toxicity to the benthic invertebrate *Hyalella azteca*

NORWOOD, W. [1]***, ALAEE, M.*** [1]***, SVERKO, E.*** [1]***, BROWN, M.*** [1] ***and GALICIA, M.*** [1]

[1]*Environment Canada*

Chronic toxicity and bioaccumulation of decamethylcyclopentasiloxane (D5) to *Hyalella azteca*, a freshwater amphipod, was examined in a series of spiked sediment exposures. Juvenile *H. azteca* were exposed for 28 days (chronic) to a concentration series of D5 in two natural sediments of differing organic carbon content (O.C.) and particle size composition. As well, adult *H. azteca* were caged in the overlay water for 7

days to determine bioaccumulation from the water. The chronic, sediment D5 LC50s were 194 and 785 µg D5•g^{-1} dry weight for Lakes Erie (0.5% O.C.) and Restoule (11% O.C.) respectively. Inhibition of growth only occurred with the Lake Restoule spiked sediment with a resultant EC25 of 821 µg•g^{-1} dry weight. Lethality was a more sensitive endpoint than growth inhibition. Bioaccumulation Factors (BAFs, 28 days) of 16,000 and 56,000 from Lakes Erie and Restoule sediments respectively indicate that siloxane D5 was bioaccumulative. However, biota sediment accumulation factors (BSAFs, 28 days) were <1 indicating that D5 did not bioconcentrate based on lipid normalized tissue concentrations and organic carbon normalized sediment concentrations. Organic carbon in the sediment was protective.

Enantioselective inhibition kinetics of crufomate with acetylcholinesterase

CHAI, T. [1] ***and QIU, J.*** [1]

[1]*Chinese Academy of Agricultural Sciences*

Generally, the enantiomers of chiral pesticides have different bioactivity for target organism or toxicity for nontarget organism. Previous studies mainly focused on the inhibition behaviors of racemate, and less works involved in that of enantiomers. This work studied the enantioselective inhibition kinetics of crufomate, an organophosphorus insecticide in the reaction with acetylcholinesterase (ATchE) from electric eel. Two enantiomers of crufomate were separated and prepared by chiral high-performance liquid chromatography, their elution orders on different chiral stationary phases were detected with an optical rotation detector. The inhibition rate constants (k_i), Michaelis-Menten parameter (K_m) and concentration of the inhibitor giving 50% inhibition under defined conditions (IC_{50}) of the racemate and two enantiomers of crufomate were respectively calculated by references. According to their k_i values, three inhibitors had different inhibitory potency with ATchE, and the inhibitory activity of racemate was most. The differences of inhibitory activity between racemate and (-)-enantiomer were less than that between racemate and (+)-enantiomer. The K_m value of (-)-enantiomer was smaller than those of racemate and (+)-enantiomer, indicated it had stronger ATchE-ATch affinity compared to racemate and (+)-enantiomer. Simultaneously, the IC_{50} value of (-)-enantiomer was smaller than those of racemate and (+)-enantiomer.

Toxicity of anthracenedione and azo dyes to *Hyalella azteca*

BARTLETT, A. [1], BROWN, L. [1], PALABRICIA, V. [1] and BALAKRISHNAN, V. [1]

[1]Environment Canada

Very little toxicological data are associated with the 2600 medium-priority chemicals listed in Canada's Chemicals Management Plan (CMP). This study assessed several of these compounds to determine effects on survival and growth of the freshwater amphipod, *Hyalella azteca*. Four-week, water-only, static-renewal toxicity tests were conducted with three anthracenedione dyes (Acid Blue 80, Acid Blue 40, Acid Blue 129) and two azo dyes (Disperse Yellow 7, Sudan Red G). The anthracenedione dyes showed little/no toxicity to *Hyalella*. Acid Blue 80 was non-toxic to survival or growth of *Hyalella* up to 10,000 µg L^{-1} (the highest concentration tested). Acid Blue 40 and Acid Blue 129 demonstrated some toxicity to *Hyalella*, with four-week LC50s estimated to be 13,500 and 6470 µg L^{-1}, respectively. Growth was not affected by Acid Blue 40, but was weakly affected by Acid Blue 129, with a four-week EC25 of 1950 µg L^{-1}. The azo dyes were much more toxic to *Hyalella*. Four-week LC50s for Disperse Yellow 7 and Sudan Red G were 117 and 30 µg L^{-1}, respectively. Neither azo dye showed an effect on growth. Dye toxicity reported here was calculated using nominal concentrations; however, based on previous data, measured dye concentrations are expected to be substantially less than nominal (30-50%). These toxicity data will be used in CMP risk assessments to determine if these dyes could impact freshwater ecosystems downstream of textile dying facilities or municipal wastewater outfalls.

Perfluorinated sulfonates and carboxylates in Canadian wastewater treatment plants

GUERRA, P. [1], KINSTMAN, L. [1], NG, T. [1], ALAEE, M. [1] and SMYTH, S. [1]

[1]Environment Canada

Perfluorinated compounds (PFCs) are a structurally diverse group of chemicals used in carpets, apparel and packaging products as processing additives during fluoropolymer production and as surfactants in consumer applications. These compounds have a dual hydrophobic and oleophobic character, resulting in both water and oil repellency. The most studied PFCs are perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), having the potential to persist in nature, bioaccumulate, and become toxic. As a result, PFOS was added to the list of persistent organic pollutants (POPs) at the Stockholm Convention in 2009. Currently, a large number of new PFCs are in use as alternatives; however they can also breakdown to PFOS and

PFOA, which would add to the reservoir of these persistent contaminants in the environment. Different studies have recognized wastewater treatment plants (WWTPs) as one of the principal routes for introducing these compounds into the aquatic environment. Consequently, there is increasing attention on understanding the fate and behavior of PFCs in WWTPs. Various studies have reported PFCs levels in the influent and effluent of WWTPs worldwide. PFCs in WWTPs are thought to originate from domestic activities, industrial discharges, or other commercial activities. This study aimed to evaluate levels, fate and distribution of different PFCs, including PFOA and PFOS in influent, effluent and biosolids from 5 WWTPs in Canada. Furthermore, seasonal variation and the effect of the different types of treatment on the concentrations of PFCs were studied.

Effects of Ibuprofen on rainbow trout *(Oncorhynchus mykiss)* following acute and chronic waterborne exposures (poster)

ROBICHAUD, M. [1]***, BEYGER, L.*** [1]***, ORREGO, R.*** [1]***, GUCHARDI, J.*** [1] ***and HOLDWAY, D.*** [1]

[1]*University of Ontario Institute of Technology*

Pharmaceuticals and personal care products are a growing concern in the aquatic environment. Compounds from the class of non-steroidal anti-inflammatory drugs are commonly detected in surface waters and have the potential to negatively affect aquatic organisms. The purpose of this experiment was to evaluate the effects of ibuprofen on rainbow trout *(Oncorhynchus mykiss)* following acute and chronic exposures. Using waterborne exposures of trout to 1 and 10 mg L^{-1} of ibuprofen in the acute exposure and 1, 32 and 1000 µg L^{-1} of ibuprofen in the chronic exposure, along with an experimental control, E2 control of 1000 µg L^{-1} and an E2-ibuprofen mixed treatment, COX activity, vitellogenin (VTG) concentration and ethoxyresorufin-O-deethylase (EROD) activity were evaluated. COX activity measured showed that ibuprofen did not inhibit the enzyme activity in gill or kidney tissue. To evaluate the estrogenic effects of ibuprofen, VTG concentrations were measured and it was found that by the end of the chronic exposure all of the ibuprofen treatments had significantly increased VTG concentrations compared to the control group. EROD activity appeared to be inhibited by ibuprofen but definitive conclusions could not be made. These findings indicate that more work needs to be done studying ibuprofen in aquatic systems.

Stereo-specific bioaccumulation of chiral PCBs in a marine food web from Cumberland Sound, Nunavut, Canada (poster)

LU, Z. [1], WONG, C. [2], SILVA, G. [2], TOMY, G. [3], ROSENBURG, B. [4], LEBLANC, B. [4], MCMEANS, B. [5], FISK, A. [5], MORRIS, A. [6], MARVIN, C. [6] and MUIR, D. [6]

[1]University of Manitoba, [2]University of Winnipeg, [3]Fisheries and Oceans Canada, [4]Fisheries and Oceans Canada, Freshwater Institute, [5]University of Windsor, [6]Environment Canada

The atropisomer composition of chiral PCBs, including PCBs 91, 95, 136, 149, 174, and 176, were measured in an aquatic food web from Cumberland Sound Bay to examine possible stereo-specific bioaccumulation and biotransformation. This food web consisted of mixed zooplankton, invertebrates (scallop, snail), pelagic forage fish (Arctic cod), benthic fish (skate, turbot, sculpin), piscivorous fish (char, Greenland shark) and marine mammals (ringed seal, harp seal, narwhal, beluga). Relatively low PCB concentrations were found in the low trophic level organisms, indicating low bioaccumulation of PCBs in these species. Non-racemic enantiomer fractions (EFs) were found in some piscivorous fish samples. Greenland sharks at Cumberland Sound had similar EFs as specimens caught a decade previous at the Northwater Polynya further north, supporting the hypothesis that organisms reach a steady-state enantiomer signature during the course of their lifetimes. Marine mammals had species-specific PCB EFs, likely due to a combination of in vivo biotransformation and stereo-specific trophic transfer.

Waterborne fluoxetine and 17α-ethynylestradiol interact to upregulate on hepatic estrogen receptor α and vitellogenin gene expression in male goldfish (poster)

SILVA DE ASSIS, H. [1], ZAMORA, J. [1], LADO, W. [1], AL- ANSARI , A. [1] and TRUDEAU, V. [1]

[1]University of Ottawa

Fluoxetine (FLX) and the synthetic estrogen 17α-ethynylestradiol (EE2) are released to aquatic systems via sewage treatment effluents with potential endocrine disrupting effects. Previous work showed that these pharmaceuticals can disrupt liver and brain function in goldfish. To better understand possible interactions between these compounds male goldfish were exposed to low nominal water concentrations of FLX (0.54 µg/L) and EE2 (5 ng/L) alone and in combination for 14 days. Ethanol (1.4 mL/70 L tank) served as the control. Real-time reverse-transcription polymerase chain reaction was used to assess pharmaceutical effects on gene expression. Data were normalized using elongation factor 1α. Both FLX and EE2 increased hepatic estrogen receptor α (ERα) and vitellogenin (VTG) by 1.9-2.4 fold, but this did not reach statistical significance

(p>0.05). In marked contrast, co-exposure increased hepatic ERα (p<0.05) and VTG (p<0.05) by 5.5- and 5.3- fold, respectively. FLX and EE2 alone did not affect ERβ, but the co-exposure decreased ERβ by 50% (p<0.05). Hepatic ERγ was not affected by any treatments. Gene expression was also assessed in testes, pituitary and telencephalon but treatments were without effect, demonstrating specificity of action at the level of the liver. Our study demonstrates that an ecotoxicologically relevant mixture of FLX and EE2 produces estrogen-like effects in the male liver.

Effects of artificial amendments and pharmaceuticals on aquatic macrophytes in outdoor mesocosms (poster)

CARDINAL, P. [1], WONG, C. [2], HANSON, M. [1], CARLSON, J. [1] and KNAPP, C. [3]

[1]University of Manitoba, [2]University of Winnipeg, [3]University of Strathclyde

Rural communities across the Canadian Prairies discharge effluent from their secondary treatment lagoons once or twice a year. These wastewaters contain excess nutrients which are above discharge guidelines, as well as pharmaceuticals and microbes containing antibiotic resistance genes. The objective of this experiment was to study the effects of artificial wastewater and artificial wastewater with amended drugs (carbamazepine, clofibric acid, fluoxetine and naproxen at 5 µg L^{-1}, and sulfapyridine and sulfamethoxazole at 100 µg L^{-1}) on aquatic macrophytes (*Typha* spp., *Myriophyllum sibiricum*), as well as the uptake of phosphorus and selected pharmaceuticals in plant tissues. Aquatic macrophytes were assessed for wet and dry root and shoot weights, as well as total biomass. The growth endpoints of aquatic plants did not show significant differences in wet and dry weights of biomass in artificially amended tanks. Over a 28 day exposure period, the macrophytes grew on average 89.0% more biomass in the controls, 89.4 % for wastewater treatments and 87.9 % for wastewater and amended drugs treatments. These results will be used to provide further insight in the removal efficiency and implementation of constructed wetlands in the rural municipalities of Morden and Winkler, Manitoba.

Direct and indirect photolysis of the sulfonamide antibiotics sulfapyridine and sulfamethoxazole in the aquatic environment (poster)

CHALLIS, J. [1], CARLSON, J. [1], FRIESEN, K. [2], HANSON, M. [3] and WONG, C. [1]

[1]University of Winnipeg, [2]Richardson College for the Environment, University of Winnipeg, [3]University of Manitoba

Direct and indirect photolysis of the sulfonamide antibiotic drugs sulfapyridine (SPYR) and sulfamethoxazole (SMXZ) were measured under both controlled laboratory settings and natural sunlight to assess the ability of SPYR and SMXZ to undergo direct and indirect photolysis, an important contributor to their degradation in the aquatic environment. Time-based direct photochemical rate constants and quantum yields were determined using a merry-go-round photochemical reactor (emitting light over a 290 – 330 nm wavelength range) for both SPYR and SMXZ in singly deprotonated, and doubly deprotonated states, as well as at an environmentally relevant pH (pH 7). Both drugs degraded quickly in pure water in laboratory with half-lives ranging from 30 min for SMXZ to several hours for SPYR, depending on the protonation state, indicating that speciation will affect direct photolysis rates. When exposed to natural sunlight SPYR and SMXZ exhibited direct photolysis half-lives in the range of 12 – 24 hours, a result attributed to the difference in light distribution and intensity of natural sunlight. Indirect photolysis was assessed by spiking samples with synthetic waste water and irradiating under natural sunlight. Indirect photolysis increased dissipation rates, due to the influence of high levels of nitrate and dissolved organic matter. These results show that natural attenuation by photolytic processes is a major removal mechanism of sulfonamide drugs in impacted aquatic systems. Understanding the aquatic fate of such contaminants is important in order to characterize exposure and potential deleterious effects to non-target aquatic organisms, as well as the development of antibiotic resistance amongst microbial communities.

Comparison of LC/QQQ and LC/QTOF direct injection analysis for Emerging Contaminants in water (poster)

HINDLE, R. [1]

[1]*Vogon Laboratory Services Ltd.*

Emerging Contaminants (ECs) are a priority issue for monitoring in North America and Europe, and include a wide class of compounds such as pharmaceuticals and personal care products (PPCPs), hormones and steroids, and perfluorinated compounds. The list of targets to include for analysis is constantly growing, and includes not only parent compounds but also degradants and metabolites, if known. HPLC/MS/MS with electrospray ionization is a useful technique for broad scope analysis, due to routine picogram sensitivity and relatively fast run times. Because of this sensitivity, there is the opportunity to analyze hundreds of compounds at toxicologically-significant levels by direct injection, i.e. no pre-extraction, cleanup, and concentration steps. Direct injection can eliminate almost all sample prep time, reduce personnel and lab supply costs, reduce analyte bias due to differing chemical behavior in extraction and concentration steps, reduce sample size requirements to milliliters instead of liters, and reduce sample transportation costs. There is also potential for lab automation to further reduce costs and time requirements. On-line SPE cleanup and concentration can be added if lower detection limits are required, without giving up all the benefits of direct injection. This work will compare the results obtained from direct injection analysis for over 120 analytes in various waters by LC/QQQ and LC/QTOF, and discuss the advantages of each technique.

