2023 Unionid Monitoring and Biodiversity Observation (UMBO) Network assessment in the Maitland River watershed, Ontario

Mandy P. Gibson, Kelly A. McNichols-O'Rourke, and Todd J. Morris

Fisheries and Oceans Canada
Great Lakes Laboratory for Fisheries and Aquatic Sciences
867 Lakeshore Rd.
Burlington, ON
L7S 1A1

2024

Canadian Data Report of Fisheries and Aquatic Sciences 1395

Fisheries and Oceans Canada | Pêches et Océans Canada

Canada

Canadian Data Report of Fisheries and Aquatic Sciences

Data reports provide a medium for filing and archiving data compilations where little or no analysis is included. Such compilations commonly will have been prepared in support of other journal publications or reports. The subject matter of the series reflects the broad interests and policies of Fisheries and Oceans Canada, namely, fisheries management, technology and development, ocean sciences, and aquatic environments relevant to Canada.

The correct citation appears above the abstract of each report. Each report is abstracted in the data base *Aquatic Sciences and Fisheries Abstracts*.

Data reports are produced regionally but are numbered nationally. Requests for individual reports will be filled by the issuing establishment listed on the front cover and title page.

Numbers 1-25 in this series were issued as Fisheries and Marine Service Data Records. Numbers 26-160 were issued as Department of Fisheries and Environment, Fisheries and Marine Service Data Reports. The current series name was changed with report number 161.

Rapport statistique canadien des sciences halieutiques et aquatiques

Les rapports statistiques servent de base à la compilation des données de classement et d'archives pour lesquelles il y a peu ou point d'analyse. Cette compilation aura d'ordinaire été préparée pour appuyer d'autres publications ou rapports. Les sujets des rapports statistiques reflètent la vaste gamme des intérêts et politiques de Pêches et Océans Canada, notamment la gestion des pêches, la technologie et le développement, les sciences océaniques et l'environnement aquatique, au Canada.

Le titre exact figure au haut du résumé de chaque rapport. Les rapports à l'industrie sont résumés dans la base de données *Résumés des sciences aquatiques et halieutiques*.

Les rapports statistiques sont produits à l'échelon régional, mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement d'origine dont le nom figure sur la couverture et la page du titre.

Les numéros 1 à 25 de cette série ont été publiés à titre de Records statistiques, Service des pêches et de la mer. Les numéros 26-160 ont été publiés à titre de Rapports statistiques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom de la série a été modifié à partir du numéro 161.

Canadian Data Report
of Fisheries and Aquatic Sciences 1395

2024

2023 Unionid Monitoring and Biodiversity Observation (UMBO) Network assessment in the Maitland River watershed, Ontario

by

Mandy P. Gibson, Kelly A. McNichols-O'Rourke, and Todd J. Morris

Fisheries and Oceans Canada
Great Lakes Laboratory for Fisheries and Aquatic Sciences
867 Lakeshore Rd.
Burlington, ON L7S 1A1

Cat. No. Fs97-13/1395E-PDF ISBN 978-0-660-71437-0 ISSN 1488-5395

Correct citation for this publication:

Gibson, M.P., McNichols-O'Rourke, K.A., and Morris, T.J. 2024. 2023 Unionid Monitoring and Biodiversity Observation (UMBO) Network assessment in the Maitland River watershed, Ontario. Can. Data. Rep. Fish. Aquat. Sci. 1395: vii + 36 p.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Gibson, M.P., McNichols-O'Rourke, K.A., and Morris, T.J. 2024. 2023 Unionid Monitoring and Biodiversity Observation (UMBO) Network assessment in the Maitland River watershed, Ontario. Can. Data. Rep. Fish. Aquat. Sci. 1395: vii + 36 p.

Unionid freshwater mussels are ecologically valuable in aquatic ecosystems and also one of the most globally imperiled taxa. Managed by Fisheries and Oceans Canada, the Unionid Monitoring and Biodiversity Observation (UMBO) Network collects and analyzes long-term monitoring data for freshwater mussel species at risk (SAR) across southwestern Ontario. The Maitland River watershed was first sampled as part of UMBO in 2008 because of the presence of two Special Concern species: *Cambarunio iris* (Rainbow) and *Lampsilis fasciola* (Wavyrayed Lampmussel). The second survey (first monitoring event) occurred in 2023 and is outlined in this report. Surveys were conducted in areas previously sampled using the same quantitative methodology: 75 m^2 were systematically excavated within 25 individual 15 m^2 blocks (375 m^2 area). A total of 642 mussels representing 10 live species were detected across all sites. Two mussel SAR were found (n = 406), accounting for ~ 63% of the mussel community. Site abundance varied from 24 to 276 mussels and live site species richness ranged from 3 to 9 species. Mean density (± standard error) at each site ranged from 0.32 (± 0.08) to 3.68 (± 0.78) mussels/m^2. Mean species richness at each site ranged between 0.21 (± 0.04) to 1.07 (± 0.10) species/m^2. The most abundant species (and most abundant SAR) was *C. iris* with 335 live mussels observed across all sites. Monitoring of the Maitland River watershed UMBO sites will be significant in tracking changes in the overall mussel community and supporting the recovery of the *C. iris* and *L. fasciola* populations.

RÉSUMÉ

Gibson, M.P., McNichols-O'Rourke, K.A., and Morris, T.J. 2024. 2023 Unionid Monitoring and Biodiversity Observation (UMBO) Network assessment in the Maitland River watershed, Ontario. Can. Data. Rep. Fish. Aquat. Sci. 1395: vii + 36 p.

Les moules d'eau douce de la famille des unionidés ont une grande valeur écologique dans les écosystèmes aquatiques et constituent l'un des taxons les plus menacés à l'échelle mondiale. Géré par Pêches et Océans Canada, le réseau UMBO (Unionid Monitoring and Biodiversity Observation – observation de la biodiversité et surveillance des unionidés) recueille et analyse des données de surveillance à long terme sur les espèces de moules d'eau douce en péril dans le sud-ouest de l'Ontario. Le bassin versant de la rivière Maitland a été échantillonné pour la première fois dans le cadre des activités du réseau UMBO en 2008 en raison de la présence de deux espèces préoccupantes : Cambarunio iris (la villeuse irisée) et Lampsilis fasciola (la lampsile fasciolée). Le deuxième relevé (première activité de surveillance) a eu lieu en 2023 et est décrit dans le présent rapport. Des relevés ont été réalisés dans les zones échantillonnées précédemment en utilisant la même méthodologie quantitative : 75 m2 ont été systématiquement excavés dans 25 blocs individuels de 15 m2 (375 m2). Au total, 642 moules représentant 10 espèces vivantes ont été détectées sur l'ensemble des sites. Deux espèces de moules en péril ont été trouvées (n = 406), représentant ~ 63 % de la communauté de moules. L'abondance dans les sites variait de 24 à 276 moules et la richesse en espèces vivantes des sites variait de 3 à 9 espèces. La densité moyenne (± erreur-type) dans chaque site variait de 0,32 (± 0,08) à 3,68 (± 0,78) moules/m2, et la richesse moyenne en espèces de chaque site était comprise entre 0,21 (± 0,04) et 1,07 (± 0,10) espèces/m2. L'espèce la plus abondante (et l'espèce en péril la plus abondante) était C. iris, avec 335 moules vivantes observées dans l'ensemble des sites. La surveillance des sites du bassin versant de la rivière Maitland visés par l'UMBO sera importante pour suivre les changements dans l'ensemble de la communauté de moules et pour soutenir le rétablissement des populations de C. iris et de L. fasciola.

