

SPECIAL STUDENT NIGHT

Featuring:

- Effects of ditch management in agroecosystems: from water quality to tadpole health

Amber Dyck, MSc Candidate, University of Ottawa

- The development and preliminary validation of an in vitro mutagenicity assay based on MutaMouse primary hepatocytes

Julie Cox, PhD Candidate, University of Ottawa

- Paleoecotoxicology: The application of novel tools to assess the toxicity of historically contaminated lake sediments

Cynthia Cheney, PhD Candidate, University of Ottawa

- 3D-Cultured Avian Cells for Chemical Screening: Metabolic and Gene Expression Profiles of the LMH Cell Line

Tasnia Sharin, PhD Candidate, University of Ottawa

- Naphthenic acids disrupt courtship behaviours in the Western clawed frog (*Silurana (Xenopus) tropicalis*)

Sue Zhang, MSc Candidate, University of Ottawa

- Reverse Q&A: Student Questions and Audience Answers

Wednesday, March 27th, 2019

5:00 pm – 7:30 pm

Clock Tower Brew Pub, 575 Bank Street

Ottawa, ON

\$1 members

\$3 non-members

Join us for an engaging talk, good eats and fine brew!

For more information contact Rebecca Dalton: becca.dalton@gmail.com

OTTAWA
PUB NIGHTS



Laurentian
SETAC
Laurentian Chapter of the Society of
Environmental Toxicology and Chemistry

Effects of ditch management in agroecosystems: from water quality to tadpole health

Amber Dyck, MSc Candidate, University of Ottawa

Industrial agriculture has caused extensive loss of natural wetlands in South and Eastern Ontario, such that vegetated ditches are some of the only remaining features in agroecosystems where wetland plants and wildlife can exist and proliferate. Dredging a ditch (i.e. removing vegetation and sediment to improve water flow) disturbs many ecological services, particularly habitat provision and water purification. My research aims to understand how water quality may change due to dredging, and how this may impact wildlife using a bioindicator, the northern leopard frog (*Lithobates pipiens*). In the first year of my study I compare mature vegetated sites to sites where woody vegetation has been removed, and in the second year I will compare un-dredged with dredged sites. I am comparing water quality parameters (including temperature, turbidity, pesticide and nutrient concentrations). In each ditch, tadpole exposures are conducted using in situ cages and I measure tadpole hatching success, survival, growth, development, stress levels (using leukocyte profiles), and tissue bioaccumulation of pesticides. Preliminary results indicate that 26 different pesticides were detected in the ditch waters, with atrazine, propazine, metolachlor, and atraton most frequently detected. Temperature was higher, and turbidity lower at treeless sites compared to maturely vegetated sites. Interestingly, there was higher tadpole survival, growth rates and development at the treeless site than at the mature site. This research expands the Watershed Evaluation of Beneficial Management Practices (Agriculture and Agri-Foods Canada) in the assessment of the economic and environmental benefits of naturalized waterways and reduced dredging. It will help guide policies to improve water quality and biodiversity without compromising crop yields.



The development and preliminary validation of an in vitro mutagenicity assay based on MutaMouse primary hepatocytes

Julie Cox, PhD Candidate, University of Ottawa

In order to evaluate the safety of different chemicals, toxicological assessments require the investigation of a substance's ability to damage genetic material. In vitro genetic toxicity tests using cultured cells provide a cost- and time-effective alternative to animal tests. Unfortunately, existing in vitro assays are not always reliable. This is, in part, due to their limited metabolic capacity, which is often critical to accurately assess chemical toxicity. In response to the worldwide trend to reduce animal testing, there is an acute need to develop novel, robust in vitro toxicity tests. The objective of this project is to develop an in vitro gene mutation test that improves upon existing tests and employs a well-validated mutation scoring system for convenient routine use. Primary hepatocytes (PHs) isolated from the MutaMouse are ideal for an in vitro gene mutation assay due to their metabolic competence, their proliferative capacity, their "normal" karyotype (i.e., neither transformed nor immortalized), and the presence of the MutaMouse transgene for rapid mutation scoring. A panel of thirteen mutagenic and non-mutagenic compounds was selected to investigate the performance of the MutaMouse PH in vitro gene mutation assay. The nine mutagens represent a range of classes of chemicals and include mutagens that are both direct-acting and requiring metabolic activation. All the mutagens tested, except for ICR 191, elicited significant, concentration-dependent increases in mutant frequency (MF) ranging from 2.6- to 14.4-fold over the control. None of the four non-mutagens, including two misleading, or "false", positives (i.e., tertiary butylhydroquinone and eugenol), yielded any significant increases in MF. The isolated hepatocytes behave like normal liver cells, are capable of metabolizing chemicals, and offer the reliable MutaMouse mutation scoring system. Cultured MutaMouse hepatocytes demonstrate great promise as a component of an improved in vitro gene mutation test.

