Proceedings of the 2016 Canadian Freshwater Mussel Research Meeting: March 30, 2016, Burlington, Ontario

Editors: T. J. Morris, K. A. McNichols-O'Rourke, and S. M. Reid

Fisheries and Oceans Canada
Great Lakes Laboratory for Fisheries and Aquatic Sciences
867 Lakeshore Rd., P.O. Box 5050
Burlington, ON L7R 4A6 CANADA

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Canadian Technical Report of Fisheries and Aquatic Sciences

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Les numéros 1 à 456 de cette série ont été publiés à titre de Rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de Rapports techniques de la Direction générale de la recherche et du 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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By

Editors:
T.J. Morris[1], K.A. McNichols-O'Rourke[1] and S.M. Reid[2]

[1]Fisheries and Oceans Canada
Great Lakes Laboratory for Fisheries and Aquatic Sciences
867 Lakeshore Rd., P.O. Box 5050
Burlington, ON L7R 4A6 CANADA

[2]Ontario Ministry of Natural Resources and Forestry
1600 West Bank Dr.
Peterborough, ON K9L 0G2 CANADA

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PREFACE

The first Canadian Freshwater Mussel Research Meeting was held at the Canada Centre for Inland Waters in Burlington, Ontario on March 30, 2016. The meeting was jointly hosted by Fisheries and Oceans Canada and the Ontario Ministry of Natural Resources and Forestry with financial support from the University of Guelph and the Freshwater Mollusk Conservation Society. The workshop included 23 platform presentations and three poster presentations. The meeting was attended by 90 individuals (65 in person and 25 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 mussel distribution and conservation, threat evaluation, conservation genetics, sampling issues, recovery efforts, and stewardship activities. With representation from the Pacific to the Atlantic, attendees came from six Canadian provinces (BC, MB, ON, QC, NB, NS) and two American states (MI, NY) and represented federal departments, provincial/state agencies, academic institutions, environmental non-governmental organizations, naturalist groups, zoos, museums, and interested citizens. There was an emphasis on building relationships so that collaborations can be used for future research opportunities.

PRÉFACE

La première réunion consacrée à la recherche sur les moules d'eau douce du Canada a eu lieu au Centre canadien des eaux intérieures à Burlington, en Ontario, le 30 mars 2016. 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 financier de l'Université de Guelph et de la Freshwater Mollusk Conservation Society. L'atelier comprenait 23 présentations de plateformes et trois présentations d'affiches. Pas moins de 90 personnes ont participé à la réunion (65 en personne et 25 par l'entremise 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. Parmi les sujets abordés, mentionnons l'aire de répartition et la conservation des moules, l'évaluation des menaces, la génétique de conservation, les problèmes d'échantillonnage, les efforts de rétablissement et les activités d'intendance. Représentant des ministères fédéraux, des organismes provinciaux et d'État, des établissements d'enseignement, des organisations non gouvernementales de l'environnement, des groupes de naturalistes, des zoos, des musées et des citoyens intéressés du Pacifique à l'Atlantique, les participants étaient originaires de six provinces canadiennes (C.-B., Man., Ont., QC, N.-B., N.-É.) et de deux États américains (MI, NY). L'accent a été mis sur l'établissement de relations afin de pouvoir utiliser les collaborations pour de futures 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 should be directed to the authors of each abstract and not 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 examinés de façon limitée et formatés par les éditeurs. Ils n'ont pas été envoyés pour examen externe. Les questions ou les commentaires 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; les éditeurs ne se sont pas prononcés sur leur acceptation ou leur rejet. L'utilisation d'une marque de commerce ou d'un produit ne constitue nullement une forme d'approbation ou de recommandation de son usage.

CANADIAN FRESHWATER MUSSEL 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

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Platform 2 9:30 - 9:45	**André L. Martel** and Jacqueline B. Madill	**Canadian Freshwater Pearly Mussels (Unionacea) and Species Discovery at the Canadian Museum of Nature**
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PLATFORM ABSTRACTS

Platform 1 - Search effort and imperfect detection: Influence on timed-search mussel (Bivalvia: Unionidae) surveys in Canadian rivers.

Scott M. Reid

Aquatic Research and Monitoring Section, Ontario Ministry of Natural Resources and Forestry, Peterborough, ON. CAN. Email: reid.scott@ontario.ca

Inventories and population monitoring are essential activities supporting the conservation of freshwater mussel diversity in Canadian rivers. Despite widespread use of timed-search methods to survey river mussels, the relationship between species detection and search effort has received limited study. In this study, repeat-sampling data from 54 Ontario river sites were used to estimate: 1) species detection probabilities; 2) the number of sampling events required to confidently detect species; and, 3) the power of timed-search surveys to detect future distribution declines. Mussels were collected using visual and tactile methods, and collection data were recorded separately for each 1.5 h of search time (up to 4.5 h). Thirteen species were collected; including two endangered species (Rainbow *Villosa iris* and Eastern Pondmussel *Ligumia nasuta*). In all cases, species detection was imperfect. However, detection probabilities (*p*) for most species were high (>0.69). Two repeat 4.5 h surveys are required to confidently assess whether most (83%) species are present at a site. Search effort had a positive effect on estimates of species richness, detection probability and site occupancy, and the power to detect future distribution declines. At all levels of sampling effort, detection probability and site occupancy estimates were positively correlated to mussel abundance.

Platform 2 - Canadian Freshwater Pearly Mussels (Unionacea) and Species Discovery at the Canadian Museum of Nature.

André L. Martel and Jacqueline B. Madill

Zoology Section, Malacology, Canadian Museum of Nature, Gatineau QC, & Ottawa, ON. CAN. Email: AMARTEL@mus-nature.ca

Canada's fauna of native freshwater pearly mussels (Superfamily: Unionacea) comprises 54 confirmed species distributed in rivers, lakes, ponds and other permanent freshwater habitats. These molluscs occur from Newfoundland to British Columbia and from the Northwest Territories to the Yukon. Although the current conservation situation of Canadian freshwater pearly mussels is not as bleak as in the United States, 15 species (28%) are on Canada's list of species at risk. The national collection of freshwater mussels at the Canadian Museum of Nature (CMN, Gatineau, QC) is Canada's largest, with 8,389 lots and approximately 70,000 dry and wet specimens. Unionid mussel collections found in provincial museums, various governmental

agencies and privately owned collections have, more than ever before, become a key component for the conservation status assessment of individual species. Field surveys contribute to the scientific value of properly curated and maintained unionid collections.