INTRODUCTION

Unionid freshwater mussels are ecologically valuable in aquatic ecosystems (Vaughn 2018). They are also one of the most globally imperiled taxa and a variety of factors are responsible for their decline including pollution, habitat degradation, invasive species, climate change and the loss of host fishes (Lopes-Lima et al. 2018; Ferreira-Rodríguez et al. 2019; Böhm et al. 2020). Of the 535 freshwater mussel species assessed by the International Union for Conservation of Nature in 2017, a total of 217 (41%) are considered at-risk and 89 (17%) are data deficient (Ferreira-Rodríguez et al. 2019). In North America, 67% of unionid species are classified as threatened and near-threatened (Lopes-Lima et al. 2018).

In Canada, there are 55 species of unionid mussels [Lower Great Lakes Unionid Database (LGLUD) 2024] and 20 species (37%) have been assessed as at-risk (Special Concern, Threatened, Endangered) by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC; Government of Canada 2023). Southwestern Ontario holds the most speciose assemblages of unionid mussels in all of Canada; there are 41 species in Ontario (Metcalfe-Smith et al. 2005), 16 of which, have been assessed as species at risk (SAR) by COSEWIC (Government of Canada 2023).

In 1999, Canada's long-term monitoring program for freshwater mussels was established by Environment Canada (currently Environment and Climate Change Canada; ECCC) within the Sydenham River (Metcalfe-Smith et al. 2007). The federal *Species at Risk Act* (S.C. 2002, c.29) (SARA) was established in 2002 and Fisheries and Oceans Canada (DFO) became the responsible jurisdiction for protecting aquatic species listed under *The Act*. Given this responsibility, the monitoring program transitioned from ECCC to DFO in 2004 and expanded to include seven additional watersheds. The goal of the program was to collect baseline information on freshwater mussel assemblages to track changes through time for a number of variables including density, richness, and habitat changes. The program is now known as the Unionid Monitoring and Biodiversity Observation (UMBO) Network.

The Maitland River flows into Lake Huron with a watershed capacity of approximately 2,500 km^2, with primary land use being agriculture (McGoldrick and Metcalfe-Smith 2004). Timed-search surveys were conducted between 1998 and 2004 by Environment Canada, determining that 12 species inhabit the river (McGoldrick and Metcalfe-Smith 2004; LGLUD 2024; Table 1). Of the sites surveyed, six sites were selected as monitoring sites across the watershed, based on accessibility, total observed abundance, presence of SAR, total abundance of SAR, species richness, and habitat type (Figure 1; Sheldon et al. 2020). The six sites were located across the watershed's tributaries: one site in the Lower Maitland River, one site in the Little Maitland River, one site in the Middle Maitland River, one site in the South Maitland

River, and two sites in the North Maitland River. These were originally surveyed in 2008 by DFO, and Sheldon et al. (2020) provides the detailed results of these surveys. In summary, a total of 443 individuals representing 12 species were found including two species of SAR: *Cambarunio iris* (Rainbow) and *Lampsilis fasciola* (Wavyrayed Lampmussel). These two species are currently listed as Special Concern species under SARA (Government of Canada 2023). The mussel assemblage in the Maitland River watershed was found to be relatively low in both density and abundance during the initial quadrat survey thus meeting criteria outlined in DFO (2006) for critical status.

The information in this data report summarizes the results of the second survey (first monitoring event) for the six UMBO sites in the Maitland River watershed which occurred from June 5 to July 12, 2023.

METHODS

SAMPLING METHODS

Surveys followed the methods of Metcalfe-Smith et al. (2007) in areas previously sampled using the same methodology. The sites surveyed were those described in Sheldon et al. (2020). The 2008 sample areas were located using coordinates, photographs, and site descriptions from the original sampling events and a visual search of the area confirmed the continued presence of mussels within the plot area prior to sampling. A 375 m^2 area was delineated and a systematic sampling approach was performed by dividing the area into 25 blocks, each 3 m x 5 m in size. Using a random number generator, three 1 m^2 quadrats were randomly selected for complete excavation for each block at a given site (Figure 2). The surveyed area was sampled in the downstream to upstream direction and each quadrat was surveyed using the following four stage process: (1) visual search with the naked eye; (2) visual search with a Nuova Rade Aquascope underwater viewer (Lalizas Italia Srl., Busalla, Genova, Italy); (3) manual excavation of the substrate to a depth of 10 – 15 cm; and, (4) final search with an underwater viewer to recover any mussels missed or dislodged during excavation. All live mussels found were identified to species, sexed visually (if sexually dimorphic; Table 1), and measured (maximum length in millimeters) using Vernier calipers. Whole shells and valves of species not found live at the site were identified to species and recorded. After processing, live mussels were returned to the 1 m^2 area from which they were collected. Vouchers (digital or shells) were collected for each species encountered following Morris et al. (2022). Ten individuals could not be positively identified in the field and genetic samples were therefore collected from these animals (9 genetic swabs and 1 whole-animal; Appendix A). Genetic samples were subsequently sent to the Hanner Lab at the University of Guelph for processing where molecular analyses were conducted and sequences compared to reference sequences in the Barcode of Life Data System (BOLD) to confirm identifications (Appendix A;

Hanner pers. comm. 2024; Ratnasingham and Herbert 2007). For genetic swabs, up to 400 µL of the lysis buffer was used; for the whole-animal voucher, a subsample of foot tissue was used (Hanner pers. comm. 2024).

ENVIRONMENTAL DATA COLLECTION

Prior to block delineation, environmental parameters were collected at each site. Air temperature was measured using a Kestrel 2000 Pocket Wind Meter (Nielsen-Kellerman Co., Boothwyn, Pennsylvania, U.S.A.). Water temperature and chemistry data were measured using a YSI EXO2 multiparameter sonde (YSI Inc., Yellow Springs, Ohio, U.S.A.). In addition, quadrat-specific data were collected before and after excavation. Data collected before excavation included: water velocity (m/s) using an OTT MF Pro flow meter (OTT HydroMet, Kempten, Germany); depth (m) using a metre stick; and water clarity (m) using a 0.60 m turbidity tube. Three depth measurements were taken in each quadrat and the average depth was recorded. Data collected after quadrat excavation included a visual assessment of substrate composition (%) using modified Stanfield (2010) substrate class categories: bedrock, boulder (> 250 mm in diameter); cobble (65 – 250 mm); gravel (2 – 65 mm); sand (grainy, 0.06 – 2 mm); silt (floury, < 0.06 mm); clay; muck (soft substrate); and detritus (plant matter). In addition, visual descriptions of the degree of siltation (high, medium, slight, absent), degree of algal growth (high, medium, slight, absent), shading (dense, partly open, open), and the presence or absence of aquatic macrophytes were also recorded for each excavated quadrat. Any errors or information not collected were not included in summaries.