Debromination of 13C-labelled Decabromodiphenyl Ether in Lake Sediments (poster)

ORIHEL, D. [1]***, BISBICOS, T.*** [2]***, DUPUIS, A.*** [3]***, PATERSON, M.*** [3]***, TOMY, G.*** [3] ***and MUIR, D.*** [2]

[1]*University of Alberta,* [2]*Environment Canada,* [3]*Fisheries and Oceans Canada*

A large legacy of the flame retardant decabromodiphenyl ether (decaBDE) can be found in the world's rivers, lakes, and oceans. The stability of decaBDE in the environment is arguably one of the most controversial issues left unresolved in our understanding of the fate of PBDEs in the environment. The debate revolves around whether decaBDE degrades into lower-brominated, more toxic congeners. It is reasonable to conclude, based on the laboratory results, that the debromination of decaBDE is likely occurring, but there is no direct evidence to confirm the significance of this phenomenon in natural ecosystems. To address this knowledge gap, we conducted

a series of in situ decaBDE loading experiments in a remote lake at the Experimental Lakes Area, Ontario. In this poster, we present the results of our microcosm experiments, in which intact columns of lake sediment were incubated with ultra-high purity ^{13}C-decaBDE in an oligotrophic lake for a period of up to one month. The main objectives of these experiments were to estimate the debromination rate of decaBDE in sediments under ambient lake conditions, and to identify the breakdown products formed by the debromination process. We also manipulated the light and oxygen conditions in some microcosms in order to probe the mechanisms of decaBDE debromination.

Integrated Ecosystem Health

Chitobiase activity and its use as an indicator of impacts on benthic production in receiving waters: Confounded by zooplankton

MACKENZIE, S. [1], LONG, J. [2] and HANSON, M. [1]

[1]University of Manitoba, [2]Manitoba Water Stewardship

A benthic survey is currently the most common means of determining sewage effluent impacts on invertebrates in streams and rivers. This is both time-consuming and expensive. We propose a complementary method that is both rapid and relatively inexpensive, making it a good candidate to be used for determining impacts to freshwater systems where routine benthic monitoring is not possible. The approach is based on quantifying the production of the arthropod moulting enzyme chitobiase. This free enzyme can be easily measured using a simple fluorescence assay, which has been used to estimate zooplankton production in marine and lentic systems.

Over the summer and autumn of 2010 until the summer of 2011, water samples were taken regularly from the Dead Horse Creek in southern Manitoba to be analyzed for chitobiase production. This creek is a useful model system because it has three communities releasing treated sewage effluent into it during specific time windows. Our samples were collected before, during, and after the release of the effluent, and from several sites along the stream above and below the release points. We found that the chitobiase production was highest directly downstream from the release points, which was contrary to our prediction that the effluent would decrease the chitobiase activity due to adverse impacts on the indigenous benthic macroinvertebrates. This increase in measure production was thought to be due to the copious zooplankton in the sewage

lagoons that were released into the creek, masking any potential acute impacts on growth and development on the benthos in the Dead Horse Creek.

Although the outcome of the study was not as expected, we were able to show that changes in environmental concentrations of chitobiase can be detected, and that its role in future monitoring scenarios should be considered.

The use of the Relative Risk Model and Bayesian Networks for the calculation of ecological risk for the Hg contamination in the South River, VA

LANDIS, W. [1], AYRE, K. [2], CAINS, M. [2], STINSON, J. [2] and SUMMERS, H. [2]

[1]Western Washington University, [2]Institute of Environmental Toxicoloy

Historic industrial activities in Waynesboro, Virginia from 1929 to 1950 resulted in mercury contamination of the South River. Despite the time that has elapsed from the mercury release, mercury concentrations in the river, fish and wildlife remain. A landscape-scale ecological risk assessment was to assess the potential impacts of mercury and other stressors to fish, wildlife and ecological services. Both the relative risk model (RRM) and Bayesian networks (BNs) are used. The structures of the BNs are based on the framework of the RRM but use BNs written using Netica software to calculate risk and uncertainty. The BNs reflect causal pathways, incorporates a broad array of data available for the site, and includes the results of opinions solicited from experts. Sources of stressors in the watershed include: mercury contaminated sites, stream modification, wastewater discharges, non-point source run-off, recreational activities and land uses such as agriculture and residential development. The results suggest that mercury contamination is an important factor but nutrients and other stressors are important contributors to the risks to a variety of endpoints. Restoration of the watershed will require a multi-stressor approach across the entire watershed. No reference sites or conventional concepts of ecosystem health were necessary in the conduct of this analysis and such constructs likely contributed to significant data gaps. Several examples are presented.

Effects of potato agriculture on the production and community composition of stream invertebrates (poster)

BENOY, G. [1], LOOMER, H. [2], KIDD, K. [2], CULP, J. [1] and CHAMBERS, P. [1]

[1]Environment Canada, [2]University of New Brunswick

Row cropping and the use of pesticides and fertilizers required for commercial production of potatoes are known to negatively affect local waterways. Grand Falls,

New Brunswick, is a major region of potato production in Canada, and streams draining the agricultural catchments have higher concentrations of pesticides and dissolved nutrients, more deposition of fine sediments on the stream bed and, as a result, greater algal production and distinct invertebrate communities compared to nonagricultural watersheds. Although agriculture alters the composition of invertebrate communities, it remains unknown how this suite of agricultural stressors simultaneously alters the productivity of the invertebrate community.

The influence of potato agriculture on invertebrate community productivity and composition was investigated through biomass estimates and the relative abundance of different families in the community. Invertebrate samples were collected with u-nets in the riffle areas of 14 small streams (watershed area 9 -30 km2) characterized by a gradient of potato agriculture (% ag = 0.7 - 83%) in August 2010. Estimates of total invertebrate biomass increased with agricultural activity until 50% of the watershed supported potato production; above this, invertebrate biomass began to decrease eventually returning to reference site levels once 80% of the watershed was in agricultural production. The proportion of pollution tolerant families within the invertebrate community, according the modified family biotic index, increased linearly with agricultural activity. At lower intensity agriculture (<50% ag) the productivity of the invertebrate community is stimulated but at higher intensities only the composition of the community changes reflecting non-linear effects of potato agriculture on invertebrate communities.

A whole-ecosystem study to determine the response of fish methyl mercury levels to changes in atmospheric deposition of inorganic mercury (poster)

BLANCHFIELD, P. [1]***, PATERSON, M.*** [1]***, HRENCHUK, L.*** [1]***, HINTELMANN, H.*** [2] ***and HARRIS, R.*** [3]

[1]Fisheries and Oceans Canada, [2]Trent University, [3]Reed Harris Environmental Ltd.

The relationship between mercury (Hg) loading to ecosystems and fish methylmercury (MeHg) concentrations is critical for assessment of potential controls on atmospheric Hg release. The METAALICUS project simulated an increase and a decrease in Hg deposition through annual additions to a pristine lake and its watershed for 7 years (loading), followed by 3 years of no additions (recovery). A different form of isotopically-enriched mercury was applied to upland, wetland and lake portions of the Lake 658 watershed to examine the relative importance of these areas to MeHg production and bioaccumulation. By the end of loading the food web appeared to reach

a new steady state for the lake spike; invertebrate and forage fish MeHg concentrations were 55-60% higher than they would have been without Hg additions and 40% higher for predatory northern pike (*Esox lucius*). Three years after Hg loading ended, the proportion of MeHg derived from experimental additions declined by 70% relative to peak levels in forage fish, and by 25% for pike. Mercury applied to upland and wetland areas surrounding the study lake have remained near or below detection levels in aquatic biota throughout the study, implying a much slower response to changes in Hg deposited to these areas. We demonstrated that fish MeHg concentrations readily respond to changes in amounts of Hg directly deposited to lakes. Our results imply that decreases in atmospheric Hg deposition will result in lower MeHg accumulation in aquatic food webs and that the response will depend on the relative contribution of the catchment to overall lake Hg budgets.

Dependance of the ambient chitobiase concentrations and the rate of chitobiase production on presence of wastewater, antibiotics and macrophytes in model wetlands (poster)

KOZLOVA, T. [1], HANSON, M. [1], WONG, C. [2], KNAPP, C. [3] and CARLSON, J. [2]

[1]University of Manitoba, [2]University of Winnipeg, [3]University of Strathclyde

The process of ecological recovery of isolated model wetlands spiked with reconstituted wastewater (RWW) and separately with an identical wastewater plus a mix of pharmaceuticals was assessed in this study and compared to the control, with and without macrophytes. The RWW mimicked the effluents of rural prairies communities in nutrient and selected human pharmaceutical loadings, as well as chemical oxygen demand (COD).

The goal of this particular study was to evaluate whether or not the spiked nutrients and pharmaceuticals and/or macrophyte communities affect secondary production in the wetlands as assessed through the use of the arthropod molting enzyme chitobiase. The results suggest that there are significantly elevated enzyme concentrations in the tanks with macrophytes ($p<0.05$), but that enzyme concentrations in the tanks without plants were more stable over time, especially in the controls. It was also found that the systems spiked with wastewater and antibiotics were significantly elevated in chitobiase activity, as compare to the controls ($p< 0.05$). The rate of chitobiase production in the presence of macrophytes was decreased significantly in all the treatments ($p<0.05$) and implies decreased secondary production in the mesocosms with macrophytes. This work shows that the molting enzyme chitobiase could be used as an indicator of ecosystem status, telling us coarsely about differences between

systems. More refinement is required, specifically relating zooplankton and benthos biomass to the measured rates of chitobiase production in the model wetlands.

Setting restoration targets at environmentally degraded areas in the Great Lakes: How clean is clean enough? (poster)

GEORGE, T. [1] ***and BOYD, D.*** [1]

[1]*Ontario Ministry of the Environment*

The Great Lakes Water Quality Agreement has resulted in efforts to restore, protect, and conserve environmental conditions at designated Areas of Concern (AOC). These efforts are based on fourteen Beneficial Use Impairments (BUIs), which include impairments to water, sediment, fish, wildlife, benthos, and plankton. A significant amount of resources is invested in assessing the status of the BUIs and the restoration of the AOC. However, generally it is not possible to return the AOC to its pre-development state, and scientists, policy makers, and the public are posed with the question "how clean is clean enough?" When are restorative actions sufficient to deem the beneficial use no longer impaired? Generally, a statistically significant difference between the impacted area and a 'suitable' reference site is enough to demonstrate improvement. However, challenges arise when 'value judgements' are involved in selecting a reference site and in determining how close to reference conditions the impacted area must be to declare success. For example, urban rivers would never be deemed restored if BUI comparisons were made to pristine rivers. A proposed approach in this situation is to include "cause-effect" metrics that would inform a discussion as to whether additional abatement actions could be implemented to further improve conditions. The scientific goal at AOCs is the implementation of an action plan. As such, it follows that if no feasible abatement actions remain, then the BUI has been restored.

Statistics and Data Analysis in Aquatic Toxicology and Assessment

Way past time to stop using NOEL/LOELs

LANDIS, W. [1] ***and CHAPMAN, P.*** [2]

[1]*Western Washington University,* [2]*Goulder Associates*

In this presentation we convey the fundamental error of the use of no observed effect levels (NOELs) and lowest observed effect levels (LOELs), alert the ecotoxicology community to the flaws inherent in this practice and push for this error to cease. The

problem with NOELs and LOELs is basic. The fundamental model of environmental toxicology is the exposure- (or concentration- or dose-) response curve describing the relationship between exposure and effect. It is a given that the curve is the best possible description of this relationship must be the keystone of the field of toxicology. NOEL and LOELs do not meet the criterion. After all, NOELs and LOELs are not data, they are not direct observations, but simple labels for experimental treatments. A clear alternative is curve fitting. Straightforward methods for the calculation of such models have existed since Wilcoxon 1949. GraphPad, SPSS and other software can perform the calculations. Bayesian techniques developed by Fox can be employed using WinBUGS. In all cases curve-fitting can produce estimates of the exposure-response curve with confidence or credibility limits. We propose three simple guidelines. (1) Curve-fitting is the preferred method for estimating cause-effect relationships. (2) Confidence or credibility limits have to be reported along with the archived raw data. (3) Work that treats LOELs, NOELs, NOECs, LOECs as data should be subject to intense statistical and scientific scrutiny. Finally, we call on journals and regulatory agencies to ban the use of hypothesis tests for the reporting of exposure-effect relationships. These technically indefensible practices must end.

Using multivariate statistics to derive biotic indices

GREEN, R. [1]

[1]*University of Western Ontario*

An index is a number derived from a formula that summarizes some quantity of data. In environmental studies indices are usually calculated from biological data (e.g., species abundances) and interpreted as responses to the environment. Indices are usually set in opposition to multivariate (MV) statistical methods used to detect and describe pollution impact, i.e. it is either indices or MV statistics. In practice biotic indices are usually derived to respond to pollution-induced changes in abundances of species that have been shown to be sensitive or resistant to specific contaminants. Ideally they should not only respond to pollution but also indicate what kind of pollution is happening. A simple ratio of abundances of a number of sensitive species to abundances of a number of resistant species may respond to pollution. This transitions into the indicator species concept. Such “targeted” approaches are good for detection of particular pollution impacts selected a priori, but may not respond interpretably if there is a different kind of impact. Many MV statistical methods involve linear additive models and thus can be used in two steps to derive biotic indices: first derive the linear additive model by MV analysis of a known pollution gradient, and then apply the model

for monitoring unknown situations. Discriminant Analysis could also be used, as it is in medicine to identify sick versus healthy patients. R.W. Smith and coauthors used ordination to quantify a pollution gradient and then the tolerance of each species was estimated from its distribution along the gradient. Their "Benthic Response Index" was derived as the abundance-weighted average pollution tolerance of species in a sample. They incorporated a procedure for separating spatial gradients of natural habitats (substrate, depth, latitude) from high versus low chemical exposure at a discharge.

Pesticide use in salmon aquaculture: Current practices, potential effects and risk

BURRIDGE, L. [1], LYONS, M. [1], WONG, D. [1] and PAGE, F. [1]

[1]Fisheries and Oceans Canada

Use of therapeutants is an integral part of salmon aquaculture activities. These compounds are used to treat disease outbreaks and infestations of ecto-parasites, also known as sea lice. Since 1994 sea lice have been a major concern and cost for salmon farmers and a number of pesticide and drug products have been prescribed to treat infested fish. In 2009 and 2010 severe infestations of sea-lice in southwest New Brunswick led to the emergency registration of several pesticide products: Salmosan®, a.i. azamethiphos, AlphaMax®, a.i. deltamethrin and Paramove®, a.i. hydrogen peroxide. Pesticides are applied as bath treatments with subsequent release of the effluent to the surrounding environment. This has led to concerns for non-target organisms such as the American lobster *(Homarus americanus)*, also a valuable, commercially-fished species in Atlantic Canada. In a series of bioassays we have determined the acute lethality of these formulations to larval and adult lobsters. AlphaMax® is the most toxic of the formulations with 1-h, 24-h and 10-day LC50s in the ng L^{-1} range, Salmosan® is lethal to lobsters in the µg L^{-1} and Paramove® is not lethal to lobsters at environmentally relevant concentrations (g L^{-1}). The authors will discuss these results in the context of use patterns and risk.

Climate-contaminant interactions: The summer stress syndrome and aquatic pollutants in cold-water teleost species

KENNEDY, C. [1] and ROSS, P. [2]

[1]Simon Fraser University, [2]Fisheries and Oceans Canada

Whether the winter stress syndrome (WSS), a condition of severe lipid depletion which has been attributed to aquatic contaminants in combination with reductions in feeding during cold weather, is a significant phenomenon in temperate and cold-water species is unclear. The combined effects of winter and summer temperatures, limited food ration, and contaminant exposure (current-use pesticide carbaryl) on health-realted endpoints in juvenile sockeye (*Oncorhynchus nerka*) and Northern pike minnow (*Ptychocheilus oregonensis*) were examined here. Body mass, body and organ condition

factor indices, as well as energy stores were reduced by food limitation in both species at cold temperatures, but these effects were only affected by pesticide exposure in the smaller sockeye. Under warmer temperatures, however, body mass, organ and body condition factor indices, and energy stores were reduced, in conjunction with significant increases in oxygen consumption in both species under limited food conditions, effects which were significantly increased when concurrently exposed to carbaryl. Our results indicate that fish bioenergetics are affected by conditions that would occur in contaminant-degraded habitats during the summer. The warming habitats of many cold water species suggests that this 'summer stress syndrome' may be important for the survival of some salmonid stocks, and highlights the need to evaluate the interactive effects of multiple human stressors.