In recent years, CMN-based studies on unionid mussels included field surveys, collecting, ecological and taxonomic research in different regions of Canada. In the Ottawa River (ON, QC), we study the communities of freshwater mussels where the endangered Hickorynut mussel (*Obovaria olivaria*) lives. In 2014 we discovered a vast population of Hickorynuts in the Lac Coulonge reach of the Ottawa River, which has an estimated mean density of 0.73 hickorynut / m^2 (1mX1m quadrat method). In the study area the Hickorynut represents one of the common species, along with *Elliptio complanata, Lampsilis cardium* and *Alasmidonta undulata*. At that site, the Ottawa River channel is 400 meters wide, substrate is mostly sand, and Hickorynuts are more prevalent far from shore, in currents of 10-15 cm/s and at a water depth of 3 to 5 m, therefore necessitating SCUBA surveys.

On Vancouver Island, BC, in collaboration with the Species-at-Risk team of the BC Ministry of Environment and the BC Conservation Data Centre, we examined the distribution and abundance of the Western Pearlshell (*Margaritifera falcata*), focusing on three rivers (Sarita, Cowichan, Nanaimo). In optimal habitats (Cowichan River, Nanaimo River) densities of over 300 pearlshells / m^2 could be observed. During these surveys, we examined the potential impact on this species of: clear-cut logging, shoreline erosion, bed load, decreasing stocks of Pacific salmon and Coastal Cutthroat trout (host fishes), as well as the recent occurrence of abnormally hot summer temperature and low water levels.

During our mussel surveys, high resolution underwater macro-photography is also used for taxonomical research by documenting the morphology of the mantle region of the different species of undisturbed live mussels, including siphonal apertures and mantle lures (or displays). Ethanol-preserved specimens in museum collections are also used to examine and compare the mantle region of mussel species observed during field surveys.

Platform 3 - Mussels at the Head of Lake Ontario.

Tys Theysmeyer[1], Paul Smith, and Todd J. Morris[2].

[1] *Head of Natural Lands, Royal Botanical Gardens, Burlington, ON. CAN. Email: ttheysmeyer@rbg.ca*
[2] *Great Lakes Laboratory for Fisheries & Aquatic Sciences, Fisheries and Oceans Canada, Burlington, ON. CAN.*

Located at the western tip of Lake Ontario, Cootes Paradise Marsh (320ha.) and Grindstone Marsh (72ha.) are part of the Royal Botanical Gardens Nature Reserves. These areas are also part of the Hamilton Area of Concern. Significant environmental management to restore the habitat include Common Carp (*Cyprinus carpio*) exclusion and reductions of combined sewer overflows. In association with the extensive environmental restoration projects monitoring has discovered a remarkable number of

mussel species that have found refuge in these marsh areas of the AOC. Several populations appear to be rising and recent surveys by RBG and DFO have confirmed additional species as well as two Species at Risk *(Toxolasma parvum*, *Quadrula quadrula)*, with another suspected (*Ligumia nasuta*). Shoreline surveys between 2005 & 2015 for fresh shells found 11 species and 11,500 shells (Grindstone Marsh 9,500 – 96hrs survey, Cootes Paradise 1,900 – 49hrs survey). The most common species include Giant Floater (*Pyganodon grandis*) and Paper Pondshell *(Utterbackia imbecillis)*. Surveys also indicate Cootes Paradise contains the largest Canadian population of Lilliput (*Toxalasma parvum*), with 2015 work finding this species at 7 out of 10 locations.

Platform 4 - Frontenac Arch Ligumia nasuta: what else hasn't been found?

Fred Schueler and Aleta Karstad

Bishops Mills Natural History Centre, Bishops Mills, ON. CAN. Email: bckcdb@istar.ca

The formerly abundant Unionid mussel, *Ligumia nasuta*, Eastern Pondmussel or Pointed Sand-Shell, has been reduced to a few known populations in Canada by mortality associated with Zebra Mussel fouling, and was classified as 'Endangered' by COSEWIC in 2007. Our 2005 discovery of a population in Lyn Creek, at the edge of the Frontenac Arch near Brockville, Ontario, combined with a previous discovery of a population in Beaver Lake on the Salmon River, and later at Loughborough Lake north of Kingston, led us to think of the Frontenac Arch of the Canadian Shield as an area occupied by the species. Before the Zebra Mussel invasion, it was unknown north of the Lake Ontario lakeside. We've searched only a few of the hundreds of lakes on the Arch, but in the three clusters of sites we've surveyed there are both Zebra-blighted sites, and waterbodies free of Dressenids which support surviving L. nasuta. If the detailed distribution of Unionids is to be documented across Canada in the absence of properly generous funding for museum exploration, we're going to need to recruit naturalist citizen scientists to notice and document Unionids in the same way they notice Birds and Dragonflies. Towards this end we've launched the 'Canadian Musselhead' facebook page, and invite everyone to participate in it.

Platform 5 - Freshwater Mussel surveys in Manitoba, 2013-2015.

Douglas Watkinson[1] and Todd J. Morris[2]

[1] *Freshwater Institute, Fisheries and Oceans Canada, Winnipeg, MB. CAN. Email: doug.watkinson@dfo-mpo.gc.ca*
[2] *Great Lakes Laboratory for Fisheries & Aquatic Sciences, Fisheries and Oceans Canada, Burlington, ON. CAN.*

To better understand Mapleleaf *(Quadrula quadrula*) distribution, relative abundance and habitat in the Lake Winnipeg watershed, Fisheries and Oceans Canada undertook

surveys within the known range of its host (Channel Catfish, *Ictalurus punctatus*) in Manitoba. After three years of directed surveys, we have increased the known range of the species. In addition, we now have a better understanding of the current distribution of all mussel species in the Lake Winnipeg watershed. This information will help inform future COSEWIC assessments. We will also briefly discuss a Channel Catfish movement study planned in 2016.

Platform 6 - Freshwater mussels in an urban watershed: Impacts of anthropogenic inputs and habitat alterations on populations.

