Proceedings of the 2019 Canadian Freshwater Mollusc Research Meeting: December 3-4, 2019, Burlington, Ontario

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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 développement, 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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ABSTRACT

Morris, T .J., McNichols-O'Rourke, K. A., and Reid, S.M. (Editors). 2020. Proceedings of the 2019 Canadian Freshwater Mollusc Research Meeting: December 3-4, 2019, Burlington, Ontario. Can. Tech. Rep. Fish. Aquat. Sci. 3352: viii + 34 p.

The third biennial Canadian Freshwater Mollusc Research Meeting was held at the Canada Centre for Inland Waters in Burlington, Ontario on December 3-4, 2019. The meeting was jointly hosted by Fisheries and Oceans Canada and the Ontario Ministry of Natural Resources and Forestry. The workshop included a plenary, 28 platform presentations, and eight poster presentations. The meeting was attended by 92 individuals (70 in person and 22 via WebEx).

The objective of this meeting was to bring together Canadian malacologists to share past, current, and ongoing research on freshwater molluscs. Topics of discussion included distribution and life history, threats and limiting factors, sampling and management tools, and conservation genetics. With representation from Alberta to Atlantic Canada, attendees from six Canadian provinces (AB, SK, ON, QC, NB, NS) and one American state (MI) represented federal departments, provincial/state agencies, academic institutions, environmental consultants, non-governmental organizations, naturalist groups, zoos, museums, and interested citizens. There was an emphasis on building relationships to promote future collaborations and research opportunities.

RÉSUMÉ

Morris, T .J., McNichols-O'Rourke, K. A., and Reid, S.M. (Editors). 2020. Proceedings of the 2019 Canadian Freshwater Mollusc Research Meeting: December 3-4, 2019, Burlington, Ontario. Can. Tech. Rep. Fish. Aquat. Sci. 3352: viii + 34 p.

La troisième réunion bisannuelle consacrée à la recherche sur les mollusques d'eau douce du Canada a eu lieu au Centre canadien des eaux intérieures à Burlington, en Ontario, les 3 et 4 décembre 2019. La réunion a été organisée conjointement par Pêches et Océans Canada et le ministère des Richesses naturelles et des Forêts de l'Ontario, avec le soutien de la Freshwater Mollusk Conservation Society. L'atelier comprenait une séance plénière, 28 présentations de plateformes et huit présentations d'affiches. Quatre-vingt-deux personnes ont participé à la réunion (70 en personne et 22 par de WebEx).

L'objectif de cette réunion était de rassembler des malacologistes canadiens pour discuter des recherches passées et en cours sur les mollusques d'eau douce. Les sujets de discussion comprenaient la distribution et le cycle biologique, les menaces et facteurs limitants, les outils d'échantillonnage et de gestion, et la génétique de la conservation. Avec de la représentation de l'Alberta au Canada atlantique, les participants de six provinces canadiennes (Alb., Sask., Man., Ont., Qc, N.-B., N.-É) et d'un État américain (MI) représentaient des ministères fédéraux, des agences

provinciales / d'État, des établissements d’enseignement, des consultants en environnement, des organisations gouvernementales, des groupes de naturalistes, des zoos, des musées et des citoyens intéressés. L’accent a été mis sur l’établissement de relations afin de promouvoir de futures collaborations et des possibilités de recherche.

EDITORS' COMMENTS

These proceedings contain all of the abstracts that were presented at the research meeting. The abstracts were reviewed in a limited capacity and formatted by the editors. They were not sent for external review. Questions or comments relating to their content should be directed to the authors of each abstract and not to the editors. The views and statements contained in these proceedings are those of the speakers and are neither condoned nor rejected by the editors. Any use of trade names or products does not constitute endorsement or recommendation for use.

REMARQUES DES ÉDITEURS

Le présent compte rendu contient tous les résumés ayant été présentés lors de la réunion de recherche. Les résumés ont été révisés en partie et formatés par les éditeurs. Ils n'ont pas fait l'objet d'un examen externe. Les questions ou les commentaires liés à leur contenu devraient être envoyés aux auteurs de chaque résumé et non aux éditeurs. Les points de vue et les affirmations exprimés dans ces comptes rendus sont ceux des conférenciers et n'ont été ni approuvés, ni infirmés par les éditeurs. L'utilisation d'une marque de commerce ou d'un produit ne constitue nullement une forme d'approbation ou de recommandation de son utilisation.

CANADIAN FRESHWATER MOLLUSC RESEARCH MEETING ORGANIZING COMMITTEE

Dr. Todd J. Morris	Fisheries and Oceans Canada
Kelly McNichols-O'Rourke	Fisheries and Oceans Canada
Dr. Scott M. Reid	Ontario Ministry of Natural Resources and Forestry

Program Schedule
Tuesday, December 3, 2019

8:30-9:00	Registration and Poster set-up	
9:00-9:20	Introductions and welcoming address	
9:20-10:20	Plenary	**Snails are molluscs too: what have we learned during nearly a quarter-century of field research on the Endangered Banff Springs Snail, *Physella johnsoni*?** Dwayne Lepitzki
10:20-10:40	BREAK	
Session 1: Threats and Limiting Factors		
10:40-11:00	Platform 1	**But first, let me take a *shell-fie*: Standardizing embryo development for novel ecotoxicological endpoints in freshwater snails using macrophotography** R.K. Osborne and R.S. Prosser
11:00-11:20	Platform 2	**Using non-targeted metabolomics to investigate the sub-lethal effects of carbaryl and chloride on freshwater mussels (*Lampsilis siliquoidea* and *Lasmigona costata*)** Brian Atkinson, Joseph Salerno, Linda Lissemore, Dyanne Brewer, and Ryan Prosser
11:20-11:40	Platform 3	**The effects of urban inputs, including municipal wastewater treatment plant effluents on the gut microbiome of invertebrates in the Grand River** Elise N. Millar, Karen A. Kidd, Patricia L. Gillis, and Michael G. Surettee
11:40-12:10	Platform 4	**The 'Clam Project', an Indigenous community led investigation of freshwater mussels in the Oil Sands Region of Alberta** Patricia Gillis, Harvey Sykes, Debra Hopkins, Tara Joly, Almer Waniandy, John Grant, Lorrie Gallagher, Leonard Hansen, Kaitlyn Wall, and Jim Bennett
12:10-13:00	LUNCH	
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13:00-13:20	Platform 5	**Influence of contaminants of emerging concern on unionid reproduction: Streamside and controlled lab exposures** Daelyn A. Woolnough, Mandy Annis, Lacey D. Rzodkiewicz, Justin C. Rappold, and Stephanie P. Gill
13:20 – 13:40	Platform 6	**Co-existence of Unionids and *Dreissena* in eastern Ontario & along the St-Lawrence River** Fred Schueler and Aleta Karstad
13:40 – 14:00	Platform 7	**Beyond lungs: the mysterious biology of air-breathing freshwater snails**

		Ève Gilroy, Émilie Montreuil Strub, Karyn Robichaud, Dasha MacKay, Maria Villella, Kara Chan, and Simon Blais
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14:20 – 14:40	Platform 9	**Multiple stressor effects on the ecophysiology of freshwater mussels: Flow, temperature and turbidity** Kirsten Luck and Josef D. Ackerman
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15:20 – 15:40	Platform 11	**Physical modelling of the dispersion and settlement of juvenile freshwater SAR mussels – Supply side ecology** Christopher R. Farrow and Josef D. Ackerman
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9:20 – 9:40	Platform 13	**The establishment of species diversity and abundance while identifying Brook Floater presence in rivers within Nova Scotia** Alana Ransome and Marie Lachance
9:40 – 10:00	Platform 14	**Distribution of freshwater mussels downstream of a large impoundment: Results of mussel surveys in the Saint John River, New Brunswick, Canada** Hilary MacLean, Jennifer Lento, Ashley DiMarco, R. Allen Curry, and Rebecca Dolson-Edge
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11:20 – 11:40	Platform 18	**Status update for native mussels in the Detroit River** Shay S. Allred, Daelyn A. Woolnough, Todd J. Morris, and David T. Zanatta
11:40 – 12:00	Platform 19	**Everything old is new again: The rediscovery of Lake Floater (*Pyganodon lacustris*) in Canada** Todd J. Morris, Fraser Gibson, Kelly McNichols-O'Rourke, Margaret N. Sheldon, and Dave T. Zanatta
12:00-13:00	LUNCH	