Parasites and pollution: A study of selenium uptake in parasite infected rainbow trout *(Oncorhynchus mykiss)*

HURSKY, O. [1] ***and PIETROCK, M.*** [1]

[1]*University of Saskatchewan*

Given that parasites demonstrate different sensitivity to contaminants and environmental stress there is an increasing interest in using parasites as biological or ecological indicators of their fish host life conditions as well as bioindicators of heavy metal pollution of aquatic ecosystems. The aim of this study was to investigate (1) whether there was a transfer of selenium (Se) from intestinal contents of rainbow trout (*Oncorhynchus mykiss*) to nematodes (*Raphidascaris acus*), (2) whether parasites bioconcentrated more Se than their host and (3) to examine whether the combined effect of parasitic infection and Se exposure had an effect on fish health (as determined by Fulton's condition factor, Hepatosomatic Index (HSI), Gross Energy (GE) content and levels of catalase and GST). Rainbow trout were infected with larval stages of nematodes and subsequently exposed to dietary Se for 70 days (15 µg kg^{-1} food dry weight). Se concentration of trout muscle tissue and intestinal parasites were determined using ICP-MS. The results indicated that compared to muscle tissue of their host, *R. acus* accumulated lower levels of Se, therefore *R. acus* is not a good bioindicator of Se accumulation in aquatic ecosystems. However, *R. acus* do have an impact on fish health: combined with Se exposure parasitic infection decreases GE content of the fish and impacts changes in enzyme activity.

Lethal and sub lethal toxicity of nitrates and phosphates in the endemic fish *Skiffia multipunctata*

RUEDA-JASSO, R. [1] ***and DELOS SANTOS-BAILÓN, A.*** [2]

[1]*Universidad Michoacana de San Nicolas de Hidalgo,* [2]*Universidad Michoacana de San Nicolas de Hidalgo*

The Mexican freshwater fish fauna is highly diverse. The Goodeinae family, is a group of viviparous fish endemic to the Mexican Central Plateau and is considered among the representative species. It is estimated that more than half of the species are endangered due to the deterioration and high levels of pollution in the water bodies they inhabit. This study examined the effect on *Skiffia multipunctata* by lethal and sub lethal concentrations of nitrates and phosphates to establish their respective tolerance levels. Concentrations of 0.0, 0.016, 0.028, 0.049, 0.085 and 0.15 mg L^{-1} of nitrites and 0.0, 1.62, 3.08, 5.86, 8.79, 13.18 and 19.77 mg L^{-1} of phosphates, with 3 replicates each (n=30 fish per concentration) were used to obtain the LC_{50} following OECD procedures. During the exposure of the fish to both toxic compounds; erratic swimming, collisions with the tank, presence of wounds and mortality were observed. The calculated LC_{50} were 0.064 mg L^{-1} for nitrates and 13.18 mg L^{-1} for phosphates at 24 and 96 hours respectively. The LC_{50} of both compounds which caused severe alterations at the gill level (hyperplasia, hypertrophy and lamellar fusion) were below the Official Mexican Norm limits (NOM-001) in order to conserve the wild aquatic fauna. The duration of exposure and concentration were decisive in the effect. The obtained results showed that the values proposed in NOM-001 do not guarantee the survival of the endemic fish *S. multipunctata*.

The effects of WAF and CEWAF on the fertilisation and hatching success of eggs collected from Atlantic cod *(Gadus morhua)*

BURRIDGE, L. [1]***, WONG, D.*** [1]***, LYONS, M.*** [1] ***and LEE, K.*** [1]

[1]*Fisheries and Oceans Canada*

Oils spills and the consequent response to those spills can occur wherever oil is produced or transported. Atlantic cod *(Gadus morhua)* may be exposed to the water accommodated fraction (WAF) and/or the chemically enhanced water accommodated fraction (CEWAF) of oil in the marine environment during spawning. We investigated the fertilisation and hatching success of cod eggs when exposed to Alaskan North Slope crude oil with SPC-1000 as the dispersant. Eggs from Atlantic cod were fertilised in the presence of WAF or CEWAF along with sea water controls and incubated for 24 hours. Fertilsation success was determined then individual fertilised eggs were transferred to

96- well plates and incubated until hatch. Results show that WAF concentrations in the range 0.5% to 50% (v/v) had no effect on fertilisation success compared to controls. For CEWAF (0.01% to 0.5% (v/v)), fertilization success was reduced compared to the controls and WAF exposures. Hatching success for eggs fertilised in WAF and CEWAF did not show any significant difference compared to the controls except for CEWAF at 0.5% (v/v), $p < 0.05$. Water analysis is ongoing and will provide data regarding dose-response.

Impact of endosulfan on growth, histology and metabolic rates in juvenile American Lobster *(Homarus americanus)*

DAOUD, D. [1], FAIRCHILD, W. [2], JACKMAN, P. [3], BENHALIMA, K. [2], COMEAU, M. [2], BRUNEAU, B. [2], CHABOT, D. [2] and MALLET, M. [1]

[1]Homarus Inc., [2]Fisheries and Oceans Canada, [3]Environment Canada

In recent years, several studies have been initiated to gather information on the decline of the American lobster *(Homarus americanus)* landings in some areas of Atlantic Canada. One of these studies, carried out by Environment Canada and Fisheries and Oceans Canada, has looked at the effect of anthropogenic environmental contaminants on lobster. The presence of some chemicals in the environment during critical developmental periods could potentially affect lobster growth and survival. Endosulfan, sold under trade names such as Thiodan, is a broad-spectrum organochlorine insecticide, widely used in agricultural areas within the Gulf of St. Lawrence, Canada. It has already been shown that an acute exposure to endosulfan has significant effects on survival and growth of stages I to IV larvae. In order to detect more subtle physiological impacts to endosulfan exposure, we investigated if metabolic rate, measured by oxygen consumption, changes for juvenile lobsters (stages 5 and 6) exposed to sub-lethal levels of this contaminant. Long-term measurements of oxygen consumption with an intermittent-flow system allowed the calculation of standard (SMR), active (AMR) and routine (RMR) metabolic rates. Histological observations of controlled and exposed animals will also be presented.

Assessing the chronic impact of urban runoff and municipal waste water effluents on wild freshwater mussels in the Grand River, ON.

GILLIS, P. [1]

[1]*Environment Canada*

The Grand River is an example of a river with diverse anthropogenic inputs. Along its 300 km reach, the river receives inputs from 29 Municipal Wastewater Treatment Plants (MWWTP) as well as industrial effluents and agricultural and urban runoff. Although the watershed is under pressure from population growth, it has historically supported a diverse population of freshwater mussels, including nine Species at Risk. However it was unknown how the chronic exposure to the complex contaminant mixture in the river affects freshwater mussels. The immune and general health of *Lasmigona costata* collected both up- and downstream of Kitchener-Waterloo and Cambridge was assessed over three field seasons (2008-2010). General health was estimated through lipid analysis, condition factor and mussel age. Immune status was assessed by quantifying hemocyte viability and phagocytosis activity. In addition metal bioavailability was determined through analysis of mussel gill tissues. Mussels collected from the furthest downstream site (downstream of 11 MWWTP) were the most impacted. Their maximum age and condition factor were significantly lower ($p<0.05$) than upstream mussels. The significant and cumulative increase in Cu, Pb, Zn, Al, Cr, and Ni in mussels moving downstream indicates that metals are bioavailable and that tissue concentrations increase with multiple MWWTP inputs. During one field season, the phagocytosis activity of mussel hemocytes was significantly stimulated in downstream mussels. Overall this study found that mussels chronically exposed to urban runoff and municipal effluents are negatively impacted.

Acute toxicity of the oil dispersant Corexit 9527 on aquatic photosynthetic organisms

JOHNSON-WORRELL, S. [1] ***and GORBUNOV, M.*** [1]

[1]*Rutgers University*

Oil dispersants are assumed to protect shorelines and accelerate petroleum biodegradation. However, dispersants are toxic and may adversely affect the ocean's ecosystem. Toxicity studies of oil dispersants were routinely evaluated using brine shrimp, bivalves, and fish as bioindicators. Yet, information pertaining to the dispersant effects on primary producers is limited. The goal of this research is to assess the toxicity of Corexit 9527 on aquatic photosynthesis utilizing the fluorescence induction and

relaxation technique. This fluorescence technique provides a comprehensive set of parameters that characterize the excitonic energy transfer in the light-harvesting antennae, the photochemistry in Photosystem II, and the electron transport to carbon fixation. The diatom, *Thalassiosira weissflogii*, (TW) and the zooxanthellae, *Symbiodinium* spp. (Zoox) were exposed to varying concentrations (.039 to 1.96 g L^{-1}) of Corexit 9527. The mean 48hr EC_{50} for the dispersant was 0.31 g L^{-1} and 1.37 g L^{-1} for TW and Zoox, respectively. A marked decline in the quantum efficiency of photosystem II and the energy transfer efficiency in the photosynthetic light-harvesting antenna was observed. The analysis revealed that Corexit 9527 adversely affects the primary photosynthetic reactions. This information can be utilized to organize dispersants by potency.

Evaluating the toxicity of petroleum in aquatic environments

CHAPMAN, P. [1], LANDRUM, P. [2], NEFF, J. [3] and PAGE, D. [4]

[1]Golder Associates Ltd., [2]Southern Illinois University, [3]Neff and Associates LLC, [4]Bowdoin College

Aquatic toxicology has evolved from an initial evaluation of individual chemicals to evaluations of mixtures of chemicals and complex substances. However, experimental designs for evaluating the toxicity of complex mixtures such as petroleum in aquatic environments can be highly variable and, if not appropriate, can and have produced data that are difficult or impossible to interpret accurately. We provide guidance for the proper design of laboratory and field assessments of the toxicity of mixtures of low-solubility organic chemicals such as comprise different petroleum products, based on two fundamental requirements: establishing a dose-response relationship; and, determining the causative agent(s) of any observed toxicity. These two requirements can be met only if exposure conditions and measurement endpoints are appropriate, modifying factors are considered (e.g., test conditions, test organism life stage, chemical transformations, mixture dilution, sorbing phases), and concentration/dose response relationships are correctly interpreted. Problems that occur when these two fundamental requirements are not met are illustrated using case studies.

Relationships between water hardness and aquatic toxicity of anions: Implications for establishing water quality benchmarks

ELPHICK, J. [1], GILRON, G. [2], CHALMERS, B. [3] and BAILEY, H. [1]

[1]Nautilus Environmental, [2]Coalhunter Mining Corporation, [3]Mining Association of BC

Recent investigations have demonstrated that the aquatic toxicity of anionic components of total dissolved solids, including sulphate, chloride and nitrate, exhibit strong relationships with water hardness; specifically, toxicity is elevated under soft water conditions as compared to higher water hardness conditions. This relationship likely either results from competition by ions for uptake sites at biological membranes, or from the effect of ions that contribute to hardness, in particular, calcium and magnesium, on cell membrane permeability. The presence of major anions at elevated concentrations almost always co-occurs with elevated hardness, since dissolved anions are typically balanced by equimolar concentrations of cations, including those that constitute hardness. Thus, elevated anion concentrations typically occur in conjunction with elevated water hardness. This presentation summarizes the relationships that have been established relating water hardness to the aquatic toxicity of anions, and outlines methods that have been, or could be, applied to incorporate water hardness as a toxicity modifying factor in the establishment of generic water quality guidelines and/or site-specific water quality objectives.

Quantitative relationship between biodegradation and sorption of phthalate esters and their metabolites in natural sediments

KICKHAM, P. [1], OTTON, V. [1], MOORE, M. [1], IKONOMOU, M. [2] and GOBAS, F. [1]

[1]Simon Fraser University, [2]Fisheries and Oceans Canada

Regulatory evaluations of commercial chemicals in Canada, the United States, the European Union and other countries aim to identify biodegradation rates of chemicals in natural soils and sediments. However, commonly used biodegradation testing methods are limited in their capacity to determine biodegradation rates under natural environmental conditions. As a result, widely varying biodegradation rates have been reported for many very hydrophobic substances. This variability causes difficulties in regulatory evaluations, potentially leading to chemical misclassification. In this study, we developed a model of the relationship between biodegradation, sorption and hydrophobicity and tested the model in experiments that measured the biodegradation rates of a range of di-phthalate and mono-phthalate esters in natural sediments. The results indicate that di-phthalate and mono-phthalate esters have the inherent capacity

to be quickly degraded by microbes in sediments at a common rate but that di-phthalate ester biodegradation rates in natural sediments decrease with increasing phthalate ester sorption to sediments. The results show that inherently biodegradable substances that undergo a high degree of sorption can be expected to exhibit long half-lives in natural sediments. The model provides a potential methodology for assessing biodegradation rates in natural sediments from inherent biodegradation rates measured in screening tests. The study further indicates that a reduced rate of biodegradation is associated with a reduced bioavailability and that the environmental significance of sorption reduced biodegradation rates needs to be viewed in the context of risk in chemical evaluations.

Oxidative stress and growth in juvenile alligator gar, *Atractosteus spatula*, exposed to environmental concentrations of the aquatic herbicide, diquat. (poster)

BEAUDRY, M. [1]***, MANDRAK, N.*** [2]***, GLEBE, B.*** [2]***, CAMPBELL, R.*** [3] ***and PALACE, V.*** [2]

[1]*University of Manitoba,* [2]*Fisheries and Oceans Canada,* [3]*US Fish and Wildlife Service*

In Canada, Spotted Gar, *Lepisosteus oculatus*, a species of freshwater fish, is known only to three bays of Lake Erie (Long Point, Point Pelee and Rondeau Bay) and is considered a threatened species. The remaining populations are subject to numerous anthropogenic stressors including contaminants, siltation, dredging, and removal of aquatic vegetation through applications of the bipyridal contact aquatic herbicide Diquat (1,1'-ethylene-2,2'-dipyridylium). A potent redox cycler, diquat can increase proliferation of reactive oxygen species leading to cell membrane damage and also potentially impairing growth. During the summer of 2010, water samples collected from a treated area of Rondeau Bay contained 40 ug L-1, 3 hours after treatment, declining to 4 ug L-1 after 24 hours. Diquat was not detected in reference areas. Because experimentation on threatened species is not possible, cultured alligator gar *(Atractosteus spatula)* were used as a surrogate Lepisosteidian species to examine potential effects of similar diquat concentrations on growth and oxidative stress parameters. Juvenile alligator gar (8g, n=50/group) were exposed to 0 (reference), 50, 100 or 400 ug L^{-1} in a static renewal system for a period of 21 days followed by depuration in untreated water. Concentrations of diquat, as well as the metabolites diquat monpyridal and diqaut bipyridal, were quantified (UV/Fluorescence HPLC) in liver immediately after exposure and at 6 weeks post-exposure (n=8/treatment and time). Concentrations of the antioxidant vitamins E (tocopherol) and A (retinoids), as well as peroxidized lipids, were determined in liver as indicators of oxidant damage.

Connecting breeding and wintering habitats of piscivorous birds: Implications for tracking contaminants using stable isotopes (poster)

OFUKANY, A. [1], HOBSON, K. [2] and WASSENAAR, L. [2]

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

Interpreting contaminant body burdens in migratory water birds is complicated by the differential accumulation and shedding of compounds among distinct biomes. Each fall, piscivorous double-crested cormorants (*Phalacrocorax auritus*) from the North American Great Lakes migrate to marine, freshwater, and aquaculture-based systems along the Gulf of Mexico. Contaminants in tissues may be transported from one feeding or nesting location to another and deposited in moulted feathers, eggs, and wastes.

By combining isotopic and contaminant data for moulted feathers, sources of contaminant loads can be interpreted. Stable isotope ratios ($\delta^{34}S$, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$) and total mercury (THg) were measured in winter- and summer-grown primary feathers from cormorants breeding on Lake Winnipeg, Manitoba, in 2009 and 2010. Sulphur ($\delta^{34}S$), nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopes were used to characterize diet and sources of THg. Correlations between feather isotopes and precipitation $\delta^{2}H$ were used to assign feathers to Lake Winnipeg, the Gulf Coast, or stopover sites. Data for $\delta^{34}S$, $\delta^{13}C$ and $\delta^{15}N$ indicate a higher propensity to feed on freshwater foods during winter, with no difference in feather THg from freshwater versus marine habitats. Feathers assigned to Lake Winnipeg ($\delta^{2}H$) have significantly higher THg concentrations than those from the Gulf Coast, indicating cormorants are accumulating more THg from their summer, rather than winter, diets.