DATA ANALYSIS

Site Density and Richness

Following methods described in Goguen et al. (2022), mean site density (mussels/m^2) was calculated for each site. Density of each block at each site was calculated using the following formula:

$$[1] \qquad D_{block} = \frac{\tau}{A}$$

where τ is the total number of freshwater mussels in the block and A is the total area sampled in the block (i.e., 3 m^2 quadrats). Equation [1] was then used to calculate the mean site density:

$$[2] \qquad D_{site} = \frac{\sum D_{block_i}}{n}$$

where $\sum D_{block_i}$ is the sum of block densities within a site and n is the total number of blocks surveyed at the site.

Species richness (live mussel species/m^2), as described in Goguen et al. (2022), was calculated for each site using the following equation:

$$[3] \qquad SR_{block} = \frac{number\ of\ species}{A}$$

where $number\ of\ species$ represents the total number of species in the block and A is the total area sampled in the block (i.e., 3 m^2 quadrats). Equation [3] was then used to calculate the mean site species richness:

$$[4] \qquad SR_W = \frac{\sum SR_{block_i}}{n}$$

where $\sum SR_{block_i}$ is the sum of the block species richness values within the sampling event and n is the total number of blocks.

Population Size Structure

Length frequency distribution plots were produced for the most abundant species (watershed abundance greater than 50 mussels). Separation between juvenile and adult life stages is indicated visually on each plot. Length axes bins were adjusted to allow for visualization of juvenile cut-offs. Juveniles were defined as individuals under an identified length that was chosen for each species using available data (i.e., preliminary DFO unpublished data, Haag 2012, Haag and Rypel 2011). Age data using shells from southwestern Ontario, including the Maitland River, were used to determine juvenile cut-off lengths for each species when available. However, when local data was not available for a species, data from other populations in North America were used. It is important to note that population size structure can vary widely among species and waterbodies (Haag 2012), and it is most useful to determine these sizes for each species at a waterbody scale.

Proportion of Juveniles

The proportion of juveniles of a species was calculated for the most abundant common species and SAR. It was calculated by dividing the number of juveniles by the total number of individuals of a species found within the watershed (i.e., the summation of all individuals of that species across all sites).

$$[5] \qquad \propto juveniles = \frac{\sum J_i}{n}$$

where $\sum J_i$ is the sum of all juveniles (individuals under the identified juvenile cut-off length) from all sites across the watershed and *n* is the total number of mussels found at all sites across the watershed.

DATA VISUALIZATION

Figures and maps were created in R version 1.1.7 (R Core Team 2022; Posit Team 2023) using the ggplot2 package (Wickham 2016). Additional packages used for map creation were cowplot (Wilke 2020), ggrepel (Slowikowski 2023), ggspatial (Dunnington 2022), maptiles (Giraud 2022), sf (Pebesma 2018), terra (Hijmans 2022), and tidyterra (Hernangomez 2024).

RESULTS

In 2023, six sites were surveyed in the Maitland River watershed through June and July (Table 2). A total of 642 mussels representing 10 live species were observed across all sites (Table 3). Two (n = 406) of these were SAR, and together these species made up ~ 63% of the community. One additional species, *Pyganodon grandis* (Giant Floater), was represented by shells and valves only and was observed at 50% of the sites (Table 3). Previous work (Layer et al. 2022) has identified confusion amongst Maitland River Lampsilini and eight individuals could not be positively identified to species. These individuals were classified as *Lampsilis* sp. and are included in abundances but not species richness values. Among sites, abundance varied from 24 to 276 individuals and live site species richness ranged between three and nine species; however, only one site had fewer than six species. The site with the highest abundance was MR-09 (with a live site species richness of eight), accounting for ~ 43% of the total number of mussels found in the surveys. The site with the highest live species richness (MR-14) had the second highest abundance (156 mussels). For detailed species information including abundance, relative abundance, density, and frequency of occurrence for each species at each site refer to Appendix B.

Mean site density (± standard error) across the Maitland River watershed ranged from 0.32 (± 0.08) to 3.68 (± 0.78) mussels/m^2 (Table 4). Mean site species richness ranged from 0.21 (± 0.04) to 1.07 (± 0.10) species/m^2 (Table 4).

As in 2008 (Sheldon et al. 2020), *Cambarunio iris* was not only the most abundant SAR but also but the most abundant species in the Maitland River watershed with 335 live individuals observed across all six sites (Table 3). The majority (210 individuals) were found at a single site, MR-09 and it was found live at all sites surveyed. Lengths (n = 334) ranged from 9.2 to 72.0 mm with ~ 4% (n = 14) determined to be juveniles (< 22.7 mm, preliminary DFO unpublished data; Figure 3). *Lasmigona costata* (Flutedshell) was the most abundant non-SAR species with 94 individuals observed and was also found live at all sites (Table 3). Lengths (n = 94) ranged from 32.6 to 113.0 mm with ~ 2% (n = 2) individuals classified as juveniles (< 49.2 mm; Appendix C; Figure 4). *Lampsilis fasciola*, a SAR, was the third most abundant species. A total of 71 individuals were found across all sites and it was found live at 5 of 6 sites.

Lengths (n = 71) ranged from 22.7 to 91.6 mm with < 9% (n = 6) classified as juveniles (< 35.8 mm, preliminary DFO unpublished data; Figure 5). There were 55 *Alasmidonta viridis* (Slippershell) individuals across all sites, with lengths (n = 55) ranging from 12.8 to 35.5 mm. No individuals were classified as juveniles (< 11.7 mm; Appendix D; Figure 6).

The mean (± standard error) for air temperature and water temperature at UMBO sites were 21 (± 1)°C and 21.1 (± 1.49)°C, respectively (Table 5). Mean YSI EXO2 water quality parameters were as follows: conductivity was 485.02 (± 18.95) µs/cm, TDS was 341.60 (± 14.55) mg/L, salinity was 0.25 (± 0.01) psu, dissolved oxygen in percent was 119.73% (± 14.96) and in mg/L was 10.53 (± 1.14) mg/L, and pH was 8.22 (± 0.12) (Table 5). Water velocity (m/s) ranged from 0.048 (± 0.004) to 0.36 (± 0.012) and depth (m) from 0.25 (± 0.008) to 0.43 (± 0.012) (Table 6). Water clarity (m) was consistently greater than the turbidity tube used for the survey, which goes to a maximum of 0.60 m, at all quadrats across all sites. Substrate in the Maitland River watershed consisted of high percentages of cobble (23.1 – 38.8%), gravel (20.0 – 34.1%), sand (10.8 – 22.3%), and boulder (6.7 – 29.9%). Descriptive ranges of siltation, algal growth, shading, and presence of aquatic macrophytes are provided in Table 6.

ACKNOWLEDGEMENTS

The authors would like to thank Livia Collinson, Samantha Fitzgerald, and Maxime Vézina-Doré for their field assistance. Thank you to Dr. Robert Hanner and his team at the University of Guelph for processing genetic swabs and whole-animals vouchers. Thank you to Natalie Elliott-Friesen for data organization and table preparation. Thanks to the reviewers, David Andrews and Stephanie Smodis, for providing feedback on this report. Financial support for this project was provided by Fisheries and Oceans Canada’s Species at Risk Program.