Patricia L. Gillis[1], Rodney McInnis[1], Joseph Salerno[1], Shane R. de Solla[1]and Erin M. Leonard[2]

[1] *Aquatic Contaminants Research Division, Water Science and Technology Directorate, Environment and Climate Change Canada, Burlington, ON. CAN. Email: patty.gillis@canada.ca,*
[2] *Department of Biology, University of Waterloo, Waterloo, ON. CAN.*

The Grand River watershed has historically supported a rich assemblage of freshwater mussels, however 30-50% of the species once found there are believed to have been lost, losses attributed at least partially to 'pollution', though no specific cause has been identified. Water quality in the central watershed is impaired from road runoff and wastewater treatment plant effluents. The impact of anthropogenic activities on mussel populations was assessed at four sites along a 60 km reach of the Grand River spanning from an upstream reference site to an urban-impacted downstream region, and in the Speed River (Grand River tributary), at six sites along a 10 km reach selected to bracket anthropogenic inputs and structures. Semi-quantitative searching (8 hr/site) revealed that catch per effort in the Grand River declined by more than 60% from the upstream site to the area downstream of the cities. The size (length) frequency distribution of the most abundant species, *Lasmigona costata*, was significantly different upstream of the urban inputs (45-130 mm) compared to downstream (85-115 mm). In the Speed River, impoundments and wastewater treatment plants reduced the diversity and catch per effort. Most striking were 84 and 95% changes in the number of mussels found on either side of two impoundments, and a 98% drop in mussels immediately downstream of a wastewater treatment plant. Comparisons with earlier (1970s-1990s) surveys indicate that on a broad scale, the mussel community in this watershed may have improved, however localized anthropogenic activities and the cumulative effects of urban inputs continue to negatively impact mussels. These population level effects of decreased mussel abundance and underrepresentation of smaller mussels downstream of the urban area correspond to previously documented negative impacts at the biochemical and whole organism level of biological organization in wild Grand River mussels. Given the complex nature of the exposure, the specific chemical(s) and/or water quality parameter(s) responsible for the observed effects has not yet been identified.

Platform 7 - Sensitivity of freshwater mussels to neonicotinoid insecticides.

Ryan S. Prosser[1], Emily Holman[1], Adrienne Bartlett[1], Shane R. de Solla.[2], Vimal Balakrishnan[1], and Patricia L. Gillis[1]

[1] *Environment and Climate Change Canada, Aquatic Contaminants Research Division, Burlington, ON. CAN. Email: ryan.prosser@canada.ca*
[2] *Environment and Climate Change Canada, Ecotoxicology and Wildlife Health Division, Burlington, ON. CAN.*

In the past year, the mussel ecotoxicology group at Environment and Climate Change Canada has investigated the effect of a number of data-poor chemicals on the health of native freshwater mussel species. Neonicotinoid insecticides are widely used in seed treatments of row crops (e.g., corn, soybean, wheat) and have been detected in surface waters in Canada. Our objective was to investigate the sensitivity of the glochidia of a native freshwater mussel species to acute exposure to neonicotinoids. Glochidia from wavy-rayed lampmussels (*Lampsilis fasciola*) were exposed to seven neonicotinoids for 48 h. The results of the experiments indicate that glochidia are relatively insensitive to exposure to neonicotinoids, with the EC_{50} values for viability of glochidia being >500 µg/L for all seven neonicotinoids. However, testing with juveniles of another mollusks species, *Planorbella pilsbryi*, has found that growth and biomass production are a more sensitive endpoint than mortality. Future work is planned to investigate the effects of neonicotinoids on more subtle endpoints (e.g., growth, biomass production, ability to bury) in the juvenile and adult life stages of freshwater mussels.

Platform 8 - Assessment of DNA damage in freshwater mussels from the Hamilton Harbour watershed.

Ève A.M. Gilroy[1], Sara Witzke[1], Sheena Campbell[1], Kelly A. McNichols-O'Rourke[2], Todd J. Morris[2], Joseph Salerno[3], Patricia L. Gillis[3], Tys Theysmeyer[4], and Shane R. de Solla[5]

[1] *Green House Science, Burlington, ON. CAN. Email: eve.gilroy@bell.net*
[2] *Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, Burlington, ON. CAN.*
[3] *Aquatic Contaminants Research Division, Environment and Climate Change Canada, Burlington, ON*
[4] *Royal Botanical Gardens, Burlington, ON. CAN.*
[5] *Ecotoxicology and Wildlife Health Division, Environment and Climate Change Canada, Burlington, ON. CAN.*

In the last century, several regions of the Great Lakes have suffered from considerable habitat degradation due to the influence of intensive industrial, agricultural

and urban development. Cootes Paradise is a shallow wetland located west and upstream of Hamilton Harbour, and is part of the Hamilton Harbour Area of Concern. Cootes Paradise receives the effluent of a secondary wastewater treatment plant (WWTP), and is also downstream of agricultural landscapes. Hence, among others, the water quality of this wetland is affected by elevated concentrations of pesticides, pharmaceuticals, and high nutrient loadings.

A variety of chemicals can induce damage to the DNA of animals, which, if not repaired, can lead to a cascade of biological effects at any level of biological organization, from cellular to community and population level. First developed in the 1980s, the single cell gel electrophoresis assay – or Comet assay - is used to visualize and quantify cellular DNA damage. In recent years, a number of studies have used this approach to evaluate DNA damage from exposure to specific chemicals (e.g., PAHs), as well as from complex mixtures of contaminants, such as industrial or municipal effluents.

The objective of the present study was to validate the Comet assay in wild freshwater mussels and to quantify the DNA damage in mussels from the Hamilton Harbour watershed in comparison with that in mussels from reference sites in southern Ontario. Giant Floater mussels (*Pyganodon grandis*) (91 ± 18 mm, $n = 68$) were collected from three sites: Cootes Paradise; a small stream directly under highway 401 near Campbellville; and in a small agricultural stream in Embro, Ontario. Although freshwater mussels were successfully collected in Cootes Paradise, the catch per unit effort at that site was considerably lower than that in Campbellville and Embro. The mussels were brought to the laboratory and held overnight, a small quantity of hemolymph (blood) was collected the next morning, and the mussels were returned to their habitat of origin within 24 hours. The density and viability of hemocytes (blood cells) were quantified by flow cytometry, and the presence and relative quantity of DNA strand breaks were quantified using the Comet assay.