Effects of the pesticide formulation AlphaMax® on various life stages of the American Lobster *(Homarus americanus)* (poster)

WONG, D. [1], LYONS, M. [1], WADDY, S. [1] and BURRIDGE, L. [1]

[1]Fisheries and Oceans Canada

The copepod *Lepeophtheirus salmonis* is a parasite of Atlantic salmon (*Salmo salar*) and is a significant problem for the aquaculture industry. AlphaMax® is an anti-sea lice formulation which contains 1% deltamethrin, a synthetic pyrethroid pesticide that affects the sodium channels of nerve membranes in affected organisms. The prescribed treatment concentration is 2 µg deltamethrin L^{-1}. The lethality of the formulation to stage I, II and IV larvae of American lobster *(Homarus americanus)* was determined. The larvae were exposed for 24h to AlphaMax® at nominal concentrations ranging from

0.00125 to 0.325 µg deltamethrin L^{-1}. Water samples were collected from each bioassay for chemical analysis by GC-ECD to confirm the exposure concentrations. Analytical results of chemical analyses showed that the concentration of deltamethrin in the water samples was approximately 50% of nominal, indicating significant loss of deltamethrin in the exposure system. The 24h LC_{50} of AlphaMax® calculated using average measured concentrations of the active ingredient was estimated to be 0.0011, 0.001 and 0.0076 µg deltamethrin L^{-1}, for larval stages I, II and IV respectively. These concentrations represent approximately 260 to 2000 fold dilutions of the recommended treatment concentration and indicate that lobster larvae are very sensitive to this pesticide formulation.

Assimilation of metals in otoliths is influenced by microstructure, evidence from Rainbow Trout *(Oncorhynchus mykiss).* (poster)

CARROLL, L. [1], THÉBEAU, N. [2], HALDEN, N. [3] and PALACE, V. [4]

[1]Golder Associates Ltd., [2]Ministry of Natural Resources, [3]University of Manitoba, [4]Department of Fisheries and Oceans/ University of Manitoba

There are three polymorphs of calcium carbonate (CaCO3) in the otoliths of teleost fish; aragonite, vaterite, and calcite. Aragonite is the most common polymorph. However, in certain circumstances the aragonite can be replaced by vaterite, creating what is referred to as a crystalline otolith. An otolith is considered crystalline when the aragonite form of CaCO3 is partially or completely replaced by vaterite. A vateritic otolith has a different crystalline structure, abnormal shape and is visibly translucent. A laboratory feeding experiment was conducted to determine how concentrations of five dietary trace metals correspond to deposition in the otoliths of juvenile rainbow trout *(Oncorhynchus mykiss)*. At the end of the experiment, otoliths were removed and processed by Laser Ablation Inductively Coupled Plasma Mass Spectrometery (LA-ICP-MS). To distinguish whether an otolith was crystalline versus non-crystalline, strontium (Sr) was also included in the analysis. No differences were detected in the dietary metal concentrations. There were a higher proportion of “crystalline” otoliths compared to non-crystalline otoliths (70%). There were no significant differences in the proportion of crystalline otoliths among the experimental groups ($p = 0.0886$). However, there were significant differences ($p < 0.001$) between the Sr and Zn concentrations among the experimental groups. The high occurrence of crystalline otoliths in the study could be associated to the rearing practices either at the hatchery prior to the study, or perhaps during the study. Vaterite (crystalline) and aragonite (non-crystalline) have different

partition coefficients which may preclude the use of otoliths with multiple polymorphs in environmental reconstructions.

A small-scale recirculating system for measuring tritium uptake and depuration in rainbow trout (poster)

SHULTZ, C. [1] ***and KIM, S.*** [1]

[1]*Atomic Energy of Canada Ltd*

Tritium is considered to be a key radionuclide in the aquatic environment around CANDU facilities. Uptake and clearance of free water tritium (HTO) is known to be very fast in aquatic organisms but organically bound tritium (OBT) formation is much slower and the rate is dependent on variables such as water temperature, water quality, organism age and feeding rates. Conditions in dynamic natural systems such as those found around CANDU reactors make OBT formation and depuration rates difficult to predict. In order to estimate OBT formation and depuration in fish under controlled conditions, two 1000 L recirculating aquaculture systems were constructed at the AECL Chalk River Laboratories. The species that was chosen for testing was rainbow trout (*Oncorhynchus mykiss*) because of its natural abundance in North America (including in areas where some CANDU facilities are operating), its history of laboratory use and the ability to use these fish at a size that is relevant to human consumption. In this controlled environment, fish can be monitored for OBT formation and depuration as well as for biological/cellular responses during exposure to tritiated water and/or while being fed tritiated feed. Experiments that have been conducted include exposing fish to tritiated water, feeding tritiated chow, and combining both treatments. Water was maintained at the Canadian Drinking Water Standard of 7000 Bq L^{-1}. Tritiated trout chow was prepared using radiolabelled essential amino acids to an OBT activity concentration of 31000 Bq L^{-1} (combusted water). This poster describes the operating conditions as well as the observations on growth and fish condition for these experiments.

IGETG - 35 years and still going strong! (poster)

WATSON-LEUNG, T. [1] ***and NORWOOD, W.*** [2]

[1]*Ontario Ministry of the Environment,* [2]*Environment Canada*

As early as 1972 when toxicity testing was to be included in new water regulations under the *Fisheries Act*, it was recognized that standardized toxicity test methods with defined quality control procedures were required for scientific credibility, defensibility and enforcement of the data. For almost 40 years ecotoxicological testing

has been an integral part of environmental conservation and protection activities in Canada. An important part of this history is the formation of the Inter-governmental Ecotoxicology Testing Group (IGETG) and the standardization of toxicity test methods. This year IGETG, a volunteer group of scientists from federal and provincial agencies, celebrates 35 years of advancing the progress of ecotoxiological testing in Canada. IGETG members have been instrumental in the development of 21 standardized aquatic, sediment and soil toxicity methods and 7 supporting guidance documents, published in both official languages by Environment Canada's Method Development and Applications Unit (MDAU). The co-operation of IGETG members has contributed to the consistency of the federal and provincial requirements in toxicity tests which allows industry to comply with regulations in both jurisdictions by conducting a single set of tests. Contributions from IGETG members have also advanced the effectiveness of environmental assessments and monitoring programs and the development of water and sediment quality standards and guidelines. IGETG members and the MDAU were instrumental in the development of a National Quality Assurance/Accreditation Program for toxicology laboratories. This poster provides a historical timeline of the evolution of ecotoxicological testing in Canada for regulatory applications. It will provide a summary of the Federal and Provincial programs supported by IGETG and discuss the future initiatives of IGETG and the challenges facing these initiatives.

The paleoecotoxicology of Ross Lake, Manitoba: Reconstructing the impacts of 80 years of industrial and municipal activities (poster)

SCHIFFER, S. [1], LIBER, K. [1] and DOIG, L. [1]

[1]University of Saskatchewan

As a result of long-term mining and smelting activities (ore processing commenced in 1930), the sediments of Ross Lake (Flin Flon, Manitoba, Canada) are highly contaminated with trace metals. Over a similar period, Ross Lake also received municipal effluent from the surrounding community. Although the yearly contaminant loads to this small northern lake were historically substantial, little information is available regarding the associated ecological impacts. An investigation was therefore conducted to reconstruct the ecotoxicological history of Ross Lake. Cores of sediment, extracted in 2009 from the southern basin of Ross Lake, were analyzed for physicochemical and radiometric variables and sub-fossil diatom, chironomid, chaoborid, and cladoceran remains. A number of ecological changes occurred in Ross Lake subsequent to the onset of mining activities. The historically diverse, mesotrophic

diatom community was replaced by a simpler community comprised mainly of planktonic meso-eutrophic diatoms. Similarly, the diverse chironomid community was replaced by a community dominated by a single genus, *Chironomus*, a genus tolerant of low dissolved oxygen concentrations. The cladoceran community greatly decreased in species richness. Changes to the cladoceran community possibly explain the demise of *Chaoborus*, a predatory midge. Although *Chaoborus* larvae were present in pre-industrial times, they largely disappeared in the post-industrial era. Using constrained cluster analysis, distinct differences were found between pre- and post-industrial diatom, chironomid and cladoceran communities. Although improvements to both mining and municipal wastewater treatment have reduced trace metals and nutrient loading into Ross Lake, the sedimentary record suggests that this lake has not yet recovered ecologically.

Recovery of wild small bodied fish populations after a multi-year exposure to a synthetic estrogen (poster)

PARK, B. [1], PALACE, V. [1], WAUTIER, K. [1], BLANCHFIELD, P. [1] and KIDD, K. [2]

[1]Fisheries and Oceans Canada, [2]Canadian Rivers Institute

Estrogenic contaminants are routinely detected in surface waters, and can disrupt reproductive endocrine system function in aquatic organisms. To address potential impacts on wild fish populations, we added the synthetic estrogen 17 α-ethynylestradiol (EE2) to an experimental lake (L.260) from spring to fall of 2001 to 2003, and monitored various physiological and population parameters from the EE2-treated lake and two reference lakes. Previously we reported that EE2-exposed fish exhibited vitellogenin induction in males, inhibited gonad development in both sexes, testicular oocytes, and histopathological changes in liver and kidney. The fathead minnow *(Pimephales promelas)* population in the treated lake collapsed due to lack of recruitment, whereas these effects were absent in reference fish populations. To assess potential recovery of fish in L.260, we continued to monitor these populations after cessation of the EE2 additions for vitellogenin, histology and population endpoints. Fathead minnow and pearl dace (*Margariscus margarita*) were collected in spring and/or fall from 2004 to 2010. Preliminary results indicate that reproductive abnormalities were present in both species two years post-exposure in L.260, but were absent in subsequent years. There was no difference among lakes in vitellogenin levels in male fathead minnows caught in the fall of 2010; analyses of samples for other dates and for pearl dace are ongoing. Catch-per-unit-effort (CPUE) of adult fathead minnow

returned to pre-experiment levels by the spring of 2007 (4 years post addition). These results indicate that, given time, fathead minnow and pearl dace recovered across all levels of biological organization from the whole lake additions of EE2.

Biochemical and molecular responses of white sturgeon *(Acipenser transmontanus)* to an aryl hydrocarbon receptor agonist (poster)

DOERING, J. [1], WISEMAN, S. [1], BEITEL, S. [1], TENDLER, B. [1], GIESY, J. [1] and HECKER, M. [1]

[1]University of Saskatchewan

Dioxin-like chemicals in some sediments can bioaccumulate into fishes to concentrations sufficient to produce toxic effects. Benthic fishes such as sturgeon might be at greater risk of exposure to these chemicals. Despite their endangered status, little research has been done to characterize the relative responsiveness of sturgeon to dioxin-like chemicals. In a first attempt to characterize the biological effects and possible associated risks of exposure to dioxin-like chemicals in sturgeon we investigated the molecular and biochemical responses of white sturgeon to a model aryl hydrocarbon receptor agonist, β-naphthoflavone (BNF). Sturgeon were injected intraperitoneally with BNF. Rainbow trout were used as a reference species since their responses have been well characterized in the past. Three days following injection with one of three doses of BNF (0 mg kg^{-1}, 50 mg kg^{-1} or 500 mg kg^{-1}) fish were euthanized and gill, intestine, and liver collected for biochemical and molecular analyses. In sturgeon all tissues had significantly greater ethoxyresorufin-O-deethylase (EROD) activity in exposed groups. Rainbow trout had EROD activity significantly greater than controls in the liver, but undetectable activity in the intestine and gill. Based on these results, sturgeon appear to be moderately responsive to dioxin-like chemical exposure. The greater responsiveness of the intestine and gill in sturgeon suggests that these tissues might be of greater importance in the biotransformation of polyaromatic hydrocarbons in sturgeon than in trout. Further research is necessary to investigate the toxicological significance of the observed intestine and gill enzyme activity in sturgeon.

Results of toxicological testing of sediment samples collected from the vicinity of fish processing plants in the Maritimes. (poster)

HOPPER, C. [1], JACKMAN, P. [1] and DOE, K. [1]

[1]Environment Canada

Most fish processing plants discharge untreated effluent directly into marine/estuarine receiving environments. In Canada these plants are subject to the Fish Processing Operations Liquid Effluent Guidelines, which recommend screening of

effluents for solids removal and a well-designed outfall, but no toxicity tests are required. Consequently, the characterization of the fish plant effluents is based mainly on physical-chemical parameters and very little is known about the effects of fish plant effluent in the receiving environment. In addition to testing the effluent for both toxicology and chemistry parameters Environment Canada is analysing the chemical and toxicological characteristics of sediments collected from the receiving environment of fish plant effluents in the Maritimes. Sediment samples were collected at different distances from the end of pipe to determine the extent, if any, of the impact in the receiving environment. Samples from each site were analysed for marine amphipod toxicity and for redox, ammonia and sulphide levels. Grain size, total organic carbon and % moisture were also analysed. In some sites elevated levels of ammonia and sulphide were found at distances from the end of pipe and these correlated to amphipod mortality. The toxicity and chemistry analysis of sediment from the receiving environment of fish plants can be used to improve the monitoring and implementation of more strict regulation for fish plant effluents.

Parasites and chemical contamination of walleye *(Stizostedion vitreum)* and pike *(Esox lucius)* from Montreal Lake, Saskatchewan (poster)

MATWEE, L. [1] ***and PIETROCK, M.*** [1]

[1]*University of Saskatchewan*

With accelerating industrial activities in Saskatchewan, there is growing debate over the potential effects of pollutants on the health of fish in local aquatic environments. Indigenous people from Montreal Lake, Saskatchewan have expressed concern with regard to the pollutants that may be accumulating in their fish populations. In addition to negative effects due to potential chemical pollution, aboriginal leaders reported changes in fish parasitism believing that these changes may be associated with airborne contaminants. In order to identify potential health risks related to fish consumption, health, condition and chemical contamination of walleye and pike from Montreal Lake were assessed. Walleye and pike collected in September 2010 were examined for the presence of metazoan parasites, and metal and organic contaminants. The present study found 4 parasite species (belonging to the taxa Cestoda, Trematoda, Acanthocephala and Nematoda) in pike and 5 species (Cestoda, Trematoda and Hirudinea) in walleye. All parasites are nonpathogenic to humans and common to Canadian lakes. The occurrence of *Ichthyocotylurus platycephalus* in pike

and walleye, and *Neoechinorhynchus rutili* in pike were not previously recorded for Saskatchewan lakes in the aforementioned fish species. Metal and organic contaminants in the muscle tissue of both species of fish were low and fell well within levels considered acceptable for consumption.

Avoidance by sand shrimp, *Crangon septemspinosa*, of sandy patches covered by hydrated lime (calcium hydroxide) deposits (poster)

REEBS, S. [1], JACKMAN, P. [2], LOCKE, A. [3] and FAIRCHILD, W. [3]

[1]Université de Moncton, [2]Environment Canada, [3]Fisheries and Oceans Canada

Proliferation of invasive tunicates in Prince Edward Island estuaries has necessitated management of tunicates that foul mussel aquaculture structures. Spraying or immersion of tunicates with saturated solutions of hydrated lime (calcium hydroxide, $Ca(OH)_2$) is effective, but can be biocidal to non-target organisms such as sand shrimp, *Crangon septemspinosa*. To investigate sublethal behavioural effects, individual sand shrimp were offered a choice between one side of a 6-L aquarium with a sandy bottom, and the other side that had the same sandy bottom covered by a layer of deposits resulting from the injection of hydrated lime, into the water column. The injection raised water pH from 7.7 to 9.4 and deposited a flocculent mixture of magnesium carbonate and calcium carbonate on the bottom. Shrimp significantly avoided the aquarium side with flocculent material in the first 24 h. Mortality was high during the ensuing three days, but the survivors continued to avoid the flocculent side. A control group (no lime injection) showed only a moderate mortality of 15% after 96 h and no avoidance of any particular side within the aquaria. Large quantities of concentrated lime solutions can be released where bio-fouling treatment is applied to mussel socks, and particles have been observed drifting to the bottom at such locations. Results suggest that such particles can reduce the preference of sand shrimp for the affected sediment. It is unknown whether deposits persist on the seafloor, and whether sand shrimp are present in areas where hydrated lime is used.