REFERENCES

Böhm, M., Dewhurst-Richman, N.I., Seddon, M., Ledger, S.E.H., Albrecht, C., Allen, D., Bogan, A.E., Cordeiro, J., Cummings, K.S., Cuttelod, A., Darrigran, G., Darwall, W., Fehér, Z., Gibson, C., Graf, D.L., Köhler, F., Lopes-Lima, M., Pastorino, G., Perez, K.E., Smith, K., van Damme, D., Vinarski, M.V., von Proschwitz, T., von Rintelen, T., Aldridge, D.C., Aravind, N.A., Budha, P.B., Clavijo, C., Van Tu, D., Gargominy, O., Ghamizi, M., Haase, M., Hilton-Taylor, C., Johnson, P.D., Kebapçı, Ü., Lajtner, J., Lange, C.N., Lepitzki, D.W.A., Martínez-Ortí, A., Moorkens, E.A., Neubert, E., Pollock, C.M., Prié, V., Radea, C., Ramirez, R., Ramos, M.A., Santos, S.B., Slapnik, R., Son, M.O., Stensgaard, A., and Collen, B. 2020. The conservation status of the world's freshwater molluscs. Hydrobiologia **848**:3231–3254. doi:10.1007/s10750-020-04385-w

DFO (Fisheries and Oceans Canada). 2006. Allowable harm analysis workshops for freshwater Species at Risk in Central and Arctic Region; October 18-19, 2005, February 8-9, 2006 and February 13-14, 2006. DFO Can. Sci. Advis. Sec. Proceed. Ser. 2006/026.

Dunnington, D. 2022. ggspatial: spatial data framework for ggplot2. R package version 1.1.7. Available from https://CRAN.R-project.org/package=ggspatial [accessed 19 January 2024].

Ferreira-Rodríguez, N., Akiyama, Y.B., Aksenova, O.V., Araujo, R., Barnhart, M.C., Bespalaya, Y. V., Bogan, A.E., Bolotov, I.N., Budha, P.B., Clavijo, C., Clearwater, S.J., Darrigran, G., Do, V.T., Douda, K., Froufe, E., Gumpinger, C., Henrikson, L., Humphrey, C.L., Johnson, N.A., Klishko, O., Klunzinger, M.W., Kovitvadhi, S., Kovitvadhi, U., Lajtner , J., Lopes-Lima, M., Moorkens, E.A., Nagayama, S., Karl-Otto Nagel, K., Nakanoad, M., Negishi, J.N., Ondina, P., Oulasvirta, P., Prié, V., Riccardi, N., Rudzīte, M., Sheldon, F., Sousan, R., Strayeran, D.L., Takeuchi, M., Taskinen, J.,Teixeira, A., Tiemann, J.S., Urbańska, M., Varandas, S., Vinarski, M.V. Wicklow, B.J., Zając, T., and Vaughn, C.C. 2019. Research priorities for freshwater mussel conservation assessment. Biological Conservation **231**:77-87. doi:10.1016/j.biocon.2019.01.002

Giraud, T. 2022. maptiles: download and display map tiles. R package version 0.4.0. Available from https://CRAN.R-project.org/package=maptiles [accessed 19 January 2024].

Goguen, M.N., McNichols-O'Rourke, K.A., and Morris, T.J. 2022. Tracking the recovery of freshwater mussels in the Saugeen River watershed: A comparison of long-term monitoring sampling events in 2011 and 2019. Canadian Manuscript Report of Fisheries and Aquatic Sciences 3242: vi + 33 p.

Government of Canada. 2023. Species at Risk Public Registry. Available from https://species-registry.canada.ca/index-en.html#/species?sortBy=commonNameSort&sortDirection=asc&currentPage=5&pageSize=10 [accessed 9 January 2024].

Government of Ontario. 2023. Species at Risk in Ontario (SARO) List. Ministry of the Environment, Conservation and Parks. Available from https://www.ontario.ca/page/species-risk-ontario?name=Fish+and+Mussels#section-6 [accessed 9 January 2024].

Haag, W.R. 2012. North American freshwater mussels. Cambridge University Press, New York, New York, U.S.A.

Haag, W.R., and Rypel, A.L. 2011. Growth and longevity in freshwater mussels: evolutionary and conservation implications. Biological Reviews **86**:225-247.

Hanner, R., pers. comm. 2024. E-mail communication with M.P. Gibson. Fisheries and Oceans Canada, Burlington, Ontario. April 2024.

Hernangomez, D. 2024. tidyterra: tidyverse methods and ggplot2 helpers for terra objects. R package version 0.4.0. Available from https://dieghernan.github.io/tidyterra/ [accessed 19 January 2024].

Hijmans, R. 2022. terra: spatial data analysis. R package version 1.7-46. Available from https://CRAN.R-project.org/package=terra [accessed 19 January 2024].

Layer, M., R., Minton, R.L., Morris, T.J., and Zanatta, D.T. 2022. Utility of shell-valve outlines for distinguishing among four Lampsiline mussel species (Bivalvia: Unionidae) in the Great lakes region. Freshwater Mollusk Biology and Conservation **25**:37-53. doi:10.31931/fmbc-d-21-00007

Lopes-Lima, M., Burlakova, L., Karatayev, A., Mehler, K., Seddon, M., and Sousa, R. 2018. Conservation of freshwater bivalves at the global scale: diversity, threats and research needs. Hydrobiologia **810(1)**:1-14. doi:10.1007/s10750-017-3486-7

LGLUD (Lower Great Lakes Unionid Database). 2024. Lower Great Lakes Unionid Database. Microsoft Access 2016. Fisheries and Oceans Canada, Great Lakes Laboratory for Fisheries and Aquatic Sciences, Burlington, Ontario.

McGoldrick, D.J., and Metcalfe-Smith, J.L. 2004. Freshwater mussel communities of the Maitland River, Ontario. Report to the Interdepartmental Recovery Fund, DFO's Species at Risk program (SARCEP), the Maitland Valley Conservation Authority and the Ontario Ministry of Natural Resources. 19 p.

Metcalfe-Smith, J.L., MacKenzie, A., Carmichael, I., and McGoldrick, D. 2005. Photo field guide to the freshwater mussels of Ontario. St. Thomas, Ontario. St. Thomas Field Naturalist Club Inc.

Metcalfe-Smith, J.L., McGoldrick, D.J., Zanatta, D.T., and Grapentine, L.C.. 2007. Development of a monitoring program for tracking the recovery of endangered freshwater mussels in the Sydenham River, Ontario. Prepared for the Sydenham River Recovery Team, the Interdepartmental Recovery Fund and Fisheries and Oceans Canada. NWRI Contribution No. 07-510.

MolluscaBase eds. 2024. MolluscaBase. Available from https://www.molluscabase.org/ [accessed 9 January 2024].

Morris, T.J., Martel, A.L., Wilson, G., Goguen, M.N., McNichols-O'Rourke, K.A., and Colm, J.E. 2022. Guidance on the collection of freshwater mussel (Bivalvia: Unionida) vouchers within Canada. Canadian Manuscript Report of Fisheries and Aquatic Sciences. 3241: v + 15 p.