The catch per unit effort was lower in Cootes Paradise compared to Campbellville and Embro. Cell density was significantly elevated in Giant Floater mussels collected in Campbellville and Cootes Paradise, compared to those collected in Embro. The hemocytes of mussels from Cootes Paradise had significantly greater DNA damage than those from Campbellville and Embro, suggesting exposure to genotoxic compounds. As it has been linked to reduced growth, abnormal development and reduced survival, DNA damage, if not repaired, could have repercussions at the individual and community level. Validation of the Comet assay in other aquatic species, and follow-up studies in Hamilton Harbour AOC are under consideration.

Platform 9 – Mapleleaf Mussel (*Quadrula quadrula*) in Canada: Status updates, phylogeography, and fine-scale genetic studies.

Jordan R. Hoffman[1], Todd J. Morris[2], and **David T. Zanatta[1]**.

[1]Department of Biology, Institute for Great Lakes Research, Central Michigan University, Mount Pleasant, MI. USA. Email: zanat1d@cmich.edu

[2] *Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, Burlington, ON. CAN.*

The Mapleleaf (*Quadrula quadrula*) is a long-lived, thick-shelled unionid mussel found in the lower Great Lakes watershed in Ontario and the Lake Winnipeg watershed in Manitoba. In 2006, COSEWIC assessed the species as Threatened in Ontario and Endangered in Manitoba. Threats to the species and its habitats in Canada include agricultural impacts, industrial spills, and impacts of fouling by dreissenid mussels. Subsequent intensive surveys since 2006 have revealed that the species has a similar distribution to historical accounts and may even be expanding its distribution in both Ontario and Manitoba. Encouragingly, further losses due to threats have not played out as feared in Ontario. In Manitoba, with the invasion of Lake Winnipeg and the Red River by Zebra Mussels in 2014, the threat of dreissenid mussel fouling has yet to play out, but is no longer a perceived threat. Phylogeographic studies using mitochondrial and microsatellite DNA analyses have revealed that Great Lakes and Manitoba populations all likely originated from a source in the Upper Mississippi. Fine-scale genetic analyses revealed significant genetic structure in the Great Lakes population of Mapleleaf, with indications that the establishment of large numbers of Mapleleaf in coastal areas of Lake Ontario may be a recent event, facilitated by the construction of the Welland canal ca. 180 years ago. Ultimately, this information has the potential to improve conservation strategies for this and other unionid species at risk.

Platform 10 - Conservation genetics of unionids in southern Ontario: comparative multispecies analysis of spatial structure, diversity, and mating systems.

Chris Wilson[1], Heather Galbraith[2], Caleigh Smith[1], and Dave Zanatta[3]

[1] *Aquatic Research and Monitoring Section, Ontario Ministry of Natural Resources and Forestry, Trent University, Peterborough, ON. CAN. Email: chris.wilson@ontario.ca*
[2] *USGS, Leetown Science Center, Norther Appalachian Research Branch, Wellsboro, PA. USA*
[3] *Department of Biology, Institute for Great Lakes Research, Central Michigan University, Mount Pleasant, MI. USA.*

Understanding the genetic diversity and structure of species and populations is extremely valuable for informed conservation. We investigated patterns of genetic structure and diversity in six unionid mussel species which vary in their conservation status, life history strategies, and dispersal capabilities in southern Ontario rivers. All species showed evidence of historical genetic connectivity within rivers was ubiquitous across species and may reflect dispersal abilities of host fish. There was little to no signature of recent disturbance events or bottlenecks, even in endangered species, likely as a function of mussel longevity and historical population sizes (insufficient time for genetic drift to be detectable). Genetic structure was largely at the watershed scale, with little to know evidence of genetic substructure or subpopulations within rivers, which again may reflect host dispersal events prior to barrier construction. These

results indicate that population augmentation via translocation within rivers may be a useful conservation tool if needed, while minimizing genetic risks to recipient sites. Other efforts have focused on the reproductive biology of several species, and have detected multiple paternity within single broods of developing glochidia in each species which has been assessed to date. Recent interest in population augmentation via translocation and propagation may rely on these results to inform conservation activities and management for unionids in the Great Lakes region.

Platform 11 - Physical ecology of juvenile and adult SAR unionid mussels

Josef D. Ackerman

Physical Ecology & Aquatic Science, Department of Integrative Biology, University of Guelph, Guelph, ON. CAN. Email: ackerman@uoguelph.ca

We have been investigating the physical ecology of recently excysted juvenile and adult unionid mussels by examining the role of hydrodynamics in their life history. High bed shear stress was found to detach juvenile mussels from surfaces, confirming its role in determining suitable habitat in the field. Importantly, juvenile mussels that adhered to the bed using their foot withstood higher bed shear stress before detachment. This indicates the importance of behavior in mussel ecology. We have also investigated potential juvenile habitat in the field. Fluid dynamics also affects the deliver (I.e., flux) of seston to mussels and their ability to feed. The clearance rate (CR) of juvenile mussels was positively related to algal flux at ecologically relevant pore water levels. The CR of adult mussels also varied with algal flux and saturated at high seston flux. CR values under moderate to high flux differed significantly from values obtained in aquaria/tanks. Ongoing research using flow cytometry has focused on selective feeding in juvenile and adult mussels.

Platform 12 - The effect of suspended sediment flux on the suspension feeding of a freshwater mussel.

Shaylah Tuttle-Raycraft and **Josef D. Ackerman**

Physical Ecology & Aquatic Science, Department of Integrative Biology, University of Guelph, Guelph, ON. CAN. Email: stuttle@uoguelph.ca

The effect of suspended sediment (SS) flux on the suspension feeding of *Lampsilis siliquoidea*, a freshwater mussel species without conservation concerns, was examined using different combinations of sediment concentration (C) and velocity (U). The response of clearance rate (the amount of water cleared of particles per unit time; CR) to SS flux (C x U) was nonlinear and varied on the response surface defined by velocity and concentration. CR generally increased with velocity whereas they were reduced with increased SS concentration. However, the response was not uniform; velocity had

a greater influence on CR when the SS concentration was low, as did concentration when velocity was low. This demonstrates that that the relationship between SS and suspension feeding is more complicated than previously demonstrated in studies that focus on concentration alone. The interaction of concentration and velocity provides an example of how multiple stressors can affect organisms in nature.