Session 3: Sampling and Management Tools		
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13:20 – 13:40	Platform 21	**Effect of *Microcystis aeruginosa*-associated microcystin – LR on the survival of two life stages of a freshwater mussel (*Lampsilis siliquoidea*)** S.M. Gene, R.S. Shahmohamadloo, X. Ortiz, and R.S. Prosser
13:40 – 14:00	Platform 22	**With age comes wisdom: Assessing growth and longevity in freshwater mussels** Kelly A. McNichols-O'Rourke, Margaret N. Sheldon, Julie Vanden Byllaardt, Maggie Fang, and Todd J. Morris
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14:20 - 14:40	Platform 24	***Clam Counter:* Zooming in on the past, present and future of the freshwater mussel reporting app** MK Whibbs and C. Lee
14:40 – 15:00	BREAK	
15:00 – 15:20	Platform 25	**Validating freshwater mussel (Unionidae) markers for environmental DNA (eDNA) detection in wetlands** Charise A. Currier, Joanna Freeland, Chris C. Wilson, and Todd J. Morris
15:20 – 15:40	Platform 26	**Community eDNA metabarcoding as a detection tool for documenting freshwater mussel (Unionidae) species assemblages** Stephanie Coghlan, Charise A. Currier, Joanna Freeland, Todd J. Morris, and Chris C. Wilson
15:40 – 16:00	Platform 27	**Absence of genetic structure reflects post-glacial history and present-day host use in *Quadrula quadrula* (Mapleleaf mussel) from Manitoba, Canada** Nichelle M. VanTassel, Caitlin E. Beaver, Douglas A. Watkinson, Todd J. Morris, and David T. Zanatta
16:00 – 16:20	Platform 28	**Development of empirically driven genetic guidelines for captive propagation of imperiled freshwater mussels** Nichelle M. VanTassel and David T. Zanatta

Poster Session
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15:40 – 17:00

Poster 1	**Invasion of the South Nation River by Banded & Chinese Mystery snails** Fred Schueler and Aleta Karstad
Poster 2	**Application of morphometric analyses and DNA barcoding for distinguishing between pigtoe mussels (*Fusconaia flava* and *Pleurobema sintoxia*) in the Great Lakes region** Julia A. Willsie, Nichelle M. VanTassel, Todd J. Morris, and David T. Zanatta
Poster 3	**Protecting Endangered Hickorynut (*Obovaria olivaria*) in Canada** Arianne Savoie, France Pouliot, and Marie-Pierre Veilleux
Poster 4	**Conservation status of *Cyclonaias tuberculata* (Purple Wartyback) in Canada.** Kelly A. McNichols-O'Rourke, Margaret N. Sheldon, and Todd J. Morris
Poster 5	**Filling in the blanks: Returning to the Canard River** Margaret N. Sheldon, Kelly A. McNichols-O'Rourke, and Todd J. Morris
Poster 6	**Assessing the toxicity of Atovaquone to freshwater invertebrates** C. James Bennett, Danielle Milani, Naomi Stock, Joseph Salerno, Jennifer Unsworth, Yaryna Kudla, Sarah Cook, and Patricia L. Gillis
Poster 7	**Comparing the toxicities of road salt alternatives to *Lampsilis fasciola* glochidia** Patricia L. Gillis, Joseph Salerno, C. James Bennett, Yaryna Kudla, and Margo Smith
Poster 8	**Sensitivity of larval and juvenile freshwater mussels (Unionidae) to ammonia, chloride, copper, potassium, and selected binary chemical mixtures** Joseph Salerno, Patricia Gillis, Hufsa Khan, Erika Burton, Jim Bennett, Lorna Deeth, Paul Sibley, and Ryan Prosser

PLATFORM PRESENTATION ABSTRACTS

Plenary: Snails are molluscs too: what have we learned during nearly a quarter-century of field research on the Endangered Banff Springs Snail, *Physella johnsoni*?

Dwayne Lepitzki

Wildlife Systems Research, Banff; contractor with Parks Canada since 1995; Co-chair Molluscs SSC of COSEWIC. Email: lepitzki@telusplanet.net

In a handful of thermal springs on Sulphur Mountain in Banff National Park, Alberta lives a small, endemic snail. In 1997 Banff Springs Snail, *Physella johnsoni*, made history by becoming the first extant mollusc to be formally assessed by COSEWIC (Committee on the Status of Endangered Wildlife in Canada). In 2000, it was uplisted from Threatened to Endangered and became the first Endangered species living entirely within Banff National Park. In 2007 it again made history by being the first wildlife species in Canada to have completed all the steps required for protection and recovery under Canada's *Species At Risk Act*: an approved Recovery Strategy, an approved Action Plan, and delineated Critical Habitat. In 2018, COSEWIC confirmed the status of Endangered. Achieving these milestones has only been possible because of continuing and intensive field study. What questions were initially asked and answered? What new questions resulted? What have been the challenges? What's been learned over nearly a quarter century of field research? What will happen to the species and its habitat in the future? How does the snail fit into the story of conservation in the 21st century?

Platform 1: But first, let me take a *shell-fie*: Standardizing embryo development for novel ecotoxicological endpoints in freshwater snails using macrophotography

R.K. Osborne and R.S. Prosser

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Despite being the most diverse class of the Molluscan phylum, very little is known about Gastropods (snails and slugs), especially those that live in freshwater. Embryonic development in freshwater gastropods has yet to be characterized thoroughly and as a result, developmental milestones are not common as ecotoxicological endpoints. Throughout embryonic development, numerous complex changes occur on timescales ranging from seconds to days and improvements in technology allow us to accurately measure these changes on increasingly finer scales. As these developmental changes reveal important underlying evolutionary and ecological processes, it is especially important now to improve our understanding of them in light of the unprecedented local and global change that many populations are experiencing. Using novel macrophotography and image processing techniques, we identified and measured

embryonic growth patterns and the timing of developmental milestones in the freshwater snail *Planorbella pilsbryi*. Variations in abiotic factors, such as temperature, were tested to assess the natural variability of embryonic development within and between egg masses, as well as the influence of culturing conditions on development. There was very little variability within and between egg masses cultured under the same conditions, which allowed us to develop a standardized growth and development curve for this species. Our previous multi-generational work has shown that embryonic milestones may reveal the influence of past parental contaminant exposure, provide insight into the complex biological responses to environmental stress, and predict the health of juveniles later in life. To explore these concepts further, testing of the influence of contaminants on embryonic development and their use in multi-generation studies is ongoing.

Platform 2: Using non-targeted metabolomics to investigate the sub-lethal effects of carbaryl and chloride on freshwater mussels (*Lampsilis siliquoidea* and *Lasmigona costata*)

Brian Atkinson[1], Joseph Salerno[2], Linda Lissemore[1], Dyanne Brewer[3], and Ryan Prosser[4]

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Sub-lethal exposure to environmental contaminants can pose a hazard to populations of aquatic organisms over time, particularly threatened and endangered freshwater mussel species in Ontario. There is a need to investigate new screening tools for describing and measuring organisms' sub-lethal response in stressors. These tools need to be able to accurately assess stress, along with being cost effective, reproducible and sensitive. This study investigated the potential for non-targeted metabolomics analysis of mussel hemolymph to be used as a tool to identify stressed individuals. Analysis of hemolymph also offers the potential to develop a non-lethal sampling alternative that is simple to conduct, non-intrusive and provides reliable data.

This research evaluated the effects of 28-day exposures of carbaryl and chloride on the metabolome of the freshwater mussels *Lampsilis siliquoidea* (Fatmucket) and *Lasmigona costata* (Flutedshell) respectively. Carbaryl is a common agricultural pesticide used to control aphids that has been detected in surface waters in Ontario. Sodium chloride is commonly used as a deicing control on roads and bridges and can be released in significant pulses after thaw and rainfall events. These studies explored the differences found in the metabolome of test animals at two environmentally relevant concentrations relative to a control group. The anticipated outcome of these results will

confirm that examining hemolymph using non-targeted metabolomics holds promise as a non-lethal tool to assess stress in threatened or endangered mussel species.

Platform 3: The effects of urban inputs, including municipal wastewater treatment plant effluents on the gut microbiome of invertebrates in the Grand River

Elise N. Millar[1,2], Karen A. Kidd[1,2,3], Patricia L. Gillis[4], and Michael G. Surettee[5]

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In humans, the composition of gut microbes has been shown to affect host weight, immune function, and disease status, and is sensitive to diet, environment, and pharmaceutical use. The gut microbiome has been shown to modulate the toxicity and bioavailability of chemical stressors, however the effects of chemicals on the gut microbiome of aquatic biota are largely unknown. The Waterloo and Kitchener wastewater treatment plants (WWTPs) release effluents containing antibiotics, pharmaceuticals, and other contaminants into the Grand River (ON) that may negatively affect the gut microbiome of downstream organisms. In this study, we collected freshwater mussels (*Lasmigona costata*), several species of insect larvae, and riparian spiders (*Tetragnathidae*) from sites upstream and downstream of these WWTPs. The gut microbiome was analyzed following the extraction, polymerase chain reaction amplification, and sequencing of bacterial DNA using the V3-V4 hypervariable region of the 16S rRNA genetic barcode. Shannon alpha diversity, which measures the variance within locations while considering the abundance and evenness of bacterial species present, differed significantly among locations for mussels (one-way analysis of variance (ANOVA): $F=7.894$, $p=0.001$) and spiders (one-way ANOVA: $F=4.788$, $p=0.01$), and decreased downstream of the Kitchener WWTP for mussels (Tukey HSD: $p=0.0008$), while increasing for spiders (Tukey HSD: $p=0.007$). Bray-Curtis beta diversity was used to assess how bacterial composition varied between samples using dissimilarity measures. This metric was significantly dissimilar among locations for mussels (permutational multivariate analysis of variance (PERMANOVA): $F=4.1586$, $p=0.0001$), spiders (PERMANOVA: $F=3.2068$, $p=0.0006$), and *Heptageniidae* insects (PERMANOVA: $F=3.3274$, $p=0.0001$). These initial results indicate that the gut microbiome of downstream organisms differ from the normal bacterial composition observed upstream of the WWTPs; such differences may lead to altered health or ecosystem level effects. This information adds to our understanding of the impact of chemical stressors on the gut microbiome of aquatic and riparian macroinvertebrates.