Food web mercury concentrations in lakes near two major atmospheric mercury emitters in western Canada (poster)

BIELEFELD, K. [1], EVANS, M. [1], MUIR, D. [1], KIRK, J. [1] and KEATING, J. [1]

[1]Environment Canada

Anthropogenic mercury (Hg) release has been increasing globally, amplifying concern over its toxicological effects. Under the Government of Canada's Clean Air Regulatory Agenda, we are studying lakes near two major atmospheric Hg emitters to

assess their impact on these systems. This study was unique as it compared two different Hg-affected environments differing in emitter technology, emission type and watershed characteristics. Lakes in the Flin Flon area (northern Manitoba and Saskatchewan) received emissions from a base metal smelter from 1930-2010 and lakes in central Alberta (Wabamun L. area) have received emissions from a cluster of 4 coal-fired power plants since the 1950s. Food web studies including water, sediments, and biota focussed on northern pike and were conducted over 2009-2010 in lakes in the two areas. Sediment Hg concentrations were high in lakes very near the Flin Flon smelter but rapidly diminished with distance while northern pike in the immediate vicinity of the smelter did not have high Hg concentrations. However, northern pike in lakes 70+ km to the NW had mean Hg concentrations greater than 0.5 $\mu g\ g^{-1}$ consumption guideline; such high levels were not observed in lakes to the southeast. In Alberta, sediment Hg concentrations were low and mean mercury concentrations in fish were <0.5 $\mu g\ g^{-1}$ in all lakes. Mercury biomagnification rates (estimated from the slopes of the δ^{15}N-Hg regression for each lake) were quantified and intercepts examined. Lakes with the highest fish Hg in the area NW of Flin Flon had high intercepts (Hg at the base of the food web) but slopes were not (Lake 1=0.15, McLurg=0.19), while lakes in Alberta with relatively low Hg in the food web and low intercepts had the highest slopes (Jackfish=0.22, Pigeon=0.26) such that pike attained similar Hg concentrations as in lakes with lower biomagnification rates. Our ongoing work will further elucidate the physical, chemical and biological factors affecting mercury levels in northern pike.

Acute toxicity of the pesticides deltamethrin and azamethiphos to two species of shrimp (poster)

LYONS, M. [1], WONG, D. [1], MACKEIGAN, K. [1] and BURRIDGE, L. [1]

[1]Fisheries and Oceans Canada

The use of pesticides in the salmon aquaculture industry has become a concern of the industry, government regulators, environmentalists and people employed within the traditional fisheries. AlphaMax®, a formulation of the pyrethroid pesticide deltamethrin, was registered in Canada in the summer of 2010 for emergency use in the treatment of sea lice on Atlantic salmon. Salmosan®, a formulation of the organophosphate pesticide, azamethiphos, is currently registered for use in Canada. The recommended treatment concentrations are: AlphaMax®, 2 µg L^{-1}, as deltamethrin and Salmosan®, 100 µg L^{-1}, as azamethiphos. Our objectives were to measure the lethality of these pesticide formulations to the marine crustaceans *Crangon septemspinosa,* the sand shrimp, and *Mysis stenolepis,* a mysid shrimp using conventional 24-hr exposures. Measured deltamethrin treatment concentrations ranged from 0.2 ng L^{-1} to 13 ng L^{-1} for *M. stenolepis* and from 8 ng L^{-1} to 47 ng L^{-1} for *C. septemspinosa*. The 24 h LC50 for deltamethrin was estimated to be 1.9 ng L^{-1} for *M. stenolepis* and 39 ng L^{-1} for C. septemspinosa. Measured azamethiphos treatment concentrations ranged from 15 µg L^{-1} to 90 µg L^{-1} for *M. stenolepis* and from 60 µg L^{-1} to 525 µg L^{-1} for *C. septemspinosa* The 24-h LC50 for azamethiphos was estimated to be 19.5 µg L^{-1} for *M. stenolepis* and 199.1 µg L^{-1} for *C. septemspinosa*. Results showed that deltamethrin was 5000 times more lethal than azamethiphos to Crangon and 10000 times more lethal to Mysids.

Watershed Management and Remediation of Degraded Lakes

Canada - Manitoba State of Lake Winnipeg Report: 1999 to 2007

PAGE, E. [1] and LEVESQUE, L. [2]

[1]Manitoba Water Stewardship, [2]Environment Canada

Lake Winnipeg is the 10th largest freshwater lake in the world and the 6th largest lake in Canada and is of great commercial, recreational, cultural, and natural importance to Manitobans. Water quality in Lake Winnipeg has deteriorated over time and algal blooms have become more frequent and intense in the offshore areas of the lake and are also noticeable in the nearshore areas. Elevated phosphorus and nitrogen loading to

Lake Winnipeg from the watershed is one of the key reasons for these algal blooms. A large amount of scientific data has been collected by federal and provincial government agencies, universities, and other researchers on Lake Winnipeg since intensive monitoring began on the lake in 1999. Since this time, chemical and biological samples have been collected from a lake wide network of 65 stations during the spring, summer, and fall. However, until now there has been no formal or comprehensive assessment of the current state of Lake Winnipeg and the changes since intensive monitoring began in late 1999. Environment Canada and Manitoba Water Stewardship, along with many others conducting research and monitoring on the lake, worked together to produce a State of the Lake report for Lake Winnipeg to summarize the scientific knowledge on the lake, focusing on the period from 1999 to 2007. The report is intended to serve as a baseline for future lake assessments and presents key information that will help to support the development of performance indicators and ecologically relevant nutrient objectives for Lake Winnipeg.

Restoration of wetlands in a Red River Valley watershed can improve water quality: Results of scenario-based modeling using SWAT

BENOY, G. [1], MELLES, S. [1] and VANROBAEYS, J. [2]

[1]Environment Canada, [2]Agriculture & Agri-Food Canada

Lake Winnipeg is Canada's 6th largest freshwater lake and is subject to an increasing rate of eutrophication as a result of nonpoint source pollution from farms and municipal wastewaters. We examine scenarios designed to compare the relative effects of wetland restoration and position on modelled nutrient loadings to Lake Winnipeg from a pilot watershed in the Lake's Basin, the La Salle watershed. Scenarios were examined using the Soil and Water Assessment Tool (SWAT). SWAT is a well-known watershed scale hydrologic model designed to assess nonpoint source pollution loadings to contributing streams across a wide range of scales. Modelled results suggested that increasing wetland cover to historic levels decreased yearly nutrient loadings by 9-21% for both total nitrogen and total phosphorus. When 25% of historic wetland areas were restored at subwatershed outlets, equivalent or better nutrient reductions were attained. But placing all wetland area at the watershed outlet did not result in as substantial nutrient reductions. There was a larger range of uncertainty when wetlands were modelled across all subwatersheds than when the entire wetland area was modelled at the outlet. These results suggest that wetland position is as important as wetland amount in terms of nutrient reductions. When combined with the

creation of artificial wetlands and small dams, land cover and hydrologic alteration strategies emerge as an important policy option for reducing exports of nutrients from watersheds.

Remediation of eutrophic lakes: How does iron treatment affect aquatic invertebrates?

WILSON, L. [1], ORIHEL, D. [1], PROCTOR, H. [1] and SCHINDLER, D. [1]

[1]University of Alberta

In some regions, internal phosphorus loading is responsible for the majority of annual inputs of phosphorus to eutrophic waterbodies. To improve water quality of these lakes, reductions in external phosphorus inputs must be accompanied by a strategy to reduce this internal loading. Iron treatment is a potential remediation technique because iron can precipitate phosphorous from the water column and inhibit phosphorous release from the sediments. However, the use of iron to remediate eutrophic waterbodies has a short history worldwide, and the effects of iron addition on aquatic biota are poorly understood. To investigate the response of aquatic invertebrate assemblages (zooplankton, zoobenthos and emerging insects) to iron addition, an in-situ mesocosm study was conducted in a eutrophic lake in Alberta, Canada. Different rates of iron (ferric chloride), ranging from 2.5g $(m^2)^{-1}$ to 225 $g(m^2)^{-1}$, were applied to the mesocosms. Our analyses thus far suggest that immediate effects of iron amendment on invertebrates are minimal. Significant delayed effects on richness, evenness and diversity of some zooplankton communities became apparent one to two months following iron application, possibly owing to profound changes in their food source (phytoplankton). The abundance, richness, evenness and diversity of zoobenthos were not affected by iron application. The total number of emergent insects increased with the highest rates of iron application. Given the minimal adverse effects of iron amendment on aquatic invertebrates, the use of ferric chloride may to be a suitable remediation technique for eutrophic lakes.

A technique to control internal phosphorus loading in eutrophic prairie lakes and reservoirs

ORIHEL, D. [1], VINEBROOKE, R. [1], BALLARD, N. [1], WILSON, L. [1] and SCHINDLER, D. [1]

[1]University of Alberta

Reductions in external phosphorus loading have been highly effective in controlling eutrophication in some lakes, but have had surprisingly little effect in

others. In many shallow lakes, phosphorus reductions have not led to improvements in water quality because of the continued release of phosphorus from lake sediments. A potential remediation technique for inhibiting internal phosphorus recycling in eutrophic waterbodies is iron treatment. Iron applications have successfully controlled internal eutrophication in a number of lakes and reservoirs, but the use of iron for remediation has a short history and the science behind this management approach is in its early stages. We are assessing the feasibility of iron treatment for controlling internal phosphorus loading in shallow prairie lakes and reservoirs. Our research spans several scales of inquiry, using in vitro sediment assays, in situ replicated lake mesocosm experiments, and whole dugout manipulations. In this presentation, we describe how iron treatment improved water quality in a shallow, hypereutrophic lake in Alberta. We applied different amounts of iron to fifteen large in-lake mesocosms and monitored changes in water quality over 10 months. Iron treatment inhibited internal phosphorus loading, reduced the biomass of phytoplankton, caused shifts in phytoplankton species composition, and reduced levels of cyanobacterial toxins.

Use of multiple lines of evidence to support sediment remediation and management decisions for the St. Marys River Area of Concern (poster)

BARRETT, C. [1]***, TAILLON, K.*** [2]***, KIM, K.*** [2]***, MILANI, D.*** [2]***, AN, S.*** [2]***, CHAMBERS, M.*** [2]***, McCHRISTIE, M.*** [3]***, HENNING, M. , FUCHSMAN, P. and ANTUNES, P.*** [1]

[1]Algoma University, [2]Environment Canada, [3]Ontario Ministry of the Environment

The St. Marys River connects Lake Superior and Lake Huron and is often referred to as the "Hub of the Great Lakes." Since the early 1900s the river has received industrial and municipal wastewater, which has resulted in sediment contamination with petroleum hydrocarbons, polycyclic aromatic hydrocarbons, oils/grease, and metals such as chromium, iron, and zinc. Because of the extensive contamination and other environmental concerns, the St. Marys River was designated as one of 43 Areas of Concern (AOC) under Annex 2 of the 1987 Canada-US Great Lakes Water Quality Agreement. The agreement requires the development of a Remedial Action Plan (RAP) for AOCs. Under the Canadian portion of the RAP, science-based evidence is being collected in support of the Canada-Ontario Decision-Making Framework for Assessment of Great Lakes Contaminated Sediment. This includes an assessment of sediment toxicity, benthic community structure, pore water chemistry, surficial and at-depth sediment chemistry, and modeling of sediment transport and fate. The results of these studies, as well as a Conceptual Site Model that schematically illustrates contaminant

sources, migration pathways, and potential impacts will be presented and discussed in the context of developing a sediment management plan for the St. Marys River.

Reconstructing the past environmental conditions of Lake Diefenbaker (poster)

LUCAS, B. [1], LIBER, K. [1], JONES, P. [1], GIESY, J. [1], SEREDA, J. [1], BHARADWAJ, L. [1], HUDSON, J. [1], DE BOER, D. [1], WHEATER, H. [1] and DOIG, L. [1]

[1]University of Saskatchewan

Lake Diefenbaker supplies drinking water to approximately 45% of Saskatchewan's population. Recent anecdotal evidence suggests that the frequency of blooms of cyanobacteria is increasing in this reservoir. Chemicals released from these blooms are potentially a threat to human health or the health of aquatic animals. Because limited empirical data are available to determine environmental trends, a paleolimnological study is being conducted to reconstruct the historical nutritional status of Lake Diefenbaker. The objective of this research is to determine if the nutrient status of Lake Diefenbaker has changed over time and whether this change is of concern. Cores of sediments were collected by gravity corer from a deep-water location near a potential point source of nutrients. One core was sectioned into 1-cm increments and these subsamples were analyzed for three sedimentary forms of phosphorous. To evaluate anthropogenic phosphorous loading over time, concentrations of apatite inorganic phosphorous, non-apatite inorganic phosphorus, and organic phosphorous were measured. Ratios of stable isotopes of carbon and nitrogen were used to identify historical sources of organic matter. In addition, subfossil remains of diatoms were isolated from each section and identified to the lowest taxonomic level. Preliminary data suggest that the diatom community in Lake Diefenbaker has typically consisted of planktonic species and that the diatom community composition has changed substantially over time. It was initially historically dominated by *Stephanodiscus parvus*, but has been more recently dominated by *Aulacoseira ambigua*. This change suggests that nutrient inputs have increased over the life of this reservoir. Additional work will evaluate whether nutrient concentrations are continuing to increase or have reached a plateau.

Reconstructing the history of sewage discharge into Ross Lake, Manitoba, Canada (poster)

TSE, T. [1], LIBER, K. [1], DOIG, L. [1], GIESY, J. [1], SEREDA, J. [1], BHARADWAJ, L. [1], DE BOER, D. [1], WHEATER, H. [1] and JONES, P. [1]

[1]University of Saskatchewan

As early as 1930, Ross Lake (Manitoba, Canada) was receiving substantial inputs of trace metals from nearby metal mining and smelting activities. Organic matter was also discharged into Ross Lake from the surrounding community, now the City of Flin Flon. Although these industrial and municipal activities have been ongoing for over 80 years, currently there is limited information on the effects of these contaminants on the ecology of Ross Lake. Changes to mine and municipal effluents over time have reduced loads of both metals and nutrients to Ross Lake. However, it is unknown whether these changes have improved the ecological state of Ross Lake. Using paleolimnological techniques, the objectives of this research were to i) reconstruct the historical timeline of sewage input into Ross Lake and ii) evaluate the subsequent effects on primary producers. Cores of sediment were collected from the southern basin of Ross Lake in 2009 by use of a gravity corer. Using an extruder, one core was sectioned at 1-cm increments and subsamples from these sections were analyzed for various faecal sterols, including coprostanol (5β-cholestan-3β-ol), which is used as a chemical marker of human sewage contamination. Due to the historically high input of nutrients and the likely stimulation of primary productivity, strong faecal sterol and phytopigment signatures are expected within those sediment sections corresponding to the post-industrial era. Preliminary sterol findings will be presented and discussed within the context of changes to mining and municipal activities over time.

Importance of internal phosphorus loading in a hypereutrophic Prairie lake and its implications for lake remediation (poster)

CALLAGHAN, D. [1], ORIHEL, D. [1], PATERSON, M. [2] and SCHINDLER, D. [1]

[1]University of Alberta, [2]Fisheries and Oceans Canada

Killarney Lake, Manitoba, has a long history of manipulations to reduce the frequency and intensity of cyanobacterial blooms. Previous remediation attempts have not been successful, and excessive algal growth continues to produce negative effects. Total phosphorus (P) concentrations are exceptionally high (>200 μg L^{-1}; Manitoba Water Stewardship) and an understanding of the primary sources of P is required to implement remediation strategies. Seasonal dynamics in P concentrations are consistent

with other lakes with high internal nutrient loading, but benthic phosphorus fluxes have not previously been explicitly quantified. We conducted a sediment core experiment to investigate sediment P release and to determine its relative contribution to high P concentrations in the overlying water column. Sediment cores were collected from three sites and incubated in the laboratory under either oxic or anoxic conditions. Fluxes of soluble reactive P from sediments were generally low under oxic conditions (< 5 mg $(m^2)^{-1}$ d^{-1}), but ranged between 10-50 mg$(m^2)^{-1}$ d^{-1} under anoxic conditions. Our measured rates of P release will be compared with the estimated mass of P in Killarney Lake to assess the overall contribution of sediment release to total P concentrations. Evaluating the importance of internal P loading will help determine the need to sequester sediment P as part of the remediation strategy for Killarney Lake.