Platform 13 - Species boundaries and levels of intermixing between *Lampsilis siliquoidea* and *L. radiata*.

Isabel Porto- Hannes[1], H. Lasker[1], and Lyubov E. Burlakova[2]

[1] *University at Buffalo, Buffalo, NY, USA. Email: isabelha@buffalo.edu*
[2] *Buffalo State College, Great Lakes Center, Buffalo, NY, USA.*

After the last glaciation, freshwater mussel species (Family Unionidae) range expansion led to secondary contact between species that were isolated in the past, and if these species had incomplete reproductive barriers, gene flow could have occurred (hybridization with or without introgression). There is some evidence that two closely related species, *Lampsilis radiata* and *L. siliquoidea* can potentially hybridize; however the prevalence, direction and geographic extent of the potential hybrid zone is not well known. Hybridization was suggested due to the presence of morphological and genetic intermediate forms where their geographic range overlap in the lower Great Lakes, St. Lawrence River and Lake Champlain watersheds in the United States and Canada. The presence of intermediate forms has also led to a long history of name confusion and debate on their phylogenetic relationship. The objective was to determine the phylogenetic relationship and levels of intermixing (e.g., non vs. limited hybridization vs. introgression) between *L. siliquoidea* and *L. radiata*. Species boundaries and potential hybridization were determined using mitochondrial cytochrome oxidase subunit I gene (COI) and 6 microsatellite loci. Maternally (m)- and paternally (p)- inherited COI haplotypes of these two species are distinct, indicating that these species are different. Incongruences between m- and p-inherited COI haplotype assignments were also found suggesting restricted hybridization in eastern Lake Ontario and St. Lawrence River tributaries. Preliminary microsatellite data suggested more frequent hybridization than the COI data predicted. Lastly, introgression has occurred on both directions, however is more frequent from *L. siliquoidea* into *L. radiata.* Correct identification of species, potential hybridization and description of hybrid zones is fundamental in developing and implementing measures to conserve and restore unionid species and populations.

Platform 14 - Environmental DNA (eDNA) as a tool for targeted detection of multiple Unionid species at-risk.

Charise A. Currier[1], Todd J. Morris[2], Chris Wilson[3], and Joanna Freeland[4].

[1] *Trent University, Peterborough, ON. CAN. Email: charisecurrier@trentu.ca*

[2]Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, Burlington, ON. CAN.
[3] Aquatic Research and Monitoring Section, Ontario Ministry of Natural Resources and Forestry, Trent University, Peterborough, ON. CAN.
[4] Biology Department, Trent University, Peterborough, ON. CAN.

Environmental DNA (eDNA) detection can be used to infer species presence by using species-specific markers to screen DNA from water samples. This technique is increasingly being used to track the spread of invasive fish species, but has largely not been applied to other taxonomic groups. However, the low environmental impact of eDNA sampling makes the method ideal for sampling sensitive populations. Here we report on research that aims to quantify the opportunities and limitations of eDNA for the detection of rare, at-risk organisms. Our study focuses on the detection of native freshwater mussel (Unionidae) species at risk using species-specific qPCR markers. We will use a multi-species approach to develop highly specific markers for each of four closely-related species. To date, species-specific qPCR primer-probe sets have been developed, validated under controlled conditions, and environmental samples have been collected across the species' ranges to test for species detections. The eDNA-derived species occurrences will be compared with existing distribution maps to evaluate the efficacy of the method. eDNA shows promise as an effective tool for the targeted detection of closely-related at-risk invertebrates; however, a thorough knowledge of the detection thresholds and limitations of the eDNA tools is crucial for meaningful interpretation of results and the eventual use of eDNA for management applications.

Platform 15 - A Risk Assessment of Golden Mussel (*Limnoperna fortunei*) for Ontario.

Gerry Mackie

Professor Emeritus, Department of Integrative Biology, University of Guelph, Guelph, ON. CAN. Email: gerry.mackie@sympatico.ca

A Risk Assessment of Golden Mussel for Ontario was performed by: (i) assessing probability of invasion by estimating the probability of arrival, survival, establishment, and spread (ii) estimating impacts of invasion, and (iii) assessing potential risk from the above two steps.

Ballast water exchange of Atlantic transoceanic vessels from South America was ranked the primary potential pathway for introduction of Golden Mussel directly into the Great Lakes. A "back door" entry into the Great Lakes was also considered via overland dispersal (e.g. trailered boats) from ballast water exchange of transoceanic vessels from Asia to the Pacific coast of North America. However, the overall probability of arrival through these two pathways was ranked low.

The probability of survival, establishment, and spread of Golden Mussel in Ontario was considered low because of its physiological intolerance of cold, winter

waters. The level of certainty was considered moderate as there has been no Golden Mussel reported from lakes that freeze over in Asia or South America. While it does survive at 4.2^{o}C, the lowest thermal threshold for Golden Mussel in different length classes needs to be empirically examined. The consequence of establishment was predicted to be of high negative ecological and economic impacts. A Climatch analysis using Japan and South America as source regions and Ontario as the target region resulted in very different climates between both sources and Ontario, also suggesting that *L. fortunei* will not likely establish in Ontario under current climate conditions. An examination of threshold temperatures needed for onset of reproduction (16-17^{o}C) and completion of development (21^{o}C) at latitudes in which *L. fortunei* occurs, shows that about 150 days at 16.5^{o}C and 75 days at 21^{o}C are required for establishment of the mussel. After examining the mean number of degree days for each Great Lake from 2010 to 2015, none of the Great Lakes has sufficient time at 16.5^{o}C for reproduction to occur; only Lake Erie has sufficient time at 21^{o}C for settlement to occur.

Platform 16 - Unionid Mussel Species at Risk vs. the Round Goby.