Platform 4: The 'Clam Project', an Indigenous community led investigation of freshwater mussels in the Oil Sands Region of Alberta

Patricia Gillis[1], Harvey Sykes[2], Debra Hopkins[3], Tara Joly[4], Almer Waniandy[2], John Grant[2], Lorrie Gallagher[2], Leonard Hansen[2], Kaitlyn Wall[3], and Jim Bennett[1]

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Indigenous Knowledge holders of the Fort McMurray Métis have noticed that freshwater mussels, referred to locally as 'clams', are not nearly as abundant and widespread as they once were. Elder, and project co-lead Harvey Skyes stated 'there was an abundance of clams at one time. I remember swimming in the Clearwater River where you couldn't go swimming without stepping on them'. While there is limited Western Science information available on freshwater mussels in Alberta, Indigenous Knowledge holders have historical and current information. The Oil Sands Monitoring Program-funded 'Clam Project' is led by the Fort McMurray Métis, in partnership with Alberta Environment and Parks, Willow Springs Strategic Solutions, and Environment and Climate Change Canada. The Clam Project has three underpinning questions: Where were the freshwater clams in the past? Where are the freshwater clams now? and Why are the freshwater clams not where they used to be? Indigenous Knowledge holders and Western Scientists have been working together for the past three years to address these questions about clam health and distribution in a locally relevant and culturally appropriate way. To investigate: *Where were the freshwater clams in the past?* The project researchers conduct and document interviews and conversations with Métis Elders and traditional land users. To investigate: *Where are the freshwater clams now*? The project team returns to sites where Indigenous Knowledge holders remember mussel populations in the Lower Athabasca watershed and we work together to find new locations to learn about current mussel abundance and distribution. The opportunities for the McMurray Elders and land users to travel in search of clams has allowed for the renewal of cultural relationships. To investigate: *Why are the freshwater clams not where they used to be?* One modality of investigation includes quantifying contaminant levels in the water, sediment, and mussel tissues at each study site to reveal spatial patterns of contaminant distribution. This presentation will provide an overview of the project and introduce how we have prioritized Indigenous Knowledge using a community-based participatory research approach to braid two knowledge systems to create new learnings together.

Platform 5: Influence of contaminants of emerging concern on unionid reproduction: Streamside and controlled lab exposures

Daelyn A. Woolnough[1], Mandy Annis[2], Lacey D. Rzodkiewicz[1,3], Justin C. Rappold[1,4], and Stephanie P. Gill[1,5]

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Chemical mixtures that are found in North American waterways and may influence aquatic life, including unionids, are understudied. Many of the exposure effects are largely unknown and unregulated and many of these chemicals are termed contaminants of emerging concern (CEC). We considered CECs at environmentally relevant concentrations in both a streamside and a laboratory setting. In the streamside setting we collected water from 8 sites in an agricultural watershed (Maumee R., OH) and 6 sites in an urban watershed (Milwaukee R., WI) over 21 day exposures to unionids. In the laboratory we considered the influence to a mixture of 8 contaminants found to be common in agricultural watersheds and an 11 contaminant urban mixture over 40 day and 100 day exposures on unionids and host fish. A variety of metrics, that would potentially affect population levels of unionids, including gamete production, glochidia viability, and unionid transformation rates among these various chemical mixtures were considered. In the streamside setting we observed many non-lethal behavioral differences between agricultural and urban watersheds that are important for fitness (e.g., movement and luring). The CEC profiles in the streamside setting had a very complex set of chemical mixtures that appears to influence sperm production in unionids. In the laboratory, glochidia viability decreased faster in urban chemicals than controls or agricultural mixtures. 40 and 100 day exposures showed variable transformation of juveniles and in some treatments a potential delay in juvenile transformation was observed. The results of this study show that managers should consider the CEC profiles of waterbodies and these data will aid in the understanding and conservation of unionids.

Platform 6: Co-existence of Unionids and *Dreissena* in eastern Ontario & along the St-Lawrence River

Fred Schueler and Aleta Karstad

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From the onset of the Zebra Mussel invasion of eastern Ontario we have been looking for populations of Unionids that have adapted to coexist with the invaders. We review the evidence of persistence of *Leptodea fragilis* in the lower South Nation River and

Hoople Creek, of *Elliptio complanata* and other species in the Mississippi, abundance of *Dreissena* in the low-calcium Ottawa River, the persistence of Unionids in the Ottawa River water along the north side of the St-Lawrence, and the potential for coexistence evolving in the mouths of direct tributaries of the St-Lawrence.

Platform 7: Beyond lungs: the mysterious biology of air-breathing freshwater snails

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In order to assess the effects of contamination on the survival, growth, and reproductive output of freshwater snails, we designed cages for deployment in the field. The design of these cages was tested with the freshwater snail (Ramshorn) *Planorbella pilsbryi* (Gastropoda Heterobranchia Planorbidae) in the summer of 2019, at sites within the Hamilton Harbour watershed.
A few weeks later, these cages were deployed using a similar design for an *in situ* assessment of heavily contaminated sediments (e.g., heavy metals, PAHs, and known endocrine disruptors tributyltins) in Contrecoeur, Québec, with the Great Pond Snail *Lymnea stagnalis* (Gastropoda Heterobranchia Lymnaeidea). An adjustment to the design needed to be made: while the cages deployed in the Hamilton Harbour watershed were secured to the substrate with tent pegs, those released in Contrecoeur floated at the water surface, as *Lymnea stagnalis* is an obligate pulmonate.
To follow up on these observations, the present study assessed the survival, growth and reproductive output of the freshwater snail *Planorbella pilsbryi* in floating and sinking cages, to determine whether access to the water surface produced better outcomes. Although no significant differences in survival, growth and reproductive output were observed, future metabolomics analyses will be used to further assess potential physiological effects.

Platform 8: Addressing the potential effects of Sea Lamprey assessment on SARA-listed freshwater mussels

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The invasion of Sea Lamprey *Petromyzon marinus* throughout the Great Lakes has led to an extensive, basin-wide, bi-national control program, which employs multiple control

(e.g., dams, chemical lampricides) and assessment methods (electrofishing, chemical assessment) to reduce the productivity of Sea Lamprey. The application of granular Bayluscide (gB) is one method utilized by government agencies to evaluate the population abundance of Sea Lamprey and inform control efforts; however, there are concerns that gB applications may result in the mortality of native biota, particularly freshwater mussels. This concern is further heightened as gB applications occur in systems, such as the Thames and Sydenham rivers, that support diverse mussel populations including multiple SARA-listed species. Despite these concerns, there has been little research conducted to quantify the risk of Bayluscide applications on SARA-listed freshwater mussels. Our study has generated a framework to quantify the potential risk of gB by identifying key uncertainties and methods needed to incorporate these uncertainties into a risk assessment. By developing this framework, our work provides researchers with tools to quantify the potential risk of gB and similar toxic applications to freshwater mussels.

Platform 9: Multiple stressor effects on the ecophysiology of freshwater mussels: Flow, temperature and turbidity

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Freshwater mussel habitats are exposed to habitat degradation, sedimentation, and erosion as a result of land-use changes, which can impact mussel feeding and respiration. Of particular concern, flow, temperature and total suspended solid (TSS) concentration play key roles in mussel ecophysiology and likely interact as multiple stressors. The goal of this research is to examine how flow, temperature and TSS affect the clearance rates (*CR*; volume of water cleared of suspended particles by a mussel per unit time) and oxygen consumption (OC) of freshwater mussels. *Lampsilis siliquoidea* (n = 50; shell length = 11.00 ± 0.21 [mean ± SEM] cm) were acclimated to three holding temperatures (12.5, 20 and 27.5 ˚C) and are currently being tested at three levels of water velocity (0, 15 and 25 cm s^{-1}) and TSS concentration (0, 10 and 20 mg L^{-1}) in a split-split-plot design. In addition, treatments acclimated to 12.5 and 27.5 ˚C are being tested at 20˚C in a 2 × 3 design with five replicates, with three levels of TSS flux: 1) 0 mg m^{-2} s^{-1}; 2) 150 mg m^{-2} s^{-1}; and 3) 500 mg m^{-2} s^{-1}. Flow rate, temperature, chlorophyll α fluorescence, and turbidity are being measured in a recirculating flow chamber over the course of one hour, with each trial involving a no-mussel control and five replicates. Oxygen (O_2) concentration is being measured using the InEx method described by Yahel et al. (2005), where the O_2 concentration of the water is measured at the aperture of the excurrent siphon of the mussel as well as ambient water. The results obtained from this study will provide insight into the interactions among these stressors and their effects on mussel ecophysiology. Ultimately, this information will enhance unionid conservation efforts and the formulation of protection guidelines, regulations and recovery plans.