The impact of iron on phosphorus cycling within alkaline eutrophic lakes, and the response of algal assemblages: Inferences from paleolimnology, porewaters and mesocosm experimentation (poster)

BALLARD, N. [1] ***and ORIHEL, D.*** [1]

[1]*University of Alberta*

Porewaters in sediment cores from seven Central Alberta alkaline lakes suggested that P retention in sediments was regulated by Fe(II). Greater P-flux from sediments occurred if pore- water concentrations of Fe(II) were below 100 µmol●L-1. Controlled Fe additions to mesocosms within Nakamun lake resulted in increased sediment P-retention relative to controls, with higher N:P ratios and lower abundances of cyanobacteria in the overlying water. The hypereutrophic lakes Lac La Nonne and Nakamun revealed historical changes in sediment chemistry indicating that lowered redox potentials and sulfide scavenging of Fe occurred synchronous with eutrophication. Recent and rapid eutrophication of the two lakes followed the onset of higher mean annual temperatures in Central Alberta. Sediment Fe- P dynamics may inform efforts towards mitigating impacts of climate and nutrient pollution on water quality, and offer potential controls on the growth of harmful algal blooms in alkaline eutrophic lakes.

Nutrient removal from sewage lagoon wastewaters in simulated constructed wetland mesocosms (poster)

ELLIOTT, A. [1], BARTEL, C. [2], BEATTIE, S. [2], LOKESH, S. [3], MONTERO-RODRIGUEZ, O. [4], LOW, J. [2], CARDINAL, P. [1], CARLSON, J. [2], HANSON, M. [1] and WONG, C. [2]

[1]University of Manitoba, [2]University of Winnipeg, [3]Biral Institute of Technology and Science, [4]Instituto Tecnológico de Costa Rica

Rural municipalities throughout the Prairies use sewage lagoons to treat their municipal waste. During the summer of 2010 it was determined, by characterization of the Dead Horse Creek watershed in southern Manitoba, that sewage lagoons provide insufficient nutrient removal from wastewater. Constructed wetlands may further remove nutrients from lagoon wastewaters before release to reduce impacts on the surrounding environment. This hypothesis was tested using simulated constructed wetlands (~ 3000 L), which were spiked with synthetic wastewater. Mesocosms with and without aquatic plants (*Typha* spp.,) were compared, and monitored for traditional water quality parameters (e.g., phosphorus, nitrogen, chlorophyll). Nutrient measurements were monitored pre-spike, as well as post-spike in order to monitor the rate of nutrient removal. High nutrient levels (e.g., 1.68mgP L^{-1}, 1.69mgN L^{-1}) were observed in treated tanks on day 0. Nutrient levels returned to pre-spike levels 7 days after treatment began. Nominal differences were observed between mesocosms with and without plants, possibly due to filamentous algae growth in all tanks.

Environmental Effects Monitoring in Canada

Embryotoxicity of Chilean pulp mill effluent extracts in Japanese medaka *(Oryzias latipes)*

HOLDWAY, D. [1], ORREGO, R. [1] and GUCHARDI, J. [1]

[1]University of Ontario Institute of Technology

Effects of Chilean pulp mill effluent extracts, steroid standards (testosterone and 17β-estradiol) and a wood extractive standard (beta-sitosterol) were evaluated using post-fertilized medaka (*Oryzias latipes*) embryos. Three waterborne semi-chronic exposure experiments using 24 hpf (unknown sex) d-rR (orange-red) mutant embryos, 24 hpf (unknown sex) FLFII (female leucophore free) and 72 hpf FLFII (phenotypic sex-identified) mutant embryos were undertaken. Chronic exposure of both 24 hpf d-rR and FLFII strain embryos showed similar delay in time to hatch and decreased hatchability in

all pulp mill extract exposed embryos. In contrast, significant early hatching and increased hatchability were observed in beta-sitosterol d-rR exposed embryos. High mortality was observed in all testosterone exposed embryos. Severe teratogenic responses were observed in medaka d-rR embryos in all treatments including optical deformities and impaired forebrain and heart development. Axis malformation and pericardial edema were observed in all treatments using 24 hpf FLFII. Sex-related mortality and teratogenic effects were observed in 72 hpf FLFII medaka embryos in all treatments and mostly related to males. High mortality was observed in male and female embryos exposed to testosterone. Furthermore, signs of later masculinisation were observed in females exposed to testosterone. Differences in the severity of teratogenic effects seem to be related to phenotype (d-rR and FLFII). Overall, our research indicated that Chilean pulp and paper mill extractives caused medaka embryotoxicity irrespective of the effluent treatment and strain used. The effects were mainly associated with delayed time to hatch, decreased hatchability, and sex-related teratogenesis and mortality.

Contaminated groundwater discharges to streams: Establishing exposure and effects to benthic invertebrates

GRAPENTINE, L. [1], ROY, J. [1] and BICKERTON, G. [1]

[1]Environment Canada

In many urban areas, streams receive groundwater contaminated with a range of substances, often from long-lasting legacy sources. Ecological impacts of these discharges are of concern but not well known. Among stream biota, invertebrates in the hyporheic and surficial sediment zones are expected to be the most exposed and show the greatest responses to groundwater contaminants. However, assessing exposure and effects of contaminants from this pathway is difficult due to the rapid dilution of upwelling groundwater in the stream, the partial confounding of contaminant and dissolved oxygen concentrations, and effects of other natural and human factors on the stream. In a study of a Nova Scotian stream exposed to chlorinated solvents, petroleum hydrocarbons, metals, and chlorides in groundwater, multiple locations were sampled and assessed for porewater contaminant concentrations, various habitat attributes (including subsurficial dissolved oxygen concentration and sediment particle sizes), surficial macroinvertebrate community composition, hyporheic invertebrate community composition, and in situ toxicity of water at the sediment-water interface to *Hyalella azteca*. Preliminary results suggest that both invertebrate communities are affected by chlorinated solvents and chlorides, and that in situ toxicity could be related to chlorinated solvents. Interactions among contaminant and habitat factors on invertebrate conditions and how they complicate the implementation of a sampling design able to detect impacts of groundwater contaminants will be discussed.

Otolith micro chemical reconstruction of fish exposure to trace elements in the environment

HALDEN, N. [1], FRIEDRICH, L. [2] and PALACE, V. [2]

[1]University of Manitoba, [2]Freshwater Institute

Otoliths are biominerals in the inner ear of teleost fish, composed of aragonite layers deposited in a protein matrix. Both the inorganic and organic portions have the capacity to incorporate a broad range of trace elements. Changes in trace metal concentrations in the environment influence the amounts available for incorporation into the otolith through food or ambient water. Otoliths may serve as continuous recorders of exposure to trace elements owing to their metabolic stability, continuous growth, and annular structure that provides a time scale. Otoliths were taken from

geologically distinct areas, influenced by metal or coal mining activity, to determine if chemical signatures related to mining activity and local geology can be detected. Elements indicative of surrounding geology or mining activity were chosen for LA-ICP-MS analyses across the annular zones. Otoliths downstream from a rare-element pegmatite mine contain signatures of Li, Cs, and elevated Rb; those from reference lakes do not have such concentrations. Otoliths taken from lakes adjacent to Cu, Pb, and Zn mining contain peaks of metals interpreted to indicate fish contact with tailings. Fish stocked in an open-pit Ni-Cu-Cr mine contain constant levels of these base metals throughout their life history. Anomalously high Se detected in otoliths collected in coal mine end pits indicated that fish from the mine-impacted system migrated from nearby reference streams. These cases indicate that otolith microchemistry can be used to evaluate the extent of mining activity affects in an environment, and provide information on fish movement into and out of affected areas.

Pulp and paper Environmental Effects Monitoring: From effects to solutions

ANOOP, P. [1]

[1]*Environment Canada*

Canadian Pulp and Paper mills are now in their 6th cycle of Environmental Effects Monitoring (EEM). Since Cycle 4, EEM has evolved from assessing the potential effects of pulp and paper effluent to determining the causes of the effects and identifying solutions to eliminate these effects. By the end of Cycle 3, approximately 70% of mills had confirmed an effect on at least one of the EEM effect indicators. Cycle 3 conclusions led industry to express an interest in improving the effectiveness and efficiency of pulp and paper EEM to focus monitoring efforts and resources where they were needed most while continuing to monitor all effects. The concept of critical effects sizes (CESs) was introduced to assist in identifying the effects of most concern. Defining risk through the use of CESs has been an important tool in accelerating mills progression in EEM. Amendments to the Pulp and Paper Effluent Regulations have also assisted mills to progress through the EEM requirements. It is expected that overall action taken by the industry toward the determination of causes and identification of solutions will be accelerated during Cycle 6. A summary of pulp and paper EEM activity, results, the causes determined and solutions identified to date will be provided.

The anti-estrogenic and liver metabolic effects of DHAA in rainbow trout *(Oncorhynchus mykiss)*

PANDELIDES, Z. [1], ORREGO, R. [1], GUCHARDI, J. [1] and HOLDWAY, D. [1]

[1]University of Ontario Institute of Technology

Recent studies have shown that dehydroabietic acid (DHAA), a resin acid present in pulp and paper mills, may have anti-estrogenic effects in fish. A chronic-exposure toxicity experiment using immature rainbow trout *(Oncorhynchus mykiss)* was conducted in order to assess the endocrine disrupting and liver metabolic effects of the wood extractives DHAA and β-sitosterol (BS) regularly present in pulp and paper mills and the model estrogen 17β-estradiol (E2). It was found that exposure to 5 mg L^{-1} of E2 significantly increased hepatosomatic index (HSI), vitellogenin (VTG) and plasma sorbitol dehydrogenase (SDH). This latter effect was reduced by mixing E2 with DHAA, indicating that DHAA does not cause its anti-estrogenic effects indirectly due to liver damage. Exposure to 5 mg L^{-1} of DHAA caused significant increases in liver citrate synthase (CS), and liver ethoxyresorufin-O-deethylase (EROD) activity after 7 days, however, the fish returned to control values by 28 days. The results of the study indicate that DHAA may alter energy metabolism as well as cause anti-estrogenic effects in female juvenile rainbow trout.

Regulation of P glycoprotein activity by oxidative stress in isolated teleost hepatocytes (poster)

GRAY, T. [1]

[1]Simon Fraser University

To investigate that hypothesis that oxidative stress (OS) can regulate the activity of P glycoprotein (Pgp) in isolated rainbow trout hepatocytes *(Oncorhynchus mykiss)*, cells were exposed in vitro to different pro oxidant treatments. OS is the cellular response that may result from exposure to a wide variety of electrophillic xenobiotics, many of which are commonly found at contaminated sites. Many xenobiotics, of natural and anthropogenic origin, are effluxed at the cellular level via the activity of Ppg, which may be affected by OS. A rapid and sustained state of oxidative stress was achieved by depleting glutathione (GSH) levels with diethyl maleate (DEM), but not hydrogen peroxide (H2O2). Hepatocyte suspensions exposed for thirty minutes to 2.5, 0.25 and 0.025 mM DEM had average GSH levels that were decreased by 35%, 24% and 14%, respectively. Cells exposed to 10 mM H2O2 had GSH levels that were 10% less than the negative control. Initial rates of rhodamine 123 (R123) accumulation were measured to

assess the activity of Pgp under conditions of oxidative stress. The initial rates of R123 accumulation measured using hepatocyte suspensions exposed to 3, 30, 300 or 1200 µM H2O2 were statistically indistinguishable. Hepatocyte suspensions treated with 2.5 mM DEM displayed initial R123 accumulation rates that were increased slightly relative to the negative control, but this increase was not statistically significant. This work has shown that DEM can effectively decrease hepatic GSH levels without causing cytotoxicity, and that initial rates of R123 accumulation are not significantly altered following brief exposure to DEM or H2O2, suggesting that the activity of Pgp is not decreased in vitro following conditions of OS.

Oil Sands Development and Remediation

Assessment of immune gene expression and *Trypanosoma carassii* infection in goldfish exposed to naphthenic acids and oil sands process water

HAGEN, M. [1]***, MITCHELL, S.*** [1]***, KATZENBACK, B.*** [1]***, OLADIRAN, A.*** [1]***, GARCIA, E.*** [1]***, KARPMAN, M.*** [1]***, EL-DIN, M.*** [1]***, SMITH, D.*** [1]***, MARTIN, J.*** [1] ***and BELOSEVIC, M.*** [1]

[1]*University of Alberta*

The separation of bitumen from the oil sands generates large quantities of oil sands process water (OSPW), which cannot be released into the environment, until it is successfully remediated. In this study, we exposed goldfish for up to 12 weeks to either commercial NAs or aged OSPW using a real- time continuous flow exposure apparatus. We measured the gene expression of three pro-inflammatory cytokines (IFNγ, IL1-β1, and TNFα-2), anti- inflammatory cytokine IL-10, and pro- inflammatory cytokine receptors (IFNR1-1, IFNR1-2, TNFR1, TNFR2), in the gill, kidney, and spleen of the exposed fish using quantitative-PCR (qPCR). We observed a general up-regulation of IFNγ, IL1- β1, and TNFα-2 gene expression after acute (one week) exposure of fish to either commercial NAs or OSPW. Sub-chronic (12 weeks) exposure of fish to NAs caused a significant down-regulation in immune gene expression in fish exposed to higher doses (10 and 20 mg L^{-1} of NAs). In contrast, gene expressions in fish exposed to aged OSPW were similar to those of non- exposed controls. In general, there were no differences in gene expression of pro- inflammatory cytokine receptors between exposed and non-exposed fish except for the kidney, where the gene expressions of TNFR1 and TNFR2 were up-regulated and IFNG1-1 was down-regulated after sub- chronic exposure to

OSPW. To determine whether goldfish host defense was affected by exposure to 5 mg L^{-1} and 20 mg L^{-1} of NAs, fish were infected with a blood parasite *Trypanosoma carassii* and their ability to control the infection and immune gene expression documented. After acute exposure to NAs (one week), fish exhibited significantly higher parasitemia compared to non-exposed controls. In contrast, after sub-chronic (60 days) exposure of fish to NAs, fish had higher parasitemia and greater mortality compared to non-exposed controls. Thus, the exposure of goldfish to NAs elicited a significant enhancement in pro-inflammatory gene expression and increased resistance to parasitic infection after acute exposure to NAs or OSPW, and down- regulation of immune gene expression and increased susceptibility to parasitic infection after sub-chronic exposure to NAs or OSPW.

In vivo and in vitro bioassays for the assessment of OSPW immunotoxicity and remediation

GARCIA, E. [1], GE, J. [2], OLADIRAN, A. [1], GAMAL EL-DIN, M. [3], MARTIN, J.[1] and BELOSEVIC, M. [4]

[1]University of Alberta, [2]Institute of Environmental Toxicoloy, [3]Department of Civil and Environmental Engineering, [4]Biological Sciences Department

We have developed in vitro and in vivo bioassays to explore the effect of OSPW on mammalian immune mechanisms. Using both in vitro and in vivo bioassays we found that a commercial preparation of naphthenic acids (NAs) and the organic fraction of oil sands process water (OSPW-OF) have different effects on mammalian immunity, when dosed at equivalents NAs amounts. Ozonation has recently been used as an efficient method for degrading NAs in OSPW, and decreasing its toxicity towards bacteria. We hypothesized that ozonation could reduce OSPW immunotoxicity, through the degradation of NAs, and possibly other unidentified OSPW contaminants. In vitro exposure to OSPW-OF decreased the ability of mouse macrophages to produce nitric oxide, reactive oxygen intermediates, and to efficiently perform phagocytosis. Decreased production of nitric oxide and reactive oxygen intermediates correlated with decreased gene expression of the inducible nitric oxide synthase and NADPH oxidase subunits, respectively. OSPW-OF also altered the expression of pro-inflammatory cytokine genes in macrophages. Oral administration of OSPW-OF altered the expression of pro-inflammatory cytokine and chemokine genes in the mesenteric lymph nodes and the liver of mice. Interestingly, OSPW ozonation abolished its ability to impair mouse immune mechanisms both in vitro and in vivo. We conclude that ozonation may be a

valuable treatment process for the remediation of the large volumes of water from oil sands tailing ponds.