Maude Tremblay[1], Todd J. Morris[2], and Josef D. Ackerman[3]

[1] *Fisheries Protection Program, Fisheries and Oceans Canada, Burlington, ON. CAN. Email: Maude.Tremblay@dfo-mpo.gc.ca*
[2] *Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, Burlington, ON. CAN.*
[3] *Physical Ecology & Aquatic Science, Department of Integrative Biology, University of Guelph, Guelph, ON. CAN.*

We investigated whether *Neogobius melanostomus*, an invader of biodiversity "hotspots" in the Laurentian Great Lakes region, facilitates or inhibits unionid mussel recruitment by serving as a host or sink for their parasitic larvae (glochidia). Infestation and metamorphosis rates of four mussel species with at-risk (conservation) status (*Epioblasma torulosa rangiana, Epioblasma triquetra, Lampsilis fasciola,* and *Villosa iris*) and one common species (*Actinonaias ligamentina*) on *N. melanostomus* were compared with rates on known primary and marginal hosts in the laboratory. All species successfully infested *N. melanostomus*, but only *E. triquetra, V. iris*, and *A. ligamentina* successfully metamorphosed into juveniles, albeit at very low rates well below those seen on even the marginal hosts. *Neogobius melanostomus* collected from areas of unionid occurrence in the Grand and Sydenham rivers (Ontario, Canada) exhibited glochidial body burdens of 39.4% and 5.1%, respectively, with up to 30 glochidia representing as many as six unionid species per fish. A mathematical model suggests that *N. melanostomus* serve more as a sink for glochidia than as a host for unionids, thereby limiting recruitment success. This represents a novel method by which an invasive species affects a native species.

Platform 17 - Two Steps Forward, One Step Back – An Overview of the Ontario Ministry of Natural Resources and Forestry's Early Efforts to Develop Expertise in the Culture of 'At Risk' Unionid Mussels.

Christopher Wilson and Kevin Loftus

Fish Culture Section, Ontario Ministry of Natural Resources and Forestry, Peterborough, ON. CAN. Email: Christopher.Wilson@ontario.ca, kevin.loftus@ontario.ca

In late 2009, Ontario Ministry of Natural Resources and Forestry's Executive Committee asked Fish Culture Section if it should or could do more to support the recovery of 'at risk' species. In response, in 2012 the Section sought expert input on the potential role of propagation and release in the recovery of aquatic 'at risk' species. It also initiated its first attempt to culture an 'at risk' species of mussel, the Wavy-rayed Lampmussel, in order to begin to develop expertise in the culture of such species and to begin to understand the labour and infrastructure costs associated with such work. That first effort ended in failure. Since that time, the sophistication or our efforts has grown as has our level of success and we are currently experiencing varying levels of success with four species: Wavy-rayed Lampmussel, Snuffbox, Northern Riffleshell and Kidneyshell. This presentation covers the highlights of those efforts, the lessons learned and our plans for the coming year.

Platform 18 - Toronto Zoo and Freshwater Mussel Conservation.

Mary-Kate Whibbs and **Cynthia Lee**

Toronto Zoo, Scarborough, ON. CAN. Email: mwhibbs@torontozoo.ca, clee@torontozoo.ca

Since 2013 Toronto Zoo has been actively involved in freshwater mussel conservation through research and public awareness. Toronto Zoo has conducted systematic sampling for freshwater mussels in Balsam Lake and in six rivers on the north side of the L. Ontario watershed stretching from Credit River in the west to Duffins' Creek in the east; the presence of seven native mussel species has been documented in these waterbodies as a result. Statistical analysis has revealed relatively low mussel densities across the six rivers and Balsam Lake, Humber River being the highest at an average 0.74 mussels/m^2. Critical data including habitat features and host-fish presence has also been collected and examined for each river surveyed. In collaboration with the Ministry of Natural Resources and Forestry, Toronto Zoo staff stationed at Normandale Fish Culture Station developed a detailed protocol and report on freshwater mussel rearing. To support mussel research, Toronto Zoo initiated the 'I am Important! I am Protected!' public awareness campaign which includes classroom outreach, public presentations, resource distribution and most recently, the development of a user-friendly mussel reporting smartphone app. Further details of the zoo's freshwater

mussel conservation work will be presented at the Canadian Freshwater Mussel Meeting to introduce Toronto Zoo to the greater freshwater mussel community.

Platform 19 - Summary of Federal Aquatic (fish/mussel) Species at Risk Program Outreach Efforts in Ontario (2008-2015).

Shelly Dunn

Species at Risk Branch, Fisheries and Oceans Canada, Burlington, ON. CAN. Email: shelly.dunn@dfo-mpo.gc.ca

Outreach with stakeholders and partner agencies continues to be an important priority within Fisheries and Oceans Canada's (DFO's) Species at Risk Program. In Ontario, there are currently 32 aquatic species listed on Schedule 1 of the *Species at Risk Act* (SARA) in the province; twenty-one fishes and 11 mussels, and a number of others under consideration for listing. For listed threatened and endangered species, critical habitat (CH) has been identified in recovery strategies, while a number of additional recovery strategies are currently in progress. The identification and protection of CH has implications for a wide variety of stakeholders and must be understood by them and partner agencies. Between 2008 and 2015, DFO-SAR program met face-to-face and provided web-based training for over 3600 members of communities, stakeholder groups, and partner agencies. Outreach has also included the installation of SAR information signs in sensitive areas, notices in industry newsletters, SAR distribution maps for early screening to avoid project impacts, and funding promotion for SAR stewardship. Across Ontario, these efforts have broadened awareness of the aquatic species at risk and critical habitat protection requirements of SARA, and have highlighted DFO's integrated process for the review and approval of projects under the *Fisheries Act* and SARA. Innovative approaches for outreach are being explored to address ongoing and emerging anthropogenic threats to the conservation of aquatic species at risk.

Platform 20 - An update on Brook Floater (*Alasmidonta varicosa*) conservation and management measures in Canada.

Diane L. Amirault-Langlais and Fabiola Akaishi

Department of Fisheries and Oceans-Gulf Region, Moncton, NB. CAN. Email: Diane.Amirault-Langlais@dfo-mpo.gc.ca

The Brook Floater (*Alasmidonta varicosa*) was added to Schedule 1 of the *Species at Risk Act* as Special Concern in 2013. Since this time, progress has been made towards involving local conservation groups in efforts to monitor and maintain the Brook Floater and its habitat. Three conservation groups in New Brunswick have been working on projects involving the Brook Floater. Their efforts have resulted in confirming

new locations, fostering stewardship initiatives and public awareness of this relatively little known species. The species is now known to occur in 15 watersheds within New Brunswick and Nova Scotia, with little potential for exchange between most watersheds. The species now appears to be absent from three rivers where it was historically present. In January 2016, a Draft Management Plan for the Brook Floater in Canada, developed with the input of many stakeholders, was shared for consultation to obtain input on proposed conservation measures and to encourage the involvement of additional partners in efforts to maintain the species.