Platform 10: Hydrodynamic shear stress as a predictor of freshwater SAR mussel settlement

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Species At Risk freshwater mussel habitats are thought to be affected by hydrodynamics, which facilitate juvenile settlement to the streambed, and substrates, which provide physical support. The purpose of this study is to identify how hydrodynamic forces (e.g., bed shear stress) at the reach and local scale affect settlement and presence of juvenile mussels in the riverbed. We took high resolution riverbed elevation measurements to estimate reach-level shear stress via the depth slope product and identify possible locations where juvenile mussels exist. Local bed shear stress was measured using the law of the wall and excavated to find juvenile mussels via an airlift system. Throughout the 2018 and 2019 field seasons, 136 locations were excavated within the Sydenham River (Southern Ontario) near Florence, Ontario. 26 juvenile unionids were recovered at 23 locations with relatively low measured local shear stress (i.e., ≤ 0.93 Pa) and higher quantities of smaller sediment size classes (i.e., ≤ 2 mm). Logistic regression produced a predictive model for finding where juvenile mussels could be found in the stream bed. Similar analysis is underway to ascertain other habitat indicators. These results are consistent with laboratory predictions that critical shear stress causes the incipient motion of juvenile mussels. These results help identify the habitats of juvenile unionid mussels and will aid in the recovery of these imperiled organisms.

Platform 11: Physical modelling of the dispersion and settlement of juvenile freshwater SAR mussels – Supply side ecology

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Juvenile unionid Species at Risk (SAR) mussels settle out of the water column from their host vertebrate (mostly fish) into viable habitat on the riverbed. The hydrodynamic conditions that disperse juvenile mussels downstream and mediate settlement are likely important determinants of recruitment rates in their populations. Particle release experiments using physical models (alginate microbeads) of juvenile mussels are underway. These physical models are similar in size and density to juvenile mussels while being biodegradable, non-toxic, and readily detectable. The current microbeads are made from a calcium alginate shell and their density is manipulated with glycerol/water solutions. We have successfully encapsulated two dye compounds (riboflavin, blueberry extract) into the microbeads. The dyes are detectable via fluorescence and pH manipulation, respectively. Acute exposures (72 h) of the dyed and undyed microbeads provided to quagga mussels (*Dreissena bugensis*) indicated

that the microbeads are not acutely toxic and present a low risk to SAR mussels/habitat. A biodegradation study at a local river is currently underway. A field study involving the release and recapture of microbeads is underway at a site where high resolution riverbed elevation survey data have been collected. Microbead releases are followed by captures in drift nets and specially designed sedimentation traps to reveal patterns of transport and entry into the riverbed. Results from this study will present information on the downstream dispersion of juvenile mussels as well as their entry into riverbed habitat.

Platform 12: Conservation and measures and targeted research plans for Brook Floater (*Alasmidonta varicosa*) in Canada

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The Brook Floater (*Alasmidonta varicosa*) was added to Schedule 1 of the *Species at Risk Act* as Special Concern in 2013. At the 2017 Canadian Freshwater Mollusc meeting a presentation was given on the progress made towards implementing conservation measures for Brook Floater. We provide a further update towards this progress as well as new distributional knowledge and planned studies for the Brook Floater and its host fish. Since 2017, 32 sites of Brook Floater have been found through the Species at Risk Habitat Stewardship and Aboriginal Fund for Species at Risk Programs. Four different Indigenous and conservation groups performed surveys in New Brunswick (NB) and Nova Scotia (NS) watersheds, and/or collected eDNA samples. Those projects have resulted in the potential discovery of Brook Floater in one new watershed through eDNA collection and analysis, and confirmation of Brook Floater in habitat with historical records. Outreach activities have been expanded through the offering of a freshwater mussel workshop provided by an Indigenous resource and conservation organization.

While basic biological and ecological information of the Brook Floater is known, much of it is also inferred from closely related freshwater mussel species. Host fish species in parts of the Brook Floater's range in the United States have been identified and tested in laboratory experiments, but no such experiments on host fish species specific to the Brook Floater's Canadian distribution have been conducted. The only information on host fish used by Brook Floater in Canada is a single record of a Ninespine Stickleback (*Pungitius pungitius*). Species at Risk Program - DFO Gulf Region is currently supporting a Master of Science project to evaluate several fish species as potential host fish for Brook Floater glochidial attachment and survival, and identify qualitative and quantitative habitat and ecological characteristics of Brook Floater habitat.

Platform 13: The establishment of species diversity and abundance while identifying Brook Floater presence in rivers within Nova Scotia

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As part of The Confederacy of Mainland Mi'kmaq (CMM) – Mi'kmaw Conservation Group (MCG) multi-year project (2017-2020) supported by the Aboriginal Fund for Species at Risk (AFSAR), a commitment was made to collect information on the population distribution, relative abundance, morphological variation, and critical habitat of the Brook Floater (*Alasmidonta varicosa*) and other freshwater bivalves. This involved the sampling, identification, and measurement of freshwater molluscs in rivers of Nova Scotia adjacent to the Inner Bay of Fundy and Southern Gulf of St. Lawrence, specifically the Shubenacadie/Stewiacke watershed, near Millbrook and Sipekne'katik Mi'kmaw Communities, and the Philip/Wallace watershed in Mi'kma'ki, traditional territories of the Mi'kmaq. In total, 20 different sites were surveyed with 11 of these sites surveyed each year over a period of three years. Live Brook Floater specimens were found on 7 sites with 3 sites having repeated Brook Floater presence over the years. Through this multi-year project, many partnerships were established, particularly with the Department of Fisheries and Oceans Canada (DFO) and with Cape Breton University (CBU). DFO has provided MCG with training and funding, while CBU has been an important partner in data sharing and in a collaborative project involving environmental DNA analysis to assess Brook Floater presence. These partnerships enabled MCG to build enough capacity and experience to successfully participate in collaborative efforts to gather and share information on Brook Floater presence/absence location and size structure, as well as to involve Mi'kmaw communities in freshwater mollusc research, monitoring and outreach activities. This presentation highlights MCG's monitoring and research efforts on Brook Floater over multiple years, and it narrates MCG's experience on building partnerships and collaborative networks in freshwater molluscs conservation and research.

Platform 14: Distribution of freshwater mussels downstream of a large impoundment: results of mussel surveys in the Saint John River, New Brunswick, Canada

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Freshwater mussels have the potential to be used as bioindicators because they have a long lifespan, are relatively immobile, and are sensitive to pollutants and disturbance. However, little is known about the distribution of mussel species and drivers of mussel abundance and diversity. In the Saint John River in New Brunswick, Canada, freshwater mussel surveys are underway to describe the distribution and abundance of mussel

species in the river. Specifically, this work is aimed at determining the habitat conditions that support mussel diversity at increasing distances from the large Mactaquac Hydroelectric Generating Station, to establish the use of these organisms as bioindicators of changes to flow and habitat downstream of the dam. Visual surveys and excavations were completed at 26 sites downstream of the dam in 2019. Of the 11 species that have been identified in the Saint John River, 7 were observed in 2019. The Eastern Elliptio, Eastern Floater, and Eastern Lampmussel were the three most common species found in the surveys, and were present at nearly every site. Two mussel species listed as special concern under the federal *Species at Risk Act*, the Yellow Lampmussel (*Lampsilis cariosa*) and Brook Floater (*Alasmidonta varicosa*), have been observed in the Saint John River in past surveys. However, in 2019, the Yellow Lampmussel was found at Oromocto/Gagetown sites far downstream of the dam, but Brook Foater was not observed. The Triangle Floater, which is not considered to be abundant or widely distributed, was observed at five sites in 2019, including four sites in Oromocto/Gagetown. Overall, mussels were most abundant in sandy sites that allowed for burrowing, and abundance generally increased with increasing distance from the dam. The results of these surveys will be combined with previous surveys of mussel distribution and habitat to establish baseline conditions in the Saint John River in support of future biomonitoring.

Platform 15: Discovery, distribution of population, juvenile habitat, and genetic studies of the Eastern Pearlshell Mussel, *Margaritifera margaritifera*, in the Kenauk Forest, near Montebello, Quebec