An examination of the toxic properties of water extracts in the vicinity of an oil sand extraction site

GAGNÉ, F. [1], ANDRÉ, C. [1], DOUVILLE, M. [1], TALBOT, A. [1], PARROTT, J. [1], MC MASTER, M. [1] and HEWITT, M. [1]

[1]Environment Canada

The industrial extraction of oil sands (OS) in northern Alberta, Canada, has raised concern about the water quality of the nearby Athabasca River. The purpose of this work was to examine the toxic properties of various water extracts on Oncorhynchus mykiss trout hepatocytes. The water samples were fractionated on a reverse-phase C18 cartridge and the levels of light-, medium- and heavy-weight polycyclic aromatic hydrocarbons (PAHs) were determined by fluorescence spectroscopy. Primary cultures of trout hepatocytes were exposed for 48 h at 15oC to increasing concentrations of the C18 fraction corresponding to 0.02, 0.1, 0.5 and 2.5X concentrations from upstream/downstream sites in the Athabasca River, groundwater samples, OS tailings and interceptor well-water samples. Changes in cell membrane permeability, activity of phase I and phase II biotransformation enzymes (cytochrome P4501A and glutathione S-transferase activities), oxidative damage (lipid peroxidation LPO) and genotoxicity (single and double DNA strand breaks) were monitored in post-exposure cells. The water samples produced minor changes in membrane permeability but did increase all the above endpoints at thresholds of between 0.02 and 0.1X the water concentration. The most responsive biomarker was DNA damage but it also offered the least discrimination among sites. LPO was stronger at sites downstream of the industrial operations compared to upstream sites. A decision tree analysis was performed to formulate a set of rules by which to identify the distinctive properties of each type of water sample. The analysis revealed that OS tailings and interceptor waters were characterized by an increased concentration in light PAHs (> 42 μg L^{-1}) and this fraction represented more than 85% of the total PAHs. These samples also inhibited GST activity, which could compromise the elimination of genotoxic PAHs present in the system. An analysis of groundwater samples revealed a contamination pattern similar to that for OS tailings. There is a need for more research into specific biomarkers of toxicity from OS tailings compounds such as naphthenic acids, light PAHs among others, which are a characteristic fingerprint of OS extraction activities.

Larval fish toxicity of snow melt waters from oil sands areas

PARROTT, J. [1], NORWOOD, W. [1], GILLIS, P. [1], HEADLEY, J. [1], HEWITT, M. [1], KIRK, J. [1], FRANK, R. [1], MUIR, D. [1] and WANG, Z. [1]

[1]Environment Canada

Embryo-larval fathead minnows (*Pimephales promelas*) were used to assess the toxicity of snow melt samples collected in the vicinity of the Canadian oil sands process facilities along the Athabasca River in the area of oil sands development and upstream. Snow samples were collected early March 2011, prior to spring melt and then shipped frozen back to the laboratory. Because the snow melt waters were low in essential ions, they were amended with CaCl2, NaHCO3, NaBr, KCl, and MgSO4 to bring the major ion levels up to those generally observed in the Athabasca River. Fertilized fathead minnow eggs were exposed (through hatch to 7 days post-hatch) to 25 %, 50 % and 100 % amended snow melt waters. Snow samples from upstream or far downstream of the oil sands were not toxic to larval fathead minnows at 100%. Two snow samples from around the oil sands mining and refining areas were toxic to larval minnows at 25-100 %. Toxicity occurred after hatch, and larval minnows showed deformities in the two toxic snow samples. The snow melt waters all had negligible concentrations of naphthenic acids. Compared to other sites, the two most toxic snow melt samples had higher concentrations of polycyclic aromatic hydrocarbons and metals, most likely from airborne deposition.

Can standard amphibian toxicity tests be used as a predictor of aquatic health downstream of an oil sands tailing pond?

DARWISH, T. [1], STEVENS, C. [1], ROBINSON, R. [1] and BAIYEWUN, F. [2]

[1]Golder Associates Ltd., [2]Syncrude Canada Ltd.

Monitoring programs evaluating the environmental impacts of oil sands operations often conduct toxicity testing with a fish, benthic invertebrates or plants to help predict what effect the operation may have on the receiving environment. Since 2004, Syncrude Canada Ltd. has been researching whether amphibians can be used as an accurate indicator of overall aquatic health in Beaver Creek. Beaver Creek is a low-flow, heavily impounded creek that is located immediately downstream of the Mildred Lake Settling Basin. To assess amphibian health in Beaver Creek, standard amphibian toxicity testing utilizing the FETAX (frog embryo teratogenesis assay – *Xenopus*) method has been conducted biannually on Beaver Creek surface water. In 2009 and 2011 we also monitored reproductive activity in Beaver Creek by measuring the frequency of

calling anurans and density of egg masses along the creek. Results from Beaver Creek were compared to those recorded at two nearby reference ponds. FETAX results have often shown statistically significant effects on survival, mortality and malformation in *Xenopus* larvae. Conversely, preliminary field surveys monitoring a local amphibian, the wood frog (*Rana sylvatica*), suggest that Beaver Creek supports a viable amphibian population. Although field studies are on-going, these preliminary results indicate that standard toxicity testing methods using FETAX by itself may not be an accurate indicator of water quality in the oil sands area.

The toxicity of oil sands sediments to Northern pike *(Esox lucius)* during early development

TURCOTTE, D. [1]***, ROMANOWSKI, L.*** [1]***, RAINE, J.*** [2]***, TUMBER, V.*** [1] ***and PARROTT, J.*** [1]

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

Northern pike *(Esox lucius)* are a commercially important fish species native to the northern hemisphere. In Alberta (Canada), these fish inhabit the Athabasca River, which flows through the Athabasca oil sands, and are exposed to natural sources of bitumen eroding from the McMurray formation. There is currently no information available to assess the early development of pike exposed to the bitumen present in the water of the Athabasca River. Pike are not easily cultured in a laboratory environment and no methods have been developed to assess the toxicity of oil sands to this fish species. Thus, the current study describes the design and implementation of a daily-renewal bioassay that assesses the potential effects of sediments from the Athabasca oil sands area to the early stages of pike development. Eggs were collected and fertilized with milt from spawning wild pike captured from Lake Diefenbaker, SK. The fertilized eggs were exposed to treatments containing different concentrations of sediments from the oil sands area, reference sediments or strictly culture water until complete yolk absorption of control fish, approximately 15 days post-hatch. Brine shrimp were fed to the pike embryos daily at the initiation of exogenous feeding and continued to the conclusion the experiment. Developing fish were examined for morphological deformities, survival, hatching success, and changes in weight and length between treatments. Preliminary results suggest that the Northern pike is less sensitive than walleye and fathead minnow to the toxicity of oil sands sediments. This study provides much needed information on development and survival of fish exposed to sediments from the Athabasca oil sands area and provides a means of assessing toxicity in species of fish that are relevant to Canadian environments.

An integrated isobolographic approach for testing the bioavailability and non-additive toxic outcomes of mixtures of oil sands wastes (poster)

GAUTHIER, P. [1], PYLE, G. [1] and PREPAS, E. [1]

[1]Lakehead University

Several recent studies propose that deposition of atmospheric effluents from oil sands (i.e., bitumen) upgrading activities increases the presence of toxic metals and polycyclic aromatic hydrocarbons (PAHs) in surrounding watersheds. It is likely that metal-PAH complexes occur in oil sands receiving waters and have the potential to facilitate or ameliorate the bioaccumulation of metals, resulting in non-additive net toxic outcomes. A reliable method for testing this phenomenon is presented in this poster. Firstly, an isobolographic approach using a 3-ratio ray design is incorporated to test for deviance from strictly additive effects. Secondly, LC50 concentrations for binary mixtures are tested against those expected of both additive and interactive models, and thirdly, metal body concentrations are compared between single and binary exposures. The combination of these analyses first makes it possible to test for non-additivity, followed by the hypothesis that non-additive toxic outcomes result from an altered bioavailability of metals likely due to metal-PAH complexation. 48-hour LC50 assays for copper, cadmium, nickel, vanadium, beryllium, phenanthrene, dibenzothiophene and binary metal-PAH mixtures are being carried out using *Hyalella azteca*. Tests contain no food to eliminate confounding interactions with analytes. Hence, acute exposures are necessary. All metals are being added as anion salts. PAHs are being added as enriched PDMS films. LC50 estimates for cadmium, copper, vanadium, nickel, and beryllium were 0.022, 0.047, 3.96, 20.6 and >75 mg L^{-1} respectively, suggesting contamination of these metals in receiving waters, when observed singly, is not acutely lethal to *H. azteca*. Investigation of PAH toxicity and binary mixtures is ongoing.

Analysis of historical and current surface water monitoring programs and activities in the Athabasca oil sands area (poster)

LINDEMAN, D. [1], HALL, S. [1] and RITSON-BENNETT, E. [1]

[1]Environment Canada

Water quality monitoring in the oil sands region can and should be informed by historic and current monitoring, focused studies, and research activities that have produced data and information over time. Evaluation of potential environmental impacts and the effectiveness of reclamation strategies depends on having something to compare to, and historical data may provide useable temporal reference information.

An analysis of current and historical water quality and quantity monitoring programs and activities was undertaken, to collect information on "who is measuring or has measured what, and where?" Past and current programs were reviewed, including monitoring by the Government of Canada, the Province of Alberta and relevant stakeholder monitoring organizations (such as RAMP), and the most relevant University-based studies on surface water quality. Analytical challenges and constraints that should be taken into consideration were included in the assessment. In addition, the current permit- and regulatory-related water quality monitoring in the Lower Athabasca oil sand region was examined. Surface water quality parameters required under the Alberta Environmental Protection and Enhancement Act were compiled, from EPEA licensing information provided by Alberta Environment. The Pulp and Paper Environmental Effects Monitoring program was also examined, as potential upstream reference information. Considerable historic water quality information exists, from programs dating back to the 1980s, which could be useful in comparisons with current conditions, and in the future assessment of mitigation and reclamation. Differences in analytical methods and detection limits would have to be taken into consideration, if comparisons are made between older and current data.

Characterization of oil sands waters by principal component analysis of accurate mass LC/QTOF data (poster)

HINDLE, R. [1], NOESTHEDEN, M. [1], HEADLEY, J. [2] and PERU, K. [2]

[1]Vogon Laboratory Services Ltd., [2]Environment Canada

Naphthenic acids (NAs) are a complex class of carboxylic acids found as contaminants in tailing pond waters in Alberta's oil sands. These natural chemicals are concentrated during the hot water extraction of bitumen from the oil sands. Identifying waters contaminated by NAs can be done by LC/QTOF with electrospray ionization and accurate mass detection, and the data further reduced by Principal Component Analysis (PCA) to help characterize the acid components and potentially identify the source of water contamination. Direct injection of water samples without prior extraction, cleanup, and re-concentration can help reduce bias of the various analytes that can be introduced by these steps, while still providing low detection limits of individual acids. The data presented here will show the effectiveness of these techniques.

How are populations of pearl dace from Fort McMurray, Alberta, affected by oil sands sediments? (poster)

TURCOTTE, D. [1], RAINE, J. [2], TUMBER, V. [1] and PARROTT, J. [1]

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

Pearl dace *(Semotilus margarita)* are a bottom-dwelling fish species native to Canada and the northern United States. Pearl dace inhabit effluents of the Athabasca River, which flow through the Athabasca oil sands, and are exposed to natural sources of bitumen eroding from the McMurray formation. The effects of the bitumen present in the water on pearl dace are not yet fully characterised. This project studied two populations of wild pearl dace collected upstream and downstream of the oil sands operations in Fort McMurray. Fish were exposed to treatments containing different concentrations of naturally bitumen-enriched sediments or control sediment for 21 days. Fish were fed lyophilized brine shrimp daily. Histological analysis of the gills, liver and gonads was performed to detect differences between treatments and fish populations. Quantitative RT-PCR analysis of isolated liver RNA was performed to determine if vitellogenin expression was altered in these fish. In addition, CYP1a induction was measured by a standard EROD test. Measurements were also recorded to calculate liver and gonad to body weight ratios. The result of this study will provide much needed information on the effects of bitumen-containing sediments from the Athabasca River on pearl dace health. This project will also compare how fish populations from sites upstream and downstream of the oil sands operations in Fort McMurray may respond to bitumen exposure.

Optimization of a microsatellite analysis of genetic variation in northern Alberta populations of fathead minnow *(Pimephales promelas)* (poster)

TURCOTTE, D. [1], SIT, M. [1], YUAN, H. [1], RAINE, J. [2], ANDRES, J. [2], TALBOT, A. [1] and PARROTT, J. [1]

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

Toxicity tests of oil sands constituents (OSC) are typically performed using animals raised for generations in laboratory settings. Consequently, these tests do not account for the potential adaptation of native fish from the Athabasca River system to naturally-occurring OSC in the water and thus may not provide a realistic measure of toxicity to native fish populations. Furthermore, the mechanisms that allow these fish populations to survive in OSC-containing environments are not yet known. The objective of this project is to investigate a potential genetic adaptation to OSC in fish native to the

Fort McMurray area, Alberta. The initial step of this project was to optimize a microsatellite assay that will be used to determine genotypic differences between fathead minnow populations in Fort McMurray. Fish from Demonstration Pond, a test pond containing oil sands process water, and Poplar Creek (a control site) were selected for preliminary evaluations. Preliminary results have shown a genetic variability between these two fish populations, as expected. Fish from Poplar Creek had more alleles for each marker than fish from Demonstration Pond. Some markers had a high diversity while some markers were more conservative. Markers with a too high or too low diversity cannot be used to efficiently compare population genotypes and new markers should be designed to meet this objective. Further work will also involve the analysis of more fish populations.

Nanotoxicology

Anything but standard: Lessons learned while developing nanoparticle testing protocols in a commercial ecotoxicology laboratory

SHORE, B. [1], KLOSCHINSKY, T. [1], GOTTSCHLING, A. [2], OOSTERBROEK, L. [1], BLAIS, E. [1], HENSON, E. [1], PETHO, E. [1], STEWART, H. [1], GREBNEVA, N. [1], MARQUES, L. [1] and CHAN-REMILLARD, S. [1]

[1]HydroQual Laboratories Ltd., [2]Clear Environmental Solutions

As consumer and industrial products containing manufactured nanoparticles continue to enter the market at an increasing rate, often with little understanding of environmental and/or health implications, ecotoxicity testing of nanoparticles is a topic of critical concern for regulatory agencies worldwide. HydroQual Laboratories recently conducted a research programme to develop procedures for assessing nanoparticle ecotoxicity consistent with Environment Canada methods. Three types of nanoparticles (nano-silver, nAg; nano-titanium dioxide, $nTiO_2$; and nano-zero valent iron, nZVI) were selected for study. Test development consisted of two phases: initial trials to determine suitable sample preparation techniques and assess effects of test media parameters on nanoparticle behaviour and toxicity, followed by full- scale definitive toxicity testing for a variety of species used in standard ecotoxicological tests. Nanoparticle behaviours in test media and toxicity to *Vibrio fischeri* (i.e., Microtox® testing) varied greatly depending on interactions between various intrinsic and test media properties. Inter-batch variation of nZVI was found to be so great as to preclude further testing, and it was subsequently dropped from the study. Trends in nanoparticle toxicity were

relatively consistent across species tested, but interpretation is hampered due to incomplete characterization of particle properties prior to and during testing. Development of ecotoxicological tests for nanoparticles fit for regulatory decision-making purposes will be dependent on better inter-batch consistency of nanoparticle properties, the availability of timely, cost-effective techniques to adequately characterize nanoparticles in test media, and development of a consensus as to appropriate test controls and endpoints.

Approaches to understanding the acute and chronic toxicity of metal containing nanoparticles.

COSTA, E. [1] ***and MCGEER, J.*** [1]

[1]*Wilfrid Laurier University*

The objective of this research program is to understand the potential for nanomaterials to impact sensitive aquatic organisms. Test materials include metal oxides of Fe, Zn and Ti and also Ag as polyacrylate coated particles of approximately 7 nm. Acute and chronic exposure with *Daphnia pulex* characterize survival and/or reproduction while testing with *Hydra attenuata* is for survival and tentacle regression. Testing approaches are directed at understanding particle toxicity in relation to dissolution and apply solution ultrafiltration (e.g., < 450nm, < 100nm, < 10nm, and < 1nm metal fractions) and dialysis methods. The studies on nanoAg toxicity compare the effect of ionic (Ag+) and nanoparticle silver (nAg) on acute and chronic toxicity as well as the uptake kinetics with *Daphnia pulex* as they were more sensitive than hydra. The acute EC50 for Ag+ (0.78 ug L^{-1}) and nAg (0.83 ug L^{-1}) were similar but uptake kinetics differed. For the uptake and depuration studies (48 h), Daphnia accumulated Ag+ slower than nAg but depurated Ag+ faster than nAg. Short-term nAg accumulation was linear (r2 = 0.95; non-carrier transport) and Ag+ accumulation was hyperbolic (r2 = 0.95; carrier-mediated transport), suggesting different uptake mechanisms for the two forms of silver. In combination with toxicity and accumulation results, ultrafiltration and dissolution of nAg LOEC and EC50 solutions supported the finding that ionic silver within nanoparticle solutions does not play a significant role in nAg toxicity. This research was supported by the NSERC-NRC-BDC Nanotechnology Initiative.