Paleoecotoxicology: The application of novel tools to assess the toxicity of historically contaminated lake sediments

Cynthia Cheney, PhD Candidate, University of Ottawa

Natural resource extraction has supported the development of Canada's far north, but in some cases has also resulted in environmental contamination at a regional scale. Ore processing techniques used at two mining operations in Yellowknife are responsible for the atmospheric release of approximately 20 000 tonnes of particulate arsenic trioxide. This rapid deposition of arsenic onto the surrounding landscape is thought to have caused ecological disturbances to aquatic food webs. Here, we develop methods in paleoecotoxicology to characterize the full extent of historic metal(loid) contamination, and assess the toxicity of historic lake sediment horizons. We present a novel approach to analyse sediment archives with an ecotoxicological perspective to reconstruct missing biomonitoring data at historically contaminated sites. This study examines lake sediment cores from 21 lakes within a 30km radius of Yellowknife. Sediments were dated using radiometric dating techniques, and the toxicity of dated sediment horizons was determined using the novel application of microbial biosensors and modified *Daphnia* toxicity testing techniques. We found that metal(loid) profiles track a peak in contaminant concentration during the height of mining operations, which decreases with distance from the city. Initial assessments of bioavailability indicate that arsenic in porewater is 30-50% bioavailable. We also show a decrease in *Daphnia* survivorship from sediments deposited during the time of mining operations. These results indicate that lake sediment archives can be used to reconstruct missing biomonitoring data in sites of legacy anthropogenic influence. Additionally, these results highlight the importance of applying techniques in paleoecotoxicology in cases of legacy contamination, and suggest that aquatic ecosystems in Yellowknife continue to show lingering contamination from past gold mining activities.



3D-Cultured Avian Cells for Chemical Screening: Metabolic and Gene Expression Profiles of the LMH Cell Line

Tasnia Sharin, PhD Candidate, University of Ottawa

There has been an increased demand for in vitro toxicity testing in order to efficiently screen and prioritize the vast number of chemicals in the environment that require assessment. Primary cell culture approaches have been the most common in vitro model for toxicity testing and chemical screening in avian species. However, the preparation of primary cells is relatively time consuming and uses animals. The use of immortalized cell lines could eliminate these requirements. Immortalized cell lines may therefore represent an alternative for rapid testing and providing insight on cellular mechanisms of actions of chemicals. In this study, we investigated if the immortalized chicken hepatocellular carcinoma cell line, LMH, could be a suitable alternative to primary chicken embryonic hepatocytes (CEH) for chemical screening and prioritization. LMH cells were grown as two-dimensional (2D) monolayer (proliferating and confluent cells) and as 3D spheroids in order to further characterize the optimal exposure conditions for chemical screening. Cytochrome P450 (CYP1A4) activity and gene expression were compared between CEH and LMH grown in all three culture conditions following exposure to the dioxin-like compound 3,3',4,4',5-pentachlorobiphenyl (PCB126). CYP1A activity was measured using the EROD assay and changes in mRNA expression, associated with the aryl hydrocarbon receptor (AhR) pathway, were determined using a custom-designed PCR array. Only LMH spheroids showed EROD induction. Similarly, spheroids had the greatest number of changes in AhR-related genes compared to proliferating and confluent cells. Overall, these results suggest that LMH cells grown as 3D spheroids have a metabolic and gene expression response that is comparable to CEH, and may make a suitable animal-free alternative for in vitro screening of chemicals.



Naphthenic acids disrupt courtship behaviours in the Western clawed frog (*Silurana (Xenopus) tropicalis*)

Sue Zhang, MSc Candidate, University of Ottawa

Naphthenic acids (NAs) are carboxylic acids naturally occurring in petroleum and are among the most toxic pollutants in the Alberta oil sands tailings. This wastewater is held in tailings ponds under a zero-discharge policy, but NAs still contaminate surrounding ecosystems. Little is known about their effects on frogs, with studies focusing on tadpoles and almost no previous research with adults. In fish, NAs have shown potential endocrine disrupting effects at sublethal doses, especially as anti-androgens. Since reproduction is governed by hormones, we examined the effects of NAs on courtship behaviours in Western clawed frogs. Adults were found to tolerate exposure of up to 20 mg/L NA (a commercial mixture) without noticeable toxic effects. Individual males were exposed to control conditions or 20 mg/L NA (n=6) for 5 days, then injected with hCG to induce vocalizations that were recorded with underwater microphones. Total calling duration was lower in the NA treatment (p=0.008). The experiment was replicated (n=12) and calling was again inhibited by NAs (p=0.01). Preliminary breeding trials show that females express receptivity (or lack thereof) to males. Individual females were exposed to control conditions or 20 mg/L NA for 5 days. Individual unexposed males were allowed to freely interact with one control and one exposed female (n=11). Males preferentially entered amplexus (the mating position of frogs) with control females over exposed (9 vs 2, p=0.009) suggesting that NAs reduce female receptivity. As courtship behaviours are disrupted, sublethal NA exposure may impact reproductive fitness. We are now testing the hypothesis NAs disrupt these behaviours via reduced sex steroid production. W