The presentation will offer an update of information currently available on the Brook Floater, conservation efforts currently underway and provide an overview of the measures identified in the Draft Brook Floater Management Plan. In order to achieve the objective of maintaining the Brook Floater population in Canada at current locations and where the species may be discovered, the management plan recommends specific conservation measures organized in four broad strategies: protection, management, research and monitoring and outreach and communication.

Platform 21 - Moving Forward with Management of *Gonidea angulata* in the Okanagan Valley of British Columbia, Canada.

Lora Nield[1], Jon Mageroy[2], Ian Walker[2], Roxanne Snook[2], Sean MacConnachie[3], Greg Wilson[4], Steven Brownlee[2], and Jerry Mitchell[5]

[1] *British Columbia Ministry of Forests, Lands and Natural Resource Operations, Penticton, BC. CAN. Email: lora.nield@gov.bc.ca;*
[2] *University of British Columbia Okanagan, Kelowna, BC. CAN.*
[3] *Fisheries and Oceans Canada, Nanaimo, BC. CAN.*
[4] *British Columbia Ministry of Environment, Victoria, BC. CAN.*
[5] *British Columbia Ministry of Environment, Penticton, BC. CAN.*

In Canada, the Rocky Mountain Ridged Mussel (*Gonidea angulata*) is only found within the Okanagan Valley, British Columbia. It is currently listed by COSEWIC as Endangered. The province of British Columbia, Fisheries and Oceans Canada, and the University of British Columbia Okanagan have been working in partnership to fill knowledge gaps and improve the management of this species. Surveys show that the mussel is present in several of the lakes and in the Okanagan River. There are some sites that have quite dense mussel beds, where juvenile recruitment is known to be occurring. Habitat studies and modelling show that, in the lakes, the mussel prefers areas with highly embedded substrate. Further, the preferred locations are typically shallow areas of points or bays, which experience high fetch (wind exposure). In the river, surveys show that the mussel prefers the banks of channelized sections, rather than natural or restored sections. Host fish field studies strongly suggest that Prickly Sculpin (*Cottus asper*) is the most important host for the mussel in the valley, although there are other potential hosts. Fish surveys suggest that limited host availability, likely due to the impacts of introduced fish species, may be a threat to the mussel in the southern part of the valley. Based on these findings, the province is taking management

actions to protect known mussel beds, mussel habitat and may move into management of the mussel's host fish if deemed necessary.

Platform 22 - Snuffbox in Michigan: Prioritizing research goals to help with conservation across borders.

Daelyn A. Woolnough and Mandi L. Caldwell.

Department of Biology and Institute for Great Lakes Research, Central Michigan University, Mt. Pleasant, MI. USA. Email: wooln1d@cmich.edu

Snuffbox is federally endangered in the United States and Canada and is a member of arguably the most imperiled genera in the Family Unionidae. In Michigan, we have prioritized research projects in order to target knowledge gaps that exist for this species in the Great Lakes region. In the last 5 years, we have quantified 4 populations of snuffbox throughout Michigan and while quantifying distribution have successfully quantified other ecological preferences. We will present host fish, co-occuring unionid assemblage, and habitat preferences for snuffbox. We have shown that snuffbox can use both logperch and blackside darter as hosts and that transformation can occur on hosts from other watersheds. Host test results suggest seasonal differences in the developmental stages of snuffbox juveniles between spring and fall. We have evidence that snuffbox is found in diverse unionid assemblages and have been able to document the species that co-occur with this rare species by using a method that has helped managers understand diverse unionid assemblages. In the populations studied, we have seen evidence of recruitment and longevity that has not been quantified before and we will present data on sex ratios, size class distributions, and how we have confirmed reproduction is occurring in these populations. We will present abiotic habitat preferences for the snuffbox. Overall, these data are important for the conservation of snuffbox and other mollusk species and the process we used for detection and quantification can be used in conservation management internationally.

Platform 23 - From spawning to attachment: A Northern Riffleshell, *Epioblasma torulosa rangiana*, life stage story from the Sydenham River.

Kelly A. McNichols-O'Rourke[1], Katherine Wright[1], Jessica M. Epp[2], and Todd J. Morris[1].

[1] *Great Lakes Laboratory for Fisheries & Aquatic Sciences , Fisheries and Oceans Canada, Burlington, ON. CAN. Email: kelly.mcnichols-o'rourke@dfo-mpo.gc.ca*
[2] *Fisheries Protection Program, Fisheries and Oceans Canada, Burlington, ON. CAN.*

Of the 54 species of freshwater mussels in Canada nine species have been listed as endangered under the Canadian *Species at Risk Act*. One of these is the Northern Riffleshell, *Epioblasma torulosa rangiana*. Information about the life history

characteristics of *E.t rangiana* in Ontario is limited and is required to facilitate the recovery of this species. Our goal was to identify the reproductive timing windows - spawning, brooding, glochidial release and host infestation - for *E.t. rangiana* in the Sydenham River in southwestern Ontario, Canada. Over 100 individuals of this species were PIT tagged and sampled weekly during the open water period in 2013-2014. During each sampling event 5-10 individuals were examined (generally 3 males and 3 females), gonad samples were taken, drift net samples were collected, and suspected host fish were vouchered. Sixty-five females and 44 males were PIT tagged. Data suggest that spawning occurs in summer (late June – late August) as males and females were observed with large amounts of sperm and eggs present. Gravid females were observed beginning in mid-August and continued until at least October. Suspected host fishes collected were Johnny Darter, Blackside Darter, and Logperch. Over 358 of these fish species (98 Johnny Darters, 139 Blackside Darters, and 121 Logperch) were examined (collected from June 27, 2013 to September 29, 2014) and glochidia were present in over 50%. Preliminary data, based on glochidial measurements, suggest that less than 1.0% of these glochidia are *E.t. rangiana*. The identification of these reproductive timing windows is vital to the successful recovery and the continued protection of *E.t. rangiana* under the *Species at Risk Act* in Canada.