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The first occurrence of the Eastern Pearlshell (*Margaritifera margaritifera*) was discovered by the senior author in the Kinonge River (Ottawa River watershed), the main river of the Kenauk Forest Institute, in September 2018. The Kenauk Forest is one of North America's largest and longest-established private fish and game reserves, encompassing 265 km^2 of wildlife reserve protected in part by the Nature Conservancy of Canada (NCC). Its pristine watershed has been kept in its natural state despite its location between two sizeable cities, Montreal and Ottawa-Gatineau. The discovery of the Eastern Pearlshell at Kenauk represents Canada's westernmost distribution for this species – this mussel is normally found further east in the Atlantic drainage. During the summer of 2019, our teams met in the study area to further document the distribution and abundance of the pearlshell in the Kinonge River, to determine whether the population was functional (ref. to juvenile recruitment and substrate quality), as well as to study the genetics of this unique population. We explored the Kinonge and its

tributaries searching for pools which exhibit conditions conducive to pearlshell recruitment: deep enough to withstand drought and summer warming, with shelter from insolation by riparian vegetation. Aside from characterizing abiotic habitat variables in these areas, substrate samples were extracted and examined for the presence of small juveniles. The aim was to determine which part of the Kinonge or West Kinonge had reproducing populations, and how these differ from areas without recruitment (Hemprich's thesis project). In doing so, we located another previously undocumented population in a unique and pristine tributary, the West Kinonge River. The latter showed the signs of a healthy habitat, a functional mussel population, with juvenile recruitment occurring and with substrate quality and temperature regime most favorable to both the mussel and a salmonid host. Also, the West Kinonge River had the highest densities of pearlshell were observed (quantitative quadrat methods), with values commonly reaching 15-40 ind/m^2. Haemolymph samples of pearlshell were also collected using a low-stress non-lethal method previously developed by Geist. Results of the genetic analyses revealed that freshwater pearlshell mussels from the Kinonge and West Kinonge formed a distinct group from all other North American populations. Genetic variability as measured by allelic richness and observed heterozygosity levels was lower compared to other North American populations which may partly enhance the genetic differentiation. Many localities in Canada where the Eastern Pearlshell thrives are in Atlantic Salmon rivers. It is unknown when, or if, Atlantic salmon were present in the Ottawa River watershed, including the Kinonge River, but they are no longer extant in the Kinonge and West Kinonge rivers. Therefore, we surmise that the Brook trout, *Salvelinus fontinalis,* may be the host fish in the Kenauk reserve, since that species is found in the West Kinonge River where the Eastern Pearlshell is most abundant and actively reproducing. This multidisciplinary study of the Eastern Pearlshell at Kenauk will continue in 2020.

Platform 16: Population ecology and preferential habitat of the SARA-listed Hickorynut in the Ottawa River: recent SCUBA surveys near Waltham, Québec

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The distribution of the Hickorynut mussel, *Obovaria olivaria*, has reduced over much of its North American historical range. In Canada, this species has recently been listed as Endangered and occurs in a small number of rivers – all inhabited by Lake Sturgeon – in the provinces of Québec and Ontario. During the summer of 2019 our team, using SCUBA diving, explored the area of the Réserve Écologique de la Chênais des Îles Finlay, an area of the lac Coulonge Reach of the Ottawa River where Martel and his team discovered a vast population of the Hickorynut in 2014. The goals of this research are to (i) determine the distribution and abundance of the population near the Finlay Islands ecological reserve, (ii) elucidate the reason(s) why the Hickorynut is abundant in

this area, thus gaining new knowledge on its preferred habitat, (iii) document via underwater photography and videos the natural habitat and sandy-bottom benthic community where this mussel seems to thrive, and (iv) make a series of recommendations to governmental agencies (federal & provincial), NGOs, as well as local communities, to ensure that this vast population remains undisturbed and preserved. During summer 2019 divers used a 1 m^2 quadrat to determine the distribution and abundance of this mussel. We were able to confirm that this population extends far off the shore, being found in high numbers in the middle of the main channel of the Ottawa River straddling the provinces of Québec and Ontario. In the study area, where the dives were conducted, the river is: 400-900 m wide, 3 to 6 m in depth, moderate current, with 1.5 to 2 m visibility. The substrate is composed entirely of granitic sand, with no rocks or macrophyte present. Large fluvial dunes are omnipresent and transversal to the river flow, with the lee side of the dunes being steep (30° to 35°) and 1.5 to 3 m high, and Hickorynuts commonly occur within the various sections of the dunes. In addition to *Obovaria olivaria*, the other species of mussels present in the study area south of Finlay Islands include the Plain Pocketbook, Eastern Elliptio, Eastern Lampmussel and Triangle Floater. Although at nearshore sites, a few tens of meters from a shoreline, the Plain Pocketbook and the Eastern Elliptio dominate in abundance, our results show that at sites located far from shore, at greater depths, among large fluvial dunes, the Hickorynut often becomes the dominant species, ranging from 0 to as many as 6 Hickorynuts within a quadrat (n = 88), with a mean of 1.26 ind/m^2. For all sites combined, half of all live mussels collected were Hickorynuts. In 2020 we plan to continue our research on the Hickorynut of the Ottawa River, including its presence within the vast sandy benthic community near Finlay Islands. The small-scale distribution of the Hickorynut within the fluvial dune habitat of the Ottawa River will also be a research focus. The Lac Coulonge reach of the Ottawa River, with no impoundment for a 142 km stretch and a healthy Lake Sturgeon population may contain one of the largest stocks of Hickoryut mussels remaining in Canada.

Platform 17: Monitoring Freshwater Mussels Populations in the Ausable River, Ontario: Tracking changes in populations at index stations over time to evaluate recovery efforts

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The Ausable River, located on the northern edge of the Carolinian Zone in southwestern Ontario, supports one of the most diverse and unique assemblages of aquatic fauna in Canada. At least 26 species of freshwater mussels have been confirmed. Six of these species (Kidneyshell, Northern Riffleshell, Snuffbox, Mapleleaf, Rainbow and Wavy-Rayed Lampmussel) have been assessed by the Committee on the Status of Endangered Wildlife in Canada as Species at Risk. In 2006, a long-term mussel monitoring program for the Ausable River was initiated with the objective to track responses of the mussel community to on-going recovery efforts aimed at reducing

threats such as sediment and nutrient inputs and river flow variability. Seven monitoring stations were surveyed with a systematic quadrat method in 2006, 2011 and 2018/2019. A total of 75 one-square-metre quadrats were excavated and searched at each site. The 2006 surveys yielded a total of 3043 mussels, representing 23 species. In 2011, a total of 2325 mussels representing 21 species were observed. The seven index stations were surveyed most recently over two summers (2018-2019) and a total of 2888 mussels were identified, representing 21 species. The results of this work inform recovery strategies and action or management plans, and begin to provide a long term approach to evaluating efforts on the landscape to reduce sediment, nutrients and flow.

Platform 18: Status update for native mussels in the Detroit River

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There is concern for freshwater mussels (Bivalvia: Unionidae) in the Great Lakes region after populations were severely reduced following the introduction of dreissenid mussels in the mid-1980s in Lake St. Clair. Several unionid refuges, or areas with relatively low dreissenid impact and surviving native mussels, have been found in coastal areas of lower Great Lakes; however, the connecting Detroit River had not been surveyed since 1998. For this study, 56 sites were surveyed in the Detroit River in the summer of 2019. Sites selected were a mixture of historical, potential refuge, and stratified randomized sites. All sites were surveyed for unionid mussels using SCUBA for 1 person-hour. Living unionids and shells were identified to species and quantified. Divers qualitatively estimated a variety of biotic and abiotic habitat characteristics at each site. Six PONAR grabs were taken at each site and used to estimate dreissenid densities and sediment particle sizes. Of the 56 sites surveyed, only five sites had living unionids totaling 220 live unionids of 11 species. 96% of the live unionids found (212/220) were from two sites immediately downstream of the River Canard. The most common live species were Mapleleaf (*Quadrula quadrula*, SC in Canada) and Fragile Papershell (*Leptodea fragilis*). Other SARA-listed species found alive were Threehorn Wartyback (*Obliquaria reflexa*) and Round Pigtoe (*Pleurobema sintoxia*). Over 2,000 unionid shells were collected (31 species) from 39 sites, confirming the large and diverse unionid assemblage present prior to the dreissenid invasion. Numerous shells of endangered Northern Riffleshell (*Epioblasma rangiana*) were found at 18 sites along the entire length river and a single valve of the endangered Clubshell (*Pleurobema clava*) was found on the U.S. side near Belle Isle. The future goal is to use the 2019 data to build a model that can be used to assist with site selection for a similar survey on the St. Clair River in summer 2020.

Platform 19: Everything old is new again: The rediscovery of Lake Floater (*Pyganodon lacustris*) in Canada

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In July 2018, several individuals of the genus *Pyganodon* were collected by an amateur malacologist in Little Sheguiandah Lake within the boundary of Killarney Provincial Park in the Lake Huron drainage of south-central Ontario, Canada. Photos of the specimen were sent to Fisheries and Oceans Canada in Burlington, Ontario for identification and it was confirmed that the specimens were not *Pyganodon grandis*, the only *Pyganodon* known to occur in the area. The images were shared with regional experts and it was determined that the shells most closely resembled *Pyganodon lacustris*, a species not presently considered to occur in Canada but known to occur nearby in Michigan and other Great Lakes States. In September 2018, Fisheries and Oceans Canada visited the site of the original collection in Little Sheguiandah Lake and two nearby sites in George Lake. Surveys were conducted at the three sites by snorkelling the nearshore habitat less than 2 m deep for either 7.5 person-hours (Little Sheguiandah Lake) or 2.5 person-hours (George Lake). At the site of the original collection in Little Sheguiandah Lake, 42 *Elliptio complanata* were collected along with 24 specimens of the unknown *Pyganodon*. No live animals of any species were collected within George Lake. *Pyganodon* specimens were measured in the field, photographed (right valve, left valve and dorsal view) and preserved in 95% ethanol after relaxation. Genetic identifications were determined by comparing CO1 mtDNA sequences from the unknown specimens with sequences available on GenBank using the Basic Local Assignment Search Tool (BLAST). Genetic identifications were successfully made for 18 individuals with 17 identified as *P. lacustris* and one as *P. grandis*. Using Canonical Variates Analysis (CVA) with genetically confirmed specimens of *P. grandis*, *P. cataracta*, *P. fragilis* and *P. lacustris*, a morphometric model was developed using the Integrated Morphometric Package (IMP) v. 8 by digitizing 22 landmarks on the left valve of each specimen. Shell morphology was an effective tool for differentiating between species in areas where *P. lacustris* is only expected to overlap with *P. grandis*. Shell morphology will have limited usefulness in areas where the species may overlap with other *Pyganodon*.