Pebesma, E. 2018. Simple features for R: standardized support for spatial vector data. The R Journal **10**(1): 439–446. doi:10.32614/RJ-2018-009.

Posit Team. 2023. RStudio: integrated development environment for R. Posit Software, PBC, Boston, MA. Available from http://www.posit.co/ [accessed 19 January 2024].

Ratnasingham, S., and Hebert, P.D. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes, **7(3)**:355-364. doi:10.1111/j.1471-8286.2006.01678.x

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from https://www.R-project.org/ [accessed 19 January 2024].

Slowikowski, K. 2023. ggrepel: automatically position non-overlapping text labels with 'ggplot2'. R package version 0.9.3. Available from https://CRAN.R-project.org/package=ggrepel [accessed 19 January 2024].

Sheldon, M.N., McNichols-O'Rourke, K.A., and Morris, T.J. 2020. Summary of initial surveys at index stations for long-term monitoring of freshwater mussels in southwestern Ontario between 2007 and 2018. Can. Manuscr. Rep. Fish. Aquat. Sci. 3203: vii + 85 p.

Stanfield, L. 2010. Ontario stream assessment protocol. Version 8. Fisheries Policy Section. Ontario Ministry of Natural Resources. Peterborough, Ontario, Canada. Available from https://docs.ontario.ca/documents/2575/226871.pdf [accessed 9 January 2024].

Vaughn, C.C. 2018. Ecosystem services provided by freshwater mussels. Hydrobiologia **810**:15–27. doi:10.1007/s10750-017-3139-x

Wickham, H. 2016. ggplot2: elegant graphics for data analysis. Springer-Verlag New York, 2016. Available from https://ggplot2.tidyverse.org [accessed 19 January 2024].

Wilke, C. 2020. cowplot: streamlined plot theme and plot annotations for 'ggplot2'. R package version 1.1.1. Available from https://CRAN.R-project.org/package=cowplot [accessed 19 January 2024].

Williams, J.D., Bogan, A.E., Butler, R.S., Cummings, K.S., Garner, J.T., Harris, J.L., Johnson, N.A., and Watters, G.T. 2017. A revised list of the freshwater mussels

(Mollusca: Bivalvia: Unionida) of the United States and Canada. Freshwater Mollusk Biology and Conservation **20(2)**:33-58.
doi:10.31931/fmbc.v20i2.2017.33-58

TABLES

Table 1. Mussel species found live (Y) and as shells (SH) in the Maitland River watershed during previous timed-searches (1998-2004), initial survey (2008) and the current survey (2023). Species information regarding the 1998-2004 timed-search surveys is from LGLUD (2024). Species information regarding the 2008 survey is from Sheldon et al. (2020). Watershed Species Richness includes species found only as shells. Species at risk (SAR) are highlighted with their current Committee on the Status of Endangered Wildlife in Canada (COSEWIC) assessment, federal *Species at Risk Act* (SARA) listing (Government of Canada 2023), and provincial *Endangered Species Act* (ESA) listing (Government of Ontario 2023) as of December 2023. Nomenclature here and throughout follows MolluscaBase eds. (2024) for scientific names and Williams et al. (2017) for common names.

Scientific Name	Common Name	1998-2004	2008	2023	COSEWIC Assessment	SARA (Federal)	ESA (Provincial)
Alasmidonta marginata	Elktoe	Y	Y	Y			
Alasmidonta viridis	Slippershell	Y	Y	Y			
Anodontoides ferussacianus	Cylindrical Papershell	Y	Y	-			
*Cambarunio iris**†	Rainbow	Y	Y	Y	Special Concern	Special Concern	Special Concern
*Lampsilis cardium**	Plain Pocketbook	Y	Y	Y			
*Lampsilis fasciola**	Wavyrayed Lampmussel	Y	Y	Y	Special Concern	Special Concern	Threatened
*Lampsilis siliquoidea**	Fatmucket	Y	Y	Y			
Lasmigona compressa	Creek Heelsplitter	Y	Y	Y			
Lasmigona costata	Flutedshell	Y	Y	Y			
Ortmanniana ligamentina	Mucket	Y	Y	Y			
Pyganodon grandis	Giant Floater	Y	Y	SH			
Strophitus undulatus	Creeper	Y	Y	Y			
Watershed Species Richness		**12**	**12**	**11**			

*Sexually dimorphic species
†Species currently listed under SARA and formerly known as: *Villosa iris*

Table 2. Unionid Monitoring and Biodiversity Observation Network sites and dates sampled in the Maitland River watershed in southwestern Ontario. Dotted lines represent divisions between sub-watersheds. Sites are presented in western to eastern order by their sub-watershed and in downstream to upstream order within each sub-watershed.

Site Code	Drainage	Waterbody	Sub-watershed	Latitude	Longitude	Original Survey Date	Date Surveyed
MR-01	Lake Huron	Maitland River	Lower Maitland River	43.7736111	-81.540278	30-Jul-2008	04-Jul-2023
MR-09	Lake Huron	South Maitland River	South Maitland River	43.6846	-81.54101	31-Jul-2008	10-Jul-2023
MR-16	Lake Huron	Middle Maitland River	Middle Maitland River	43.85998	-81.31941	12-Aug-2008	13-Jun-2023
MR-14	Lake Huron	Little Maitland River	Little Maitland River	43.80927	-81.14052	22-Jul-2008	07-Jun-2023
MR-02	Lake Huron	Maitland River	North Maitland River	43.8872	-81.28238	17-Jul-2008	27-Jun-2023
MR-21	Lake Huron	Maitland River		43.85602	-80.98267	16-Jul-2008	05-Jun-2023

Table 3. Species information for each UMBO site surveyed in the Maitland River watershed in 2023. Sites are presented in western to eastern order by their sub-watershed and in downstream to upstream order within each sub-watershed. Species at risk (SAR) are highlighted in grey. S(#) represents species found as complete shells and the number of shells found. V(#) represents species found as valves (one half of a complete shell) and the number of valves found. *Lampsilis* sp. is included in the abundance totals but is not included in the species richness totals. Total Site Species Richness includes both live species and species found only as shells.

Scientific name	Common Name	MR-01	MR-09	MR-16	MR-14	MR-02	MR-21	Watershed Abundance
Alasmidonta marginata	Elktoe	7	2	3	12	1	(V)1	25
Alasmidonta viridis	Slippershell	22	21	1	4	5	2	55
Cambarunio iris	Rainbow	9	210	65	19	13	19	335[+]
Lampsilis cardium	Plain Pocketbook	6	8	8	23	2	(V)3	46
Lampsilis fasciola	Wavyrayed Lampmussel	6	26	11	24	4	0	71
Lampsilis siliquoidea	Fatmucket	0	0	1	0	0	0	1
Lampsilis sp.			3	1	3			8
Lasmigona compressa	Creek Heelsplitter	0	2	0	1	0	(V)1	3
Lasmigona costata	Flutedshell	7	3	12	68	1	3	94
Ortmanniana ligamentina	Mucket	0	1	1	1	0	0	3
Pyganodon grandis	Giant Floater	0	S(1)	0	0	(V)1	(V)2	(V)3, (S)1
Strophitus undulatus	Creeper	0	V(1)	0	1	0	0	1
	Site Abundance	**57**	**276**	**103**	**156**	**26**	**24**	**642**
	Live Site Species Richness	**6**	**8**	**8**	**9**	**6**	**3**	**10**
	Total Site Species Richness	**6**	**10**	**8**	**8**	**7**	**7**	**11**

[+]Total number of individuals observed is higher than the number of individuals in the length frequency distribution (Figure 3) because length data were not available for all individuals.

Table 4. Density (mussels/m^2) and species richness (Richness, live mussel species/m^2) ± standard error for each UMBO site sampled during 2023 surveys in the Maitland River watershed, Ontario. Species richness does not include *Lampsilis* sp. Sites are presented in western to eastern order by their sub-watershed and in downstream to upstream order within each sub-watershed.

	MR-01		**MR-09**		**MR-16**		**MR-14**		**MR-02**		**MR-21**	
Block	Density	Richness	Density	Richness	Density	Richness	Density	Richness	Density	Richness	Density	Richness
1	1.00	0.67	1.33	1.00	1.67	0.67	0.33	0.33	0.33	0.33	0.00	0.00
2	0.00	0.00	1.67	0.67	1.67	0.67	1.00	1.00	0.00	0.00	1.33	0.33
3	0.33	0.33	5.33	1.33	3.67	1.00	5.33	2.00	0.67	0.67	0.67	0.33
4	0.33	0.33	1.67	0.67	2.33	1.33	1.33	0.67	0.67	0.67	0.67	0.33
5	1.00	0.67	3.00	1.00	1.67	0.33	2.33	1.00	0.67	0.67	0.00	0.00
6	1.67	1.00	1.33	0.67	1.00	0.33	4.00	1.67	0.00	0.00	0.00	0.00
7	0.33	0.33	0.00	0.00	2.00	1.00	2.00	1.00	0.33	0.33	0.33	0.33
8	0.33	0.33	1.00	0.33	1.00	0.67	2.00	1.33	0.33	0.33	0.00	0.00
9	1.33	0.67	2.33	0.67	1.00	0.67	3.00	1.00	1.00	0.33	0.33	0.33
10	0.00	0.00	3.00	0.67	1.00	0.33	1.00	1.00	0.33	0.33	0.33	0.33
11	0.33	0.33	3.67	0.67	2.33	0.67	4.00	1.67	0.00	0.00	0.33	0.33
12	0.00	0.00	4.67	1.33	1.00	0.33	2.33	1.33	0.33	0.33	1.33	0.67
13	0.33	0.33	1.33	0.33	0.33	0.33	3.00	1.33	0.00	0.00	0.00	0.00
14	0.33	0.33	1.00	0.33	1.00	0.67	1.00	0.33	0.33	0.33	0.00	0.00
15	0.67	0.33	4.33	1.00	2.00	1.33	1.33	1.00	1.00	0.67	0.33	0.33
16	1.33	1.00	16.67	1.67	1.33	1.00	1.00	0.67	0.00	0.00	0.00	0.00
17	1.33	1.00	0.67	0.67	0.67	0.67	2.67	1.00	0.00	0.00	0.33	0.33
18	1.00	1.00	1.33	0.67	0.00	0.00	1.00	1.00	0.00	0.00	0.33	0.33
19	2.33	1.33	3.33	1.33	1.33	0.33	2.33	1.00	0.33	0.33	0.67	0.33
20	0.00	0.00	9.33	1.33	0.00	0.00	2.67	1.67	0.67	0.67	0.33	0.33
21	0.33	0.33	1.33	0.67	0.33	0.33	3.33	1.33	0.33	0.33	0.33	0.33
22	0.67	0.67	2.00	1.00	1.33	1.00	1.33	0.67	0.67	0.33	0.33	0.33
23	2.33	1.67	9.00	1.67	3.00	1.00	3.00	2.00	0.00	0.00	0.00	0.00
24	1.00	1.00	2.00	0.67	0.33	0.33	0.33	0.33	0.67	0.67	0.00	0.00
25	0.67	0.67	10.67	1.67	2.33	1.00	0.33	0.33	0.00	0.00	0.00	0.00
Mean	**0.76**	**0.57**	**3.68**	**0.88**	**1.37**	**0.64**	**2.08**	**1.07**	**0.35**	**0.29**	**0.32**	**0.21**
Standard Error	**0.13**	**0.09**	**0.78**	**0.09**	**0.18**	**0.07**	**0.26**	**0.10**	**0.07**	**0.05**	**0.08**	**0.04**

Table 5. Environmental parameters collected at each UMBO site during 2023 surveys in the Maitland River watershed, Ontario. Sites are presented in western to eastern order by their sub-watershed and in downstream to upstream order within each sub-watershed.

Environmental Parameter	MR-01	MR-09	MR-16	MR-14	MR-02	MR-21	Mean (± Standard Error)
Air Temperature (°C)	25.0	23.0	20.0	19.0	19.0	20.0	**21.0 ± 1.0**
Water Temperature (°C)	25.9	23.29	15.2	21.773	21.27	19.225	**21.11 ± 1.49**
Conductivity (µs/cm)	569.0	431.6	488.7	456.0	482.5	482.3	**485.02 ± 18.95**
Total Dissolved Solids (mg/L)	363.55	289.92	390.6	315.53	338.08	351.9	**341.60 ± 14.55**
Salinity (psu)	0.27	0.21	0.29	0.23	0.25	0.26	**0.25 ± 0.01**
Dissolved Oxygen (%)	143.1	149.7	76	162.8	85.7	101.1	**119.73 ± 14.96**
Dissolved Oxygen (mg/L)	11.63	12.76	7.6	14.28	7.58	9.32	**10.53 ± 1.14**
pH	8.29	8.37	7.88	8.7	7.99	8.09	**8.22 ± 0.12**

Table 6. The mean ± standard error and descriptive range of environmental data collected during quadrat excavation at each UMBO site during 2023 surveys in the Maitland River, Ontario. Sites are presented in western to eastern order by their sub-watershed and in downstream to upstream order within each sub-watershed.