POSTER ABSTRACTS

Poster 1 - Remnant freshwater mussel diversity in Lake Ontario and Upper St. Lawrence River coastal wetlands

Victoria Kopf[1], Scott Reid[1], and Todd J. Morris[2]

[1] *Aquatic Research and Monitoring Section, Ontario Ministry of Natural Resources and Forestry, Trent University, Peterborough. email: reid.scott@ontario.ca*
[2] *Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, Burlington, ON. CAN.*

The dreissenid invasion of the Great Lakes resulted in substantial and widespread declines in native mussel diversity. Recent studies have demonstrated the importance of Great Lakes coastal wetlands as refugia for freshwater mussels. Identifying and understanding these refugia and remnant mussel populations is crucial to maintaining mussel diversity and recovering species-at-risk. To date, most research has occurred in Lake St. Clair and other Great Lakes nearshore habitats found in United States waters. The main objectives of this study were to: 1) identify important habitats for mussel species-at-risk, targeting endangered Eastern Pondmussel (*Ligumia nasuta)*, and 2) compare mussel assemblages at three wetland types; open and closed embayments, barrier beach and drowned river mouths. From August 2011 to July 2015, 41 wetlands were sampled along the Lake Ontario and upper St. Lawrence River shorelines in Ontario. At each wetland, 12 randomly selected sites were sampled using visual-tactile and clam-rake methods. 1636 live mussels were collected from 33 of the 41 sites. Thirteen species were detected, all of which were represented by live specimens. The most common species was Giant Floater (*Pyganodon grandis,* at 85% of sites with mussels), followed by Fatmucket (*Lampsilis siliquoidea*) and Eastern Elliptio (*Elliptio complanata*). Eastern Pondmussel was found at seven wetlands, threatened Mapleleaf (*Quadrula quadrula*) at two wetlands, and endangered Liliput (*Toxolasma parvus*) at one wetland. Individual wetlands contained between one and five species; no statistical differences were found in the number of species or individuals found between wetland types. Evidence of dreissenids was present at more than half of the wetlands and dreissenids were attached to 56% of live mussels. Ongoing and future research is focused on Lake Erie coastal wetlands, and assessing the long-term viability of remnant populations of mussels at risk.

Poster 2 - Assessing the risk of Black Carp (*Mylopharyngodon piceus*) invasion to native freshwater mussels (Unionidae) in the Laurentian Great Lakes.

Todd J. Morris[1], Daelyn A. Woolnough[2], and Justin Barbati[1]

[1] *Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, Burlington, Ontario, Canada. Todd.Morris@dfo-mpo.gc.ca*

[2] *Department of Biology and Institute for Great Lakes Research, Central Michigan University, Mount Pleasant, Michigan, U.S.A.* wooln1d@cmich.edu

Black Carp (*Mylopharyngodon piceus*) is a large (up to 1.5m and 70 kg) molluscivorous cyprinid, native to eastern Asia including China, eastern Russia and possibly northern Vietnam. It was first introduced to North America accidentally in the 1970s as a contaminant in Grass Carp (*Ctenopharyngodon idella*) shipments arriving in Arkansas. Subsequent deliberate introductions occurred during the 1980s when the fish was used as a food fish and as a biological control for yellow grub (*Clinostomum margaritum*). The first known escape of Black Carp into the wild occurred in 1994 when as many as 30 escaped during a flood event from an aquaculture facility into the Osage River in Missouri. Black Carp are now known in the wild from Arkansas, Illinois, Louisiana, Mississippi and Missouri and are within 1000 km of the Great Lakes Basin. Black Carp are voracious predators with the potential to have strong negative impacts on native freshwater mussels (Unionidae) should they become established within the Great Lakes basin. We have identified 51 species of Unionidae including 15 federally listed (Canada or U.S.) and 34 provincial or state listed species occurring within the region of the Great Lakes basin likely to be impacted by a Black Carp invasion. Through an analysis of gape limitation, unionid size distribution, and growth patterns we will attempt to evaluate the potential impact on these imperiled freshwater mussel stocks.

Poster 3 – Preliminary fine-scale analyses of genetic diversity, colonization, and population structure of the Eastern Pondmussel, *Ligumia nasuta*, in the Great Lakes Region.

Mariah W. Scott[1], Todd J. Morris[2], and David T. Zanatta[1].

[1] *Department of Biology, Institute for Great Lakes Research, Central Michigan University, Mount Pleasant, MI. USA. Email:* scott2mw@cmich.edu
[2] *Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, Burlington, ON 4A6 Canada.*

The freshwater mussel *Ligumia nasuta* (Eastern Pondmussel, Bivalvia: Unionidae) is known from the Atlantic slope and Great Lakes regions of eastern North America, but is considered imperiled within many states and provinces it inhabits. This expansive range was colonized by *L. nasuta* dispersing to the Great Lakes region, following the retreat of Wisconsin glaciers. While many of the known populations appear to have followed natural colonization routes, some populations in northern Michigan and western New York are hypothesized to be the result of anthropogenic introductions. Over the last few decades, this species has declined in abundance and range, due to invasive dreissenid mussels and changes in habitat. As a result, the species is considered imperiled across large portions of its range, especially in the Great Lakes Region. In this study, the genetic diversity and structure of the remnant populations in the Great Lakes region will be assessed using microsatellite DNA loci. An understanding of the genetic diversity and structure of remaining populations to inform

future management projects, examine the colonization history of the species, and determine if the remnant populations have experienced a genetic bottleneck or founder effect. Samples from 54 sites in 22 sampled waterbodies across the range of *L. nasuta* (n=295) have already been collected for this study, with additional sampling planned. A subset of 10 to 15 of the 29 newly developed microsatellite loci for *L. nasuta* will be genotyped and scored. The genetic diversity found will be used to determine population structure among sampling locations and test for significant genetic differentiation within and among populations. Mantel tests of isolation by water, road, and Euclidean distances between populations will be compared to assess the likelihood of natural or anthropogenic colonization history. Also, analyses of genetic diversity will be used to test if a past genetic bottleneck event or strong founder effect has occurred. This study will deepen our understanding of the genetic past and present of this imperiled species.

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