Platform 20: Lower Grand River freshwater mussels: Results from brail sampling of non-wadeable habitats

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To date, mussel surveys of southern Ontario rivers have largely been limited to habitats suitable for wading or snorkelling (i.e., less than 1 m deep). For these habitats, sampling protocols have been developed to survey and monitor mussel species at risk populations. However, methods are required for deeper habitats so that critical habitat descriptions (extent and functional attributes) can be refined, and the status of mussel populations associated with these habitats can be monitored. In this study, transect-based surveys using a mussel brail were undertaken at 51 non-wadeable (mean water depth = 3.6 m) sites along the lower Grand River (Brantford to Port Maitland) during the summer and fall of 2019. Each site was sampled with five tows (50m long) of the brail. In total, 777 live individuals were collected by the brail, representing 15 species. Live individuals of three mussel species at risk were collected from non-wadeable river habitats: Mapleleaf (87 individuals), Round Pigtoe (45 individuals) and Threehorn Wartyback (7 individuals). The most abundant and widespread species were Mapleleaf, Mucket, and Threeridge. Compared to the fall, significantly more individuals (12.6 vs 2.9 individuals per site) and species (3.0 vs 1.5 species per site) were collected during summer sampling. In addition to seasonal differences, longitudinal variation in mussel abundance and species richness were revealed by brail sampling. Mussel abundance and species richness were both higher upstream of Caledonia than downstream of Cayuga. Results from this study indicate that brail sampling can complement existing survey and monitoring methods used in southern Ontario rivers.

Platform 21: Effect of *Microcystis aeruginosa*-associated microcystin –LR on the survival of two life stages of a freshwater mussel (*Lampsilis siliquoidea*)

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Microcystin-LR is a toxin commonly produced by the cyanobacterium *Microcystis aeruginosa.* It is present in harmful algal blooms and is a concern for both human and environmental health in Canadian freshwater systems. Previous studies have investigated the toxicity of microcystin-LR to other organisms such as fish, however it is important to assess its toxicity to native freshwater mussels (family Unionidae), which are considered imperiled. This study examined the toxicity of microcystin-LR to Fatmucket mussels (*Lampsilis siliquoidea*) at two different life stages. Juvenile mussels were exposed to microcystin-LR in a 28-day chronic test, while glochidia underwent a 72-hour acute toxicity test. There was no significant relationship between glochidia viability and microcystin-LR concentration. The median lethal concentration (LC_{50}) value for juvenile mussels after 28 days of exposure was 2.1 µg/L. To determine the environmental relevance of the observed toxicity, an environmental exposure

distribution was created using Canadian and Canadian-U.S. Great Lakes microcystin measurements. The 28-day LC_{50} value (2.1 µg/L) was greater than those values that occurred in the environment 95% of the time, however, the LC_{10} (0.45 µg/L) and LC_{25} (0.97 µg/L) values were not greater than the measured microcystin environmental values. This indicates that microcystins may exert toxic effects on juvenile mussels at environmentally relevant concentrations. Further investigation should be considered in terms of prolonged exposure to persistent microcystin-LR, and toxicity to sensitive species at different life stages.

Platform 22: With age comes wisdom: Assessing growth and longevity in freshwater mussels

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Freshwater mussels of the unionid family are one of the planet's most imperilled groups, and they provide a number of ecosystem services within their aquatic ecosystems. Despite an increased awareness of their importance, much of the baseline information regarding their growth and longevity remains unknown. Freshwater mussels are usually viewed as organisms that are long-lived and slow growing, but determining just how long (or short) their lives really are takes a lot of time and effort. In an effort to understand growth and longevity of species at risk, Fisheries and Oceans Canada (DFO) has been ageing mussels using shell sections and internal annuli since 2010. After collecting a sample representative of the size range of the population, a single valve of each specimen is sectioned (< 0.5 mm) using a Buehler Isomet linear precision saw. Annuli are then counted with the aid of a microscope, resulting in an estimate of age for each individual. To date, 13 species, including 7 Species at Risk (SAR), have been aged. Preliminary maximum ages for mussels have ranged from up to 13 (*Toxolasma parvum*) and 28 (*Lampsilis fasciola*) years old on the shorter end of the lifespan and 45 (*Quadrula quadrula*) to 65 (*Cyclonaias tuberculata*) on the longer end. Although thin-sectioning can produce reliable age estimates in many cases, problems can arise when false annuli or disturbance lines are present, when ageing very large or very old specimens, or when there is a lot of noise within the section making it difficult to observe the true annuli. Modified ageing methods including changing the location or angle of the cut are being investigated to determine if a cleaner image of the internal annuli and therefore a more accurate age estimate can be obtained with these modified approaches. Understanding the growth and longevity of these organisms is vital to understanding their life history and these age estimates will give us the wisdom to accurately assess and effectively manage these species.

Platform 23: Development of alternative methods to detect *Simpsonaias ambigua*

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Simpsonaias ambigua, the Salamander Mussel, was designated as Endangered in Canada in 2001 and the status was re-examined and confirmed in 2011. The Salamander Mussel was historically known from several locations from the province of Ontario; currently it is only known to occur in the East Sydenham River. Traditional surveys (e.g., timed searches) to find extant populations of *S. ambigua* at large scales (e.g., within a river) can be time consuming and expensive since this species is very hard to find. There is a critical need for less costly and more effective methods to find locations where *S. ambigua* is present at larger scales. The goals of this project are 1) to develop two additional methods to identify sites (e.g., reach) where live *S. ambigua* are present and 2) compare their effectiveness of each method in detecting *S. ambigua* individuals in contrast with traditional survey methods. The first method consists of finding *S. ambigua*'s host; Mudpuppy (*Necturus maculosus*), and inspecting for signs of encysted glochidia. The second method is based on detection of *S. ambigua* environmental DNA (eDNA). A total 51 live *S. ambigua* individuals were found at eight of the nineteen sites that were surveyed during 2018-2019 using timed searches at the Sydenham River, ON. No *N. maculosus* was found during the months of July and August (2018) at any of the surveyed locations; however, *N. maculosus* was trapped in November (2018) and March (2019). *Simpsonaias ambigua* species-specific primer-probes were designed from mitochondrial DNA sequences and were tested *in silico* and *in vitro* to validate specificity. Detection of live *S. ambigua* from populations in the wild using eDNA still needs to be conducted, but these two alternative methods seem promising at finding locations where *S. ambigua* is present.

Platform 24: *Clam Counter:* Zooming in on the past, present and future of the freshwater mussel reporting app

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In 2012 Toronto Zoo initiated a 5-year field study of freshwater mussel populations in the inland watersheds of Lake Ontario and concurrently, a public campaign entitled, 'I am Important! I am Protected' with the goal of promoting awareness and conservation

of native freshwater mussel species. Stemming from the Zoo's field research and public campaign, the *Clam Counter* app for freshwater mussel identification and reporting was developed in partnership with Fisheries and Oceans Canada and officially launched in 2017. Now three field seasons in, we want to hear from you as we continue to develop the app to make *Clam Counter* the go-to resource for freshwater mussel identification in Canada. This session will tour the app, its main features, progress to date and ideas to future development. Download *Clam Counter* in advance on your Apple or Android device, in English or French, and come prepared with questions and feedback!

Platform 25: Validating freshwater mussel (Unionidae) markers for environmental DNA (eDNA) detection in wetlands

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The detection of environmental DNA (eDNA) to infer target species presence provides the opportunity to detect aquatic species with potentially increased efficiency and sensitivity compared to conventional methods. Rapid uptake of eDNA methods, however, has resulted in many publicly available markers that have only been field-tested in a narrow range of environmental conditions; it is unknown how the effectiveness of these tools may be impacted by their use in alternate habitats or conditions. In this study, eDNA markers that were previously developed to detect freshwater mussel species-at-risk (SAR) from lotic habitats were investigated as potential tools for detecting mussels from wetland and nearshore habitats where their densities were near the limit of detection for conventional sampling. The three objectives of the study were: 1. to assess the effectiveness of eDNA sampling for species detection in wetland habitats; 2. to expand our understanding of eDNA marker sensitivity (i.e., limits of detection); 3. to clarify species distributions for two mussel species at risk in southwestern Ontario wetlands. Environmental DNA detections of one of the species, *Quadrula quadrula*, correlated well with predictions based on conventional surveys. *Ligumia nasuta*, by contrast, was not detected at any sites with suspected populations, including one site where live mussels were found in 2015. A series of supplemental diagnostic analyses (i.e., BSA x dilution experiment, qPCR reaction optimization, DNA spike-in experiment) collectively suggested that the lack of *L. nasuta* eDNA detection is attributable to the absence of target DNA. While the *Q. quadrula* analyses demonstrated that eDNA sampling can be an effective and sensitive tool for species detection in wetlands, the unexpected results for *L. nasuta* emphasize the need for species-by-species assessment of eDNA detection tools including their utility across diverse environments.