		MR-01	MR-09	MR-16	MR-14	MR-02	MR-21
	Water Velocity (m/s)	0.048 ± 0.004	0.11 ± 0.007	0.34 ± 0.01	0.21 ± 0.03	0.36 ± 0.01	0.28 ± 0.02
	Depth (m)	0.33 ± 0.01	0.43 ± 0.01	0.38 ± 0.02	0.3204 ± 0.01	0.36 ± 0.009	0.25 ± 0.008
	Water Clarity (m)	> 0.6	> 0.6	> 0.6	> 0.6	> 0.6	> 0.6
Substrate Composition (%)	Bedrock	0.33 ± 0.24	4.37 ± 1.09	22.31 ± 3.98	0.00 ± 0.00	0.20 ± 0.11	0.00 ± 0.00
	Boulder (> 250 mm)	9.79 ± 0.98	29.87 ± 1.51	20.89 ± 2.16	8.66 ± 1.19	6.73 ± 0.57	16.69 ± 0.99
	Cobble (65-250 mm)	29.74 ± 1.21	26.07 ± 0.94	23.13 ± 1.65	29.86 ± 1.57	38.81 ± 1.58	29.36 ± 1.13
	Gravel (2-65 mm)	30.04 ± 1.25	20.4 ± 0.83	20.04 ± 1.27	34.14 ± 1.28	29.63 ± 1.14	31.88 ± 0.88
	Sand (0.06-2 mm)	19.52 ± 0.92	16.03 ± 0.99	10.8 ± 1.07	22.27 ± 1.42	18.7 ± 1.08	21.41 ± 1.10
	Silt (< 0.06 mm)	7.49 ± 0.64	1.93 ± 0.66	0.00 ± 0.00	1.67 ± 0.45	0.13 ± 0.09	0.08 ± 0.08
	Clay	0.067 ± 0.067	0.067 ± 0.07	0.07 ± 0.07	1.07 ± 0.47	0.13 ± 0.13	0.15 ± 0.10
	Muck	1.027 ± 0.43	0.93 ± 0.40	0.00 ± 0.00	1.67 ± 0.45	0.2 ± 0.2	0.16 ± 0.16
	Detritus	0.67 ± 0.22	0.33 ± 0.17	0.09 ± 0.09	0.67 ± 0.26	0.13 ± 0.09	0.27 ± 0.16
	Siltation	medium-heavy	slight-heavy	slight-heavy	slight-heavy	slight-heavy	slight-heavy
	Algal Growth	slight-medium	slight-medium	absent-medium	absent-heavy	slight-medium	absent-heavy
	Shading	open	open	open	open	open	open-dense
	Aquatic Macrophytes	absent-present	absent-present	absent	absent-present	absent-present	absent

FIGURES

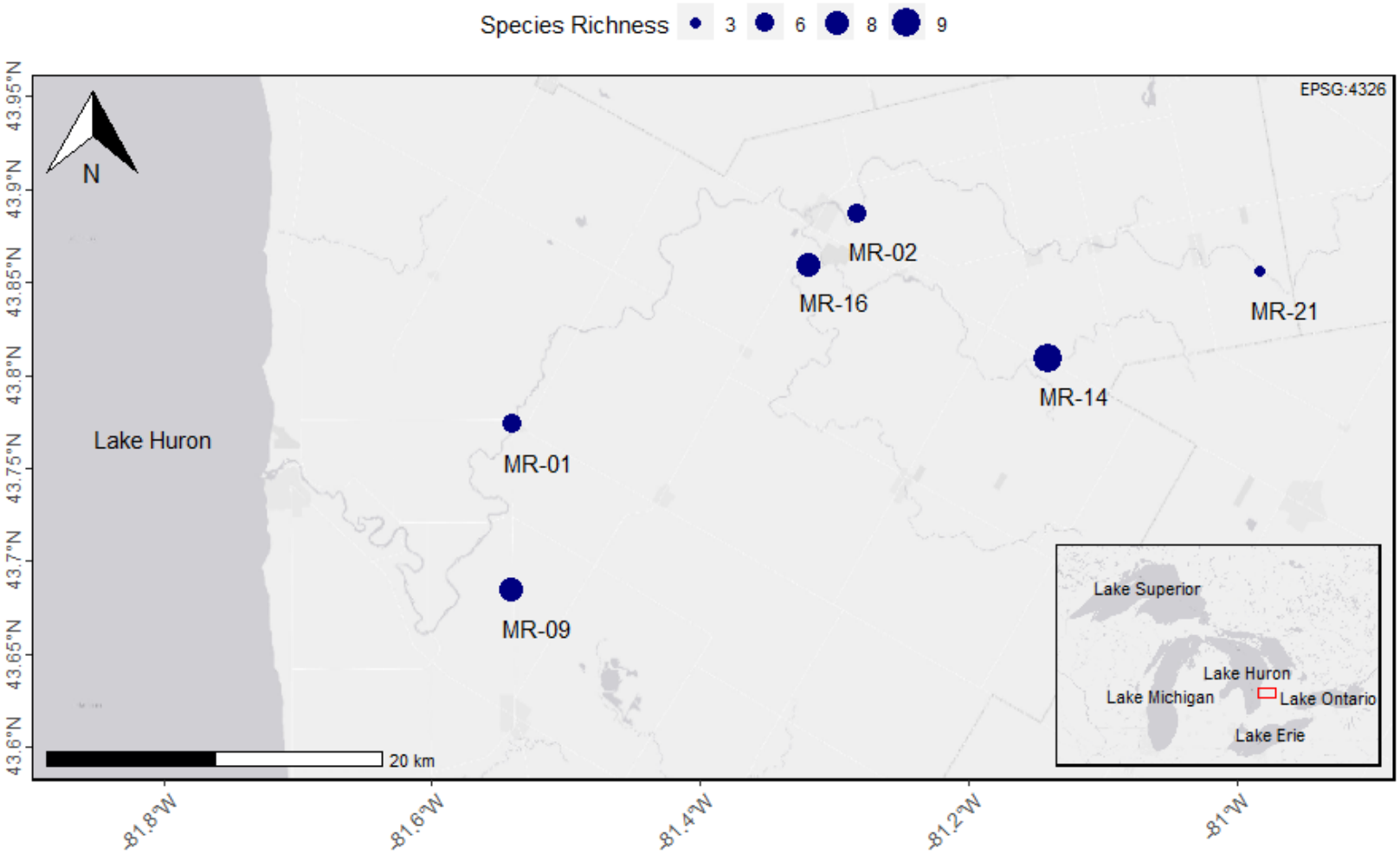


Figure 1. Six Unionid Monitoring and Biodiversity Observation (UMBO) Network sites surveyed in the Maitland River watershed in 2023. Originally established and surveyed in 2008 (Sheldon et al. 2020). Species Richness represents live site species richness (number of live mussel species) from the 2023 surveys. Map tiles by Esri, under CC BY 4.0. Data by OpenStreetMap, under ODbL.