Nanotoxicology: Not your average toxicology

GOSS, G. [1], ORTEGA, V. [1], FELIX, L. [1], EDE, J. [1] and ONG, K. [1]

[1]University of Alberta

The toxicological responses of aquatic animals resulting from exposure to manufactured nanoparticles (NPs) are a major issue in environmental risk assessment. One of the more frustrating and confusing aspects of working with nanomaterials is that as charged colloids, they can directly interact with proteins, dyes and other small molecules resulting in aberrant readings in both physicochemical and toxicological assays. This necessitates the use of extensive control and characterization regimes to effectively measure and interpret toxicological data involving nanomaterials. In this talk, I will highlight these effects, outline some of the required steps our lab uses to effectively control/interpret these colloid: solute interactions. Finally, I will be demonstrating specific nano and non-nano effects on such diverse functions as zebrafish development, in vitro immunological assays and physico- chemical properties of common nanotoxicological assays.

Increased glyphosate toxicity by dendrimer nanoparticles to *Chlamydomonas reinhardtii* (poster)

PETIT, A. [1], DEBENEST, T. [2] and GAGNÉ, F. [2]

[1]Arkema, [2]Environment Canada

The PAMAM dendrimers have been developed in biomedicine due to their high drug-carrier potential. When released in the environment, these nanoparticles have the potential to bind existing contaminants and increase their bioavailability. Glyphosate is one of the most used pesticides and contaminates many aquatic bodies. This study sought to investigate changes in glyphosate toxicity in the presence of cationic PAMAM dendrimer (4th generation) to the algae *Chlamydomonas reinhardtii*. Toxicity was examined by following decreases of cell viability and photosystem efficiency to absorb photon energy. Results showed a significant increase of glyphosate toxicity in presence of dendrimer in comparison with culture exposed to glyphosate alone. The cell viability decrease was 38.1 % for the mixture with the lowest glyphosate concentration (7.2 mM) and 60.7 % for the one with the highest glyphosate concentration (14.5 mM). The photosystem efficiency was not affected by either the pesticides or the drug carrier. The increase in toxicity with the glyphosate-dendrimer mixture could be related with the formation of dendropores which increases membrane permeability in algae.

Cell line development from model aquatic organisms: Their characterization, uses and limitations in environmental toxicology

LEE, L. [1], VO, N. [2], MACLEOD, M. [1], MIKHAEIL, M. [1], WAY, C. [1], SPITERI, K. [1], HANNER, R. [3], REN, C. [2] and BOLS, N. [2]

[1]Wilfrid Laurier University, [2]University of Waterloo, [3]University of Guelph

Predicting pollutant effects, especially in aquatic environments, is difficult and costly to assess using whole organisms. This is usually because the polluting chemicals generally occur as complex mixtures or are found at particularly low concentrations such that chemical analysis becomes challenging, and whole-animal experimental designs become cumbersome and demanding. Cell- based in vitro assays, for the most part, can circumvent these drawbacks albeit with some limitations. They can be fast, reliable, reproducible, inexpensive, sensitive, specific, and selective, depending on the cells and type of assays performed. However, they have yet to be accepted/validated with regulatory agencies. The need to detect and assess the impact of pollutants on environmental quality in real time has led to an increasing interest in developing a variety of combined in vitro chemical and biological assays that are being miniaturized and made portable for field assays. A wide assortment of biomarkers and bioindicators had been developed over the past thirty years and newer more sensitive molecular markers are currently being researched. The latter can rely on automated high-throughput technologies and mass screening of chemicals on a wide variety of test specimens that could be performed at a fraction of time and cost than the more conventional assays. In this presentation, we highlight the uses, development and characterization of fish cell lines from model aquatic organisms including zebrafish, killifish, fathead minnow, trout, salmon, walleye, eel, goldfish, perch, cod and long term cultures from some aquatic invertebrates, for their applicability in aquatic toxicology, and possible uses with microchip technologies.

Is *Hyalella* reproduction a practical endpoint to use in toxicity testing?

BARTLETT, A. [1] ***and BROWN, L.*** [1]

[1]*Environment Canada*

Reproduction in *Hyalella* can be more sensitive and environmentally relevant than survival or growth; however, its variability makes interpretation of results difficult and brings into question the practicality of conducting reproduction tests on a routine basis. The objective of this study was to conduct static-renewal sediment tests to characterize reproduction in *Hyalella*, and then to manipulate test parameters (test duration, frequency of renewal, sediment:water ratio, test containers, density of *Hyalella*) to reduce variability in reproduction. Experiments were conducted in 250-mL beakers with a 9:1 ratio of sediment:water and 10 replicates per treatment. Reproduction was characterized over 20 weeks by measuring survival, growth, and reproduction every 2 weeks starting at week 4: juvenile:adult ratios were stable from 6-20 weeks, reproduction was variable (CVs = 43-110%), and longer exposures did not reduce this variability. Effects of test duration and frequency of renewal were then investigated: higher juvenile:adult ratios occurred with fewer renewals, and survival was lower and more variable with deterioration of water quality that occurred in 10-week static tests. Experiments are currently being conducted in 1-L Imhoff settling cones to stabilize overlying water quality. Test parameters, including sediment:water ratio and number and density of *Hyalella*, are being varied. The results of these tests will be presented and compared to those from 250-mL beakers. The practicality of using *Hyalella* reproduction as an endpoint in toxicity testing and directions for future research will be discussed.

Forensic toxicity assessment on power plant effluent using rainbow trout

HENSON, E. [1]***, BLAIS, E.*** [1]***, OOSTERBROEK, L.*** [1]***, KLOSCHINSKY, T.*** [1]***, STEWART, H.*** [1] ***and PETHO, E.*** [1]

[1]*HydroQual Laboratories Ltd.*

HydroQual was commissioned to conduct a forensic toxicity assessment, designed to isolate and identify the toxic constituent of a power plant effluent pond sample. Multiple water samples were collected from the pond and were submitted to HydroQual Laboratories Ltd. (HydroQual) for toxicity testing using rainbow trout *(Oncorhynchus mykiss)*. Several undiluted samples caused lethal effects and stressed behaviour in rainbow trout. The effects were fast acting (with stressed behaviour observed within 24-hours of test initiation) and >50% mortality observed within 96-

hours of test initiation. Numerous common toxicants for rainbow trout including ammonia, chlorine, metals, and commercial products were investigated. The forensic toxicity assessment involved a literature review, an investigation into correlations between field measurements and toxicity data, and confirmatory testing to determine what proportion of the observed acute toxicity could be attributed to the potential toxicant alone. The primary toxicant causing lethal effects to rainbow trout was successfully identified. This compound would not likely have been detected using conventional methods for identifying toxic constituents (as outlined in the USEPA). Detailed methods and results of this investigation will be presented.

Establishment of continuous cell cultures from the embryos of mummichog, *Fundulus heteroclitus*, for in vitro toxicity assessments

LEE, L. [1]***, VO, N.*** [1]***, GIGNAC, S.*** [1]***, KWON, H.*** [1]***, CHEHADE, I.*** [1] ***and MACLATCHY, D.*** [1]

[1]*Wilfrid Laurier University*

The mummichog or killifish *(Fundulus heteroclitus)* has become a popular model in aquatic toxicology, especially for euryhaline environments. This species has been noted for its tolerance to environmental extremes such as high temperature, salinity, and oxygen deprivation that allows it to survive in various water bodies including degrading coastal environments. *F. heteroclitus* is thus a good teleost model for environmental effects on human health. While much work has been done at the organismal and ecological levels, studies at the cellular, molecular and genomic levels have been comparatively few, possibly due to a lack of readily available mummichog cell lines. Cell lines are integrative tools, bridging whole organismal and molecular studies, that can be easily manipulated and physiological and environmental conditions can be rigorously controlled. Although mummichog cells have been cultured in vitro as far back as the 1920's, there are no available cell lines established from this species, thus limiting research in gene manipulation/expression studies, cellular mechanism analysis, or cytotoxicity assays. In this study, we report on the successful development of cell cultures from mummichog embryos and the initial characterization of a cell line, MCE-3. These cells have been passaged over 10x, grow well at a temperature range of 25- 30°C, and can withstand holding temperatures up to 34°C. Early passaged cultures show many alkaline phosphatase- positive cells, indicating the presence of embryonic stem/progenitor cells that could be useful for potential differentiation studies. Growth characteristics, cytotoxicity and cellular responses to estrogenic compounds, salinity tolerance levels, and identifying molecular data will be presented.

It's in the bag! Toxicity identification and evaluation of plastic bag toxicity: Implications for aquatic toxicity testing and changes to testing procedures to avoid this issue (poster)

RKMAN-FILIPOVIC, S. [1]***, MOURZAEVA, K.*** [1]***, HERRMANN, O.*** [1] ***and RODGERS, D.*** [2]

[1]*Kinectrics,* [2]*Aberfoyle Aquascience*

Unexpected mortality in trout and Daphnia acute toxicity tests in normally nontoxic samples triggered a review of procedures. The only discrepancy was a change in the food grade plastic bags used in sample collection and testing; other parameters including organism health and control water quality remained constant. In follow-up tests, trout and Daphnia mortality was observed in samples collected and stored in these plastic bags, but not in parallel samples collected in glass carboys. Mortality was also observed when random bags taken from the suspect lot were soaked in control water. The soaking water was extracted under basic and acidic conditions, the extracts combined, concentrated and reconstituted in hexane for GC/MS analysis. Nonyl phenol compounds were detected at concentrations up to 240 ng L^{-1} in treated water from the toxic bags, but were not detected in control water or samples from non-toxic bags. Existing toxicity testing protocols thus appear to have a significant weakness as they lack specific tests for effects of sample storage and transport. This screening procedure is now part of our standard methods and we suggest similar procedures should be added to aquatic toxicity testing protocols.

Can field-derived periphyton communities be used to assess the impacts of herbicides? (poster)

PROSSER, R. [1]***, BRAIN, R.*** [2]***, HOSMER, A.*** [2]***, SOLOMON, K.*** [1] ***and HANSON, M.*** [3]

[1]*University of Guelph,* [2]*Syngenta Crop Protection,* [3]*University of Manitoba*

The use of periphyton in toxicity testing and risk assessment has seen resurgence in recent years. Due to the inherent biological and ecological complexity of periphyton communities, the development of a standardized, laboratory-based effects characterization tool is challenging. It is the current practice to bring natural assemblages of periphyton into the laboratory as opposed to attempting to culture representative communities (though this approach also clearly has deficiencies that will be discussed in the poster). In order to assess the suitability of field-derived communities for testing, a series of studies were conducted with communities collected from both lentic and lotic systems via colonization tiles. Using the endpoint of light-adapted photosynthetic yield as measured by pulse-amplitude modulation fluorometry

(PAM), the viability of collected communities under varying storage conditions was assessed. Community responses to varying acute durations of exposure (≤24 h exposure) to a herbicide were also measured using PAM fluorometry. The collected communities were found to be viable and stable in storage for several days, allowing for the possibility of samples being collected and tested at later dates. Sensitivity to herbicides varied depending on the site-specific community composition and exposure duration implying that data generated by this method are context dependent and suitable for risk assessment of natural assemblages in similar lotic systems. Periphyton recovery occurred rapidly upon removal from exposure (≤24 h), implying that pulsed exposures, even at relatively high concentrations, are unlikely to have a lasting impact on these communities. Overall, periphyton communities can present some unique challenges for testing, but may also be a valuable tool in assessing the ecological risks of herbicides.

Development of an in vitro medium extraction technique and LC/MS/MS analysis for sex hormones and their sulfate/glucuronide conjugates (poster)

PETERS, L. [1] ***and TOMY, G.*** [2]

[1]*University of Manitoba,* [2]*Fisheries and Oceans Canada*

As research on endocrine disruptors advances, the importance of hormone metabolites and the ability to measure them has increased. High performance liquid chromatography (HPLC) coupled with electrospray ionization tandem mass spectrometry (ESI/MS/MS) for the analyses of steroid hormones has many advantages over the more common methods of radioimmunoassay (RIA) and enzyme-linked immunoassay (EIA). LC/MS/MS is more sensitive than immunoassay (IA) methods, and multiple hormones and metabolites can be measured simultaneously. This method also does not suffer from issues of antibody cross-reactivity inherent to some IAs. This paper discusses the development and optimization of a sex hormone extraction technique from Cortland's gonadal tissue in vitro incubation medium in preparation for analysis by LC/MS/MS. Extraction efficiency of the sex steroid hormones and their conjugates from the incubation buffer were optimized by using different extracting solvents, pH adjustment, addition of an ion pair and by increasing solvent volumes. The final method for the extraction and LC/MS/MS analysis of 16 parent and sulfate/glucuronide conjugated androgens and estrogens will be presented.

Identifying cause in laboratory toxicity tests with confounding effects of multiple stressors (metals and other water quality parameters) (poster)

MILANI, D. [1] ***and NORWOOD, W.*** [1]

[1]*Environment Canada*

Benthic studies performed in 2003 in the Spanish Harbour Area of Concern (Spanish, Ontario) identified sediments as being toxic to benthic invertebrates in laboratory bioassays. The cause of toxicity, however, was inconclusive. Several metals (Ni, Mn, Zn) were elevated in the sediments and poor water quality (low pH, high conductivity and ammonia) may have confounded results. Sites were re-sampled in 2009 to update status of conditions. To address previous concerns, the overlying water stability was evaluated by conducting toxicity tests using the standard method of a 4:1 ratio of water to sediment in 250 mL beakers as well as a 67:1 ratio used in the 1 L Imhoff settling cone setup. To examine the contribution of each metal to observed effects, metal bioavailability in the epibenthic amphipod, *Hyalella azteca*, was quantified using bioaccumulation-effect models and compared to critical body concentrations. Both water quality and survival was improved in the 2009 beaker tests compared to the 2003 beaker tests. However, toxicity was still evident in 2009 tests with greater toxicity observed in the beaker tests relative to the cone tests. Survival was significantly improved by 7 to 70% in the cone tests. The two methods are evaluated and the identification of the potential cause of observed toxic effects is discussed.

Rooting for Lemna: The toxicological and statistical sensitivity of an under-utilized endpoint (poster)

ANTUNES, P. [1] ***and HANSON, M.*** [2]

[1]*Algoma University,* [2]*University of Manitoba*

Duckweeds *(Lemna spp.)* are floating macrophytes used extensively in ecotoxicology for regulatory testing, although their utility as a standard test species has been scrutinized due to their lack of an extensive rooting system from which toxicity can be assessed. Despite criticism, root length is a valuable endpoint to use in the development of risk assessment models such as the Biotic Ligand Model (BLM) for plants, where metal accumulation on the root is empirically related to toxicity. The purpose of this study was to assess the toxicological and statistical sensitivity of root length as an endpoint compared to the standard endpoints of plant dry weight and frond number. To achieve this, the toxicity of copper, nickel and lead to Lemna minor

was assessed using a standard bioassay. Testing was performed with between 5 to 7 surface waters per metal (collected from various American locations) which ranged in water quality characteristics (e.g., DOC, pH, and $CaCO_3$ L^{-1} hardness). To assess the toxicological sensitivity of each endpoint, ICx values (10%, 20% and 50%) were calculated using non-linear regression analysis to describe the relationship between the measured concentration of total dissolved Cu, Ni or Pb in solution and new growth for each of the endpoints. To assess statistical sensitivity of each endpoint, the coefficient of variation, the minimum detectable difference, and the replication required to detect a 20% changed were calculated. In terms of statistical sensitivity, root length was found to be intermediate of frond number and dry mass, with dry mass being the most sensitive endpoint evaluated. In terms of toxicological sensitivity, all ICx values for comparable scenarios (i.e., same metal and surface water) were within 10-fold of each other, with no one endpoint being consistently more responsive. Overall, root length was found to be as statistically sensitive as the standard endpoints and at times significantly more toxicologically responsive. In contrast to the critique of duckweed lacking of a testable rooting system, this study shows that root elongation can be a valuable complement to currently recommended endpoints. These results allow for enhanced ERAs for aquatic macrophytes, since the endpoint is well suited to in-situ field measurements and consistent with the development of the BLM for plants.

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