Platform 26: Community eDNA metabarcoding as a detection tool for documenting freshwater mussel (Unionidae) species assemblages

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Documenting species occurrences and habitat occupancy of unionid mussels can often be challenging. Environmental DNA (eDNA) has been shown to be a reliable tool for detecting unionids with comparable or greater sensitivity than conventional sampling, and has the added advantages of not disturbing individuals or occupied habitats. Despite this, single-species eDNA assays are limited to targeting individual species of interest and are functionally blind to the presence of other species. Community eDNA assays have the potential to characterize local species assemblages simultaneously, but are currently more expensive and at a lesser stage of development and implementation than single-species eDNA testing. We tested the effectiveness of community eDNA markers to identify unionid species assemblages at DFO reference sites, using conserved primers that target the mitochondrial 16S rDNA region. In contrast to species-specific eDNA markers, the primers were intended to amplify the mitochondrial 16S rDNA region from any mussel species that were present and identify species by next-generation parallel sequencing. Optimization of two overlapping primer pairs tested their utility for simultaneous amplification of DNA from multiple species using mock communities of mixed DNA from native Ontario species: both primer sets successfully amplified the majority of species with largely consistent results between the primer sets and across replicates, although not all species were detected. Following optimization, eDNA from water samples from DFO reference monitoring sites was amplified and sequenced to quantify species richness and diversity within and among sites. The sequencing data from the monitoring sites mirrored those from the mock communities, with good consistency for species detections among replicates within sample sites. The results were broadly consistent with species data from quadrat-based manual field surveys, although both community eDNA and traditional sampling detected some species that the other method did not. These results demonstrate that community eDNA assays using conserved primers and next-generation sequencing have the potential to simultaneously target eDNA from multiple unionid species, and provide a powerful tool for complementing or augmenting traditional field surveys to characterize and monitor unionid species assemblages.

Platform 27: Absence of genetic structure reflects post-glacial history and present-day host use in *Quadrula quadrula* (Mapleleaf mussel) from Manitoba, Canada

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Our study documents the genetic diversity and analyzes the genetic structure of the freshwater mussel (Bivalvia: Unionidae) *Quadrula quadrula*, Mapleleaf, in the Lake Winnipeg, Assiniboine River, and Red River drainages (Manitoba, Canada). Previous studies have revealed patterns of genetic diversity and structure in the Mississippi and Ohio river drainages, and the Laurentian Great Lakes drainage. Genotypes from six variable microsatellite loci show that the *Q. quadrula* population in Manitoba is significantly differentiated from the population in the Great Lakes drainage (Ontario), supporting the existence of two Designatable Units in Canada. Conversely, there is no evidence of genetic structure within the sampled range of the Manitoba population. The lack of genetic structure in *Q. quadrula* across its distribution in Manitoba is reflective of their post-glacial history and use of a vagile host and necessitates that efforts should be made to ensure connectivity and maintain gene flow across the region. Because *Q. quadrula* in Manitoba belong to a single genetic population, should the need arise, movement of hatchery-propagated juvenile *Q. quadrula,* adult *Q. quadrula*, or glochidia-carrying host catfish sourced from any river in Manitoba could be used to augment declining or at-risk locations.

Platform 28: Development of empirically driven genetic guidelines for captive propagation of imperiled freshwater mussels

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Our knowledge of genetic diversity and structure of numerous wild stock unionid species has advanced extensively in the past ten years, but there has not been the same advancement in our understanding of captive bred unionids in comparison to wild populations. This study provides empirical data critical for effective, responsible propagation efforts. Null hypothesis: wild and captive bred mussel populations have equivalent genetic diversity. Mantle tissue biopsies or foot swabs were collected from

adult mussels from each species from the Grand and Sydenham rivers in Ontario, Canada. Captively propagated juveniles of each species grown and harvested at the White Lake Fish Culture Station in Ontario. Genotypes of 8 microsatellite loci for *Lampsilis fasciola* (Wavy-rayed Lampmussel, SC in Canada) and 9 microsatellite loci for *Ptychobranchus fasciolaris* (Kidneyshell, EN in Canada) were obtained. No significant differences were detected between wild and propagated *L. fasciola* based on genetic diversity metrics (allelic richness, rarefacted allelic richness, observed and expected heterozygosity, and inbreeding coefficient). Pairwise F_{ST} values among wild caught specimens, hatchery raised juveniles, and across time (2008 to 2018) were significant ($P<0.0001$), but low (0.014-0.026). Pairwise D_{est} values were significant and somewhat higher (0.027-0.140) suggesting some fixation of alleles and limited differentiation. STRUCTURE analysis corroborated these findings with strong support for a single genetic population (K=1). Based on the data from *L. fasciola*, there is no evidence of reduced genetic diversity between wild and propagated individuals. We hypothesize that with at least 10 females contributing to brood stock, the contribution of multiple males to each glochidial brood help maintain the genetic diversity reflecting the natal population. Research is ongoing with *P. fasciolaris* genotypes, and a new hatchery study on *L. fasciola* is underway with genotypes from source females and propagated juveniles grown out separately to assess genetic variation within broods from single females.

POSTER PRESENTATION ABSTRACTS

Poster 1: Invasion of the South Nation River by Banded & Chinese Mystery Snails

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From the onset of our monitoring of the molluscan fauna of the South Nation River in 1995, until 2010 the Viviparidae were represented only by the native *Campeloma decisum* (Brown M.S.). In 2010 South Nation Conservation staff found *Cipangopaludina chinensis* (Chinese M.S.) in Henderson Creek in Winchester and two summers of 'Mystery Snail Snagaroos' removed thousands of snails from the creek. In 2012 we found *Viviparus 'georgianus'* (Banded M.S.) in the Castor River at Russell, and then in 2016 a few in the main river downstream of there at High Falls. Amie Ivany found another population of *C. chinensis* in Hess Creek in 2017, where the shells were mostly broken as if predated. Our monitoring has been sporadic, but we're encouraging closer attention, especially to the signs of predation by Mammals.

Poster 2: Application of morphometric analyses and DNA barcoding for distinguishing between pigtoe mussels (*Fusconaia flava* and *Pleurobema sintoxia*) in the Great Lakes region

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Wabash Pigtoe, *Fusconaia flava*, and the related species Round Pigtoe, *Pleurobema sintoxia*, are freshwater mussels native to the Great Lakes region. Wabash Pigtoe is considered widespread and relatively common across its distribution while Round Pigtoe is considered an imperiled species in parts of its distribution (Endangered in Canada). These species are similar in both shell shape and coloration and have confounded many freshwater malacologists, likely resulting in misidentifications and potentially impacting accuracy of status assessments and recovery efforts. We used geometric morphometric analysis and DNA barcoding to distinguish between these species. In the field, a genetic sample was collected from 133 of 246 specimens, a preliminary identification was made, shell measurements were recorded, foot colour (orange or white) was documented, and photos of the left shell valve were taken. The genetic samples had DNA extracted and COI mtDNA sequenced. The COI sequence was used for species identification by comparing to sequences available on GenBank. Landmarks (21) outlining the shell margin were analyzed using canonical variates analysis and compared to the results of the DNA sequence data. The two species were

significantly different in shape. Analysis of the landmark data correctly assigned 99.2% of specimens to their DNA-confirmed species identity. Foot colour was only 77.4% accurate in identifying species, with both colours being found in both species. Compared to our field identifications and a quiz administered to trained malacologists and an untrained group of undergraduate biology students, these species were frequently confused (77% accuracy in identifications). An identification quiz showed that training to identify unionid mussels significantly improved accuracy in differentiating between the species (trained: 83% vs. untrained: 59% correct), but additional years of experience with unionid mussels did not show any significant improvement in differentiating between the species (1-5 years experience: 82% vs. >5 years: 84% correct). Using geometric morphometrics calibrated with DNA barcoding appears have potential in improving accuracy in identifying these species in the Great Lakes region.

Poster 3: Protecting Endangered Hickorynut (*Obovaria olivaria*) in Canada

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Hickorynut is a freshwater bivalve mollusc of the Unionidae family. Recently, this species has been added to the list of wildlife species at risk (Schedule 1) under the federal *Species at Risk Act*. Although under-sampled, all known Hickorynut populations in Canada appear to have declined, but it is unclear if this is due to degraded water quality from industrial and agricultural pollution, infestation by exotic Zebra and Quagga mussels or reduced abundance of the Hickorynut's likely host, the Lake Sturgeon. DFO has the legislated responsibility and mandate for the protection and recovery of all aquatic species at risk in Canada and the Species at Risk Division is committed to support any initiative relative to the protection of Hickorynut.

Research/Recovery: our priorities in the following 5-10 years will be to 1) understand Hickorynut demographics with an emphasis on range and abundance, 2) characterize habitat requirements, 3) refine the understanding of threats to Hickorynut survival and recovery, 4) confirm host fish species and 5) increase public awareness and engagement in Hickorynut recovery and protection actions. DFO will implement the recovery of Hickorynut using a collaborative approach involving all interested stakeholders.