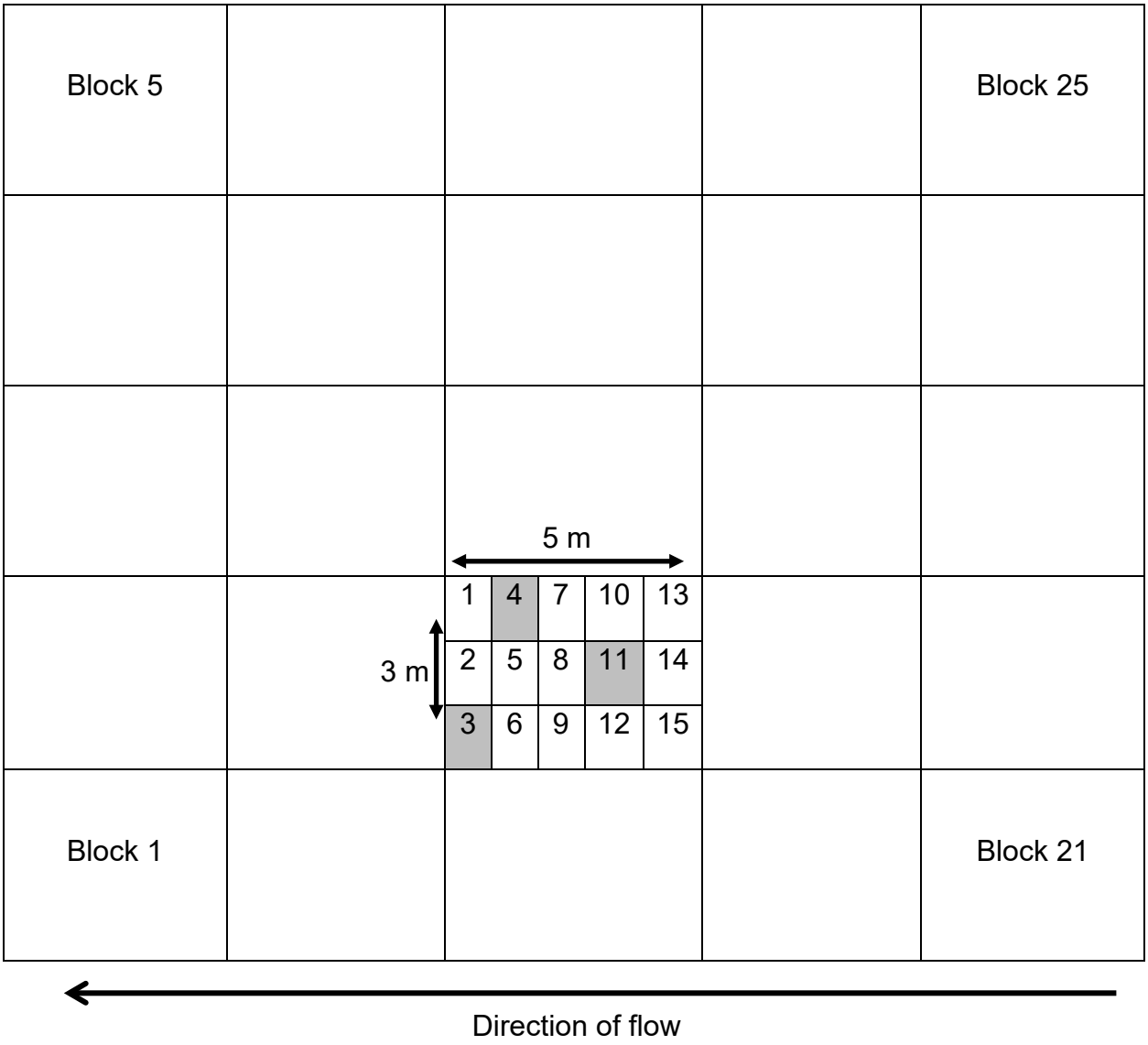


Figure 2. Systematic sampling design (Metcalfe-Smith et al. 2007) implemented at UMBO sites surveyed in the Maitland River watershed using 1 m^2 quadrats in a block setup. The shaded boxes represent the three randomly selected quadrats for excavation, which were excavated within each block.

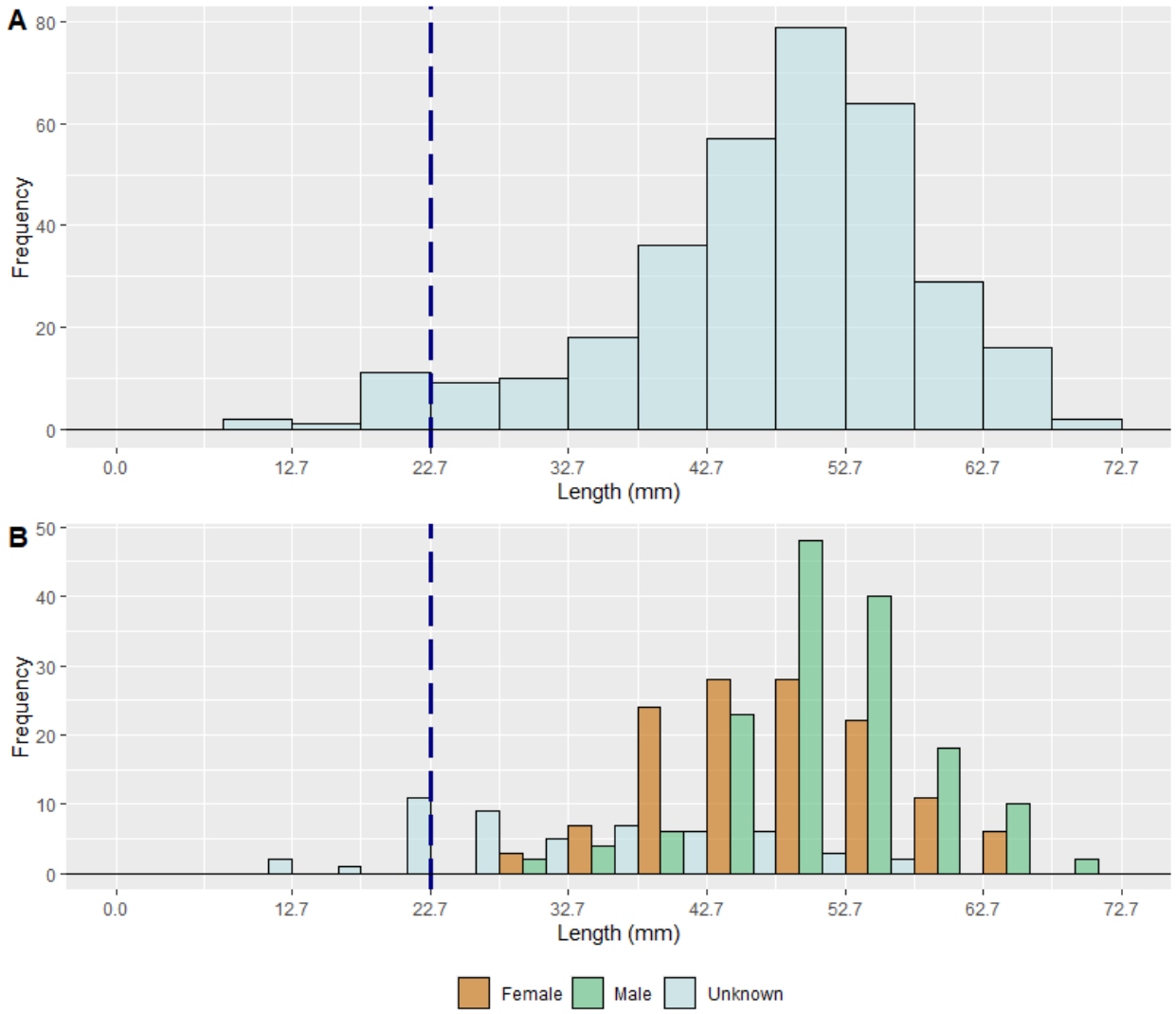


Figure 3. Length distribution of *Cambarunio iris* (Rainbow) found in the Maitland River watershed (n = 334) during 2023 UMBO surveys with (A) all individuals combined and (B) sexes separated. Unknown represents individuals that could not be sexed. Dashed line represents individuals classified as juveniles (< 22.7 mm, preliminary DFO unpublished data).