Poster 4: Conservation status of *Cyclonaias tuberculata* (Purple Wartyback) in Canada

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Cyclonaias tuberculata is a candidate species for assessment by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). Currently the species is limited to three subpopulations in Canada, the Ausable, Sydenham and Thames rivers in southwestern Ontario – the northern limit of their range. The distribution of this species has decreased by approximately 60% since 1997, as it was historically also found in Lake Erie and the Detroit River. Very little is known about this species in Canada, and studies were initiated at Fisheries and Oceans Canada (DFO) in 2018 to provide insight into the age and reproductive timing windows of the species. Although the species is dioecious, the shell does not exhibit sexual dimorphism, therefore, gonad fluid was taken from a minimum of five individuals at nine different sampling events from early June until mid-October. Sperm were observed at all but one sampling event (Aug. 8, 2018), however the highest amounts were in October. Eggs were observed at five of the sampling events with the highest number in October. Host fish(es) for *C. tuberculata* have not been confirmed in Canada, however the assumption is that they are similar to those confirmed in the USA – Channel Catfish, Black and Yellow bullheads. These fishes are wide spread throughout southwestern Ontario and do not appear to be limiting the mussel population. Fifty-three shells were collected for ageing purposes ranging from 12.58 mm to 138.04 mm and preliminary data suggest an age of up to 46 years. The main threats affecting *C*. *tuberculata* include pollution from agriculture and urban runoff and climate change. These are chronic threats that most likely interact with each other, however specific studies that address either the short or long-term effects on *C*. *tuberculata* have not been completed.

Poster 5: Filling in the blanks: Returning to the Canard River

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While extensive surveys for freshwater mussels have been occurring in Ontario since the 1990s, there are many waterbodies that remain unexplored, under-surveyed, or which have only been surveyed historically. The Canard River, located in Essex County near Windsor, Ontario, is an example of such a waterbody in which three sites on the main branch were formally surveyed in 1993. These surveys found 15 individuals of five common species. Despite the lack of significant findings during these surveys, the attention of Fisheries and Oceans Canada (DFO) was drawn back to the Canard River in 2019 as it is the only significant Canadian tributary of the Detroit River. Once home to a diverse unionid community including many Species at Risk (SAR), the Detroit River marks one of the initial points of introduction of the invasive dreissenid mussels (*Dreissena bugensis*, Quagga Mussel; *D. polymorpha*, Zebra Mussel). By 1998, unionids were considered extirpated from the Detroit River due to the deleterious effects of dreissenids. However, as other medium to large sized rivers have been shown to act as refuges for native mussels from the impacts of dreissenids, DFO completed surveys in the Canard River to investigate the status of native mussel populations that could

contribute to the potential reestablishment of unionids in the Detroit River. Surveys were completed at a total of nine sites on both the south and main branch of the Canard River over three days. Two of these sites were selected from the three sites previously surveyed. At each site, a 4.5 person-hour timed-search survey using mussel scoops and tactile searching was completed. A total of 362 live unionids were found across eight sites including 119 individuals of two SAR, Mapleleaf and Lilliput. This represents the first record of SAR in the Canard River and a new location for both species. The results of the 2019 Canard River surveys provide current data on the waterbody, contribute information used to delineate new critical habitat for the SAR, and also provide insight into the influence of the Canard River on the potential recovery of unionids in the Detroit River.

Poster 6: Assessing the toxicity of Atovaquone to freshwater invertebrates

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Atovaquone is a pharmaceutical used as both an anti-malarial and anti-protozoal drug and is found in anti-HIV cocktails. Little is know about the environmental fate of Atovaquone in waterbodies receiving municipal wastewater effluents. This work was part of a Health Canada-funded study to investigate the effect of Atovaquone on two freshwater aquatic invertebrates, *Chironomus riparius* (midge larvae), and *Eurynia dilatata* (freshwater mussel). Because no analytical methods were available to quantify Atovaquone in environmental samples, methods were developed to quantify Atovaquone in water, mussel tissues and in sediment. *C. riparius* was exposed to sediment-associated Atovaquone for 10 days using a field-collected reference sediment spiked with Atovaquone as per standard methods. Two chironomid exposures were conducted (range finding and definitive). The definitive test included six (nominal) concentrations (from 0 to 2000 mg/kg) and a solvent control (SC). There were no significant differences in chironomid survival across treatments; however multiple comparison tests indicated that growth was significantly reduced at 2000 mg/kg compared to controls. Adult *E. dilatata* were exposed to aqueous solutions of Atovaquone for 14 d with exposure solution renewal every 24 hours. Because Atovaquone was largely insoluble in water the effectiveness of various solvents were explored. The first mussel range finding toxicity test included six treatments (SC and 0 to 20000 µg/L Atovaquone). Atovaquone was toxic to *E. dilatata* at concentrations of 1000 µg/L (nominal). Additional range finding exposures with mussels are underway as is analysis of Atovaquone in surface water and municipal wastewater effluents. Lab-derived toxicity metrics will be compared to environmental levels of Atovaquone to determine if this pharmaceutical poses a risk to aquatic ecosystems.

Poster 7: Comparing the toxicities of road salt alternatives to *Lampsilis fasciola* glochidia

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In some temperate regions the increasing salinity of freshwater has been shown to be related to the use of road salt for winter road maintenance. Negative effects of elevated salinity on freshwater ecosystems, including salt-sensitive biota have been reported. Road salt alternatives such as brine and beetroot juice are used in some municipalities in an effort to reduce the amount of salt entering aquatic ecosystems. Beetroot products have a low freezing temperature which makes them effective as de-icers at low temperatures. Compared to traditional road salt and brine applications, the higher viscosity of a beetroot solution can translate into fewer applications even during peak seasonal events. The early life stages of freshwater mussels have a heightened sensitivity to salt (NaCl) and laboratory studies have shown that they are negatively impacted by the high salt load in winter road runoff. To determine the relative toxicity of road salt alternatives to freshwater mussels, the toxicities of salt brine, beetroot juice, and a brine-beetroot juice solution were assessed. *Lampsilis fasciola* glochidia were exposed to dilutions (0-2%) of three commercial products. Based on the percent of liquid road salt alternative product in the exposure, the concentrations that resulted in a loss viability (mortality surrogate) in 50 % of exposed glochidia were; salt brine, 0.42 %; beetroot juice, 0.03 %; and brine-beetroot juice solution, 0.06 %. Overall, exposures to beetroot products were more toxic than brine on a per volume basis. The toxicity of beetroot juice-containing solutions does not appear to be solely related to the chloride concentration of the exposure, or to general water quality parameters (e.g. ammonia, dissolved oxygen). Additional research is needed to identify the mechanism of toxicity in glochidia exposed to beetroot juice products. As per standard methods, toxicity tests with glochidia were conducted at 20^{o}C. Exposures will also be conducted at a lower temperature to determine the relative toxicities of these products at a temperature more typical of a winter exposure.

Poster 8: Sensitivity of larval and juvenile freshwater mussels (Unionidae) to ammonia, chloride, copper, potassium, and selected binary chemical mixtures

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In aquatic environments, organisms such as freshwater mussels are likely exposed to complex contaminant mixtures. This investigation focused on the effects of various waterborne contaminants (ammonia, chloride, copper, and potassium) and selected binary mixtures of these chemicals following a fixed-ratio design using *Villosa iris* glochidia and juvenile *Lampsilis fasciola*. In individual exposures, the chemical concentration resulting in 50% viability after 48 hours (EC_{50}) was determined for *V. iris* glochidia exposed to ammonia chloride (7.4 [95% confidence interval (CI) 6.6-8.2] mg N/L), ammonia sulfate (8.4 [7.6-9.1] mg N/L), copper sulfate (14.2 [12.9-15.4] µg $Cu2^{+}$/L), potassium chloride (12.8 [11.9-13.7] mg K^{+}/L), potassium sulfate (10.1 [8.9-11.2] mg K^{+}/L), and sodium chloride (480.5 [435.5-525.5] mg Cl^{-}/L). The chemical concentration resulting in 50% survival (LC_{50}) after 7 days for juvenile *L. fasciola* was determined using potassium sulfate (45.0 [18.8-71.2] mg K^{+}/L), and sodium chloride (1738.2 [1418.6-2057.8] mg Cl^{-}/L). In Ontario, these waterborne contaminants have been reported to co-occur, with concentrations exceeding the EC_{10} for both life stages at some locations. Data from binary mixture exposures for *V. iris* glochidia (chloride-ammonia, chloride-copper, and copper-ammonia) and juvenile *L. fasciola* (chloride-potassium) were analyzed using a regression-based, dose-response mixture analysis modeling framework. Results from the mixture analysis were used to determine if an additive model for mixture toxicity [concentration addition (CA) or independent action (IA)] best described the toxicity of each mixture and if deviation towards dose-ratio (DR) or dose-level (DL) synergism/antagonism (S/A) occurred. For all glochidia binary mixture exposures, CA was the best fit model with DL deviation reported for the chloride-copper mixture and DR deviation reported for the copper-ammonia mixture. Using the model deviation ratio (MDR), the observed toxicity in all three glochidia mixture exposures were adequately described by both CA (mean = 0.71) and IA (mean = 0.97) whereas the juvenile mixture exposure was only adequately described by CA (mean = 0.64; IA mean = 0.05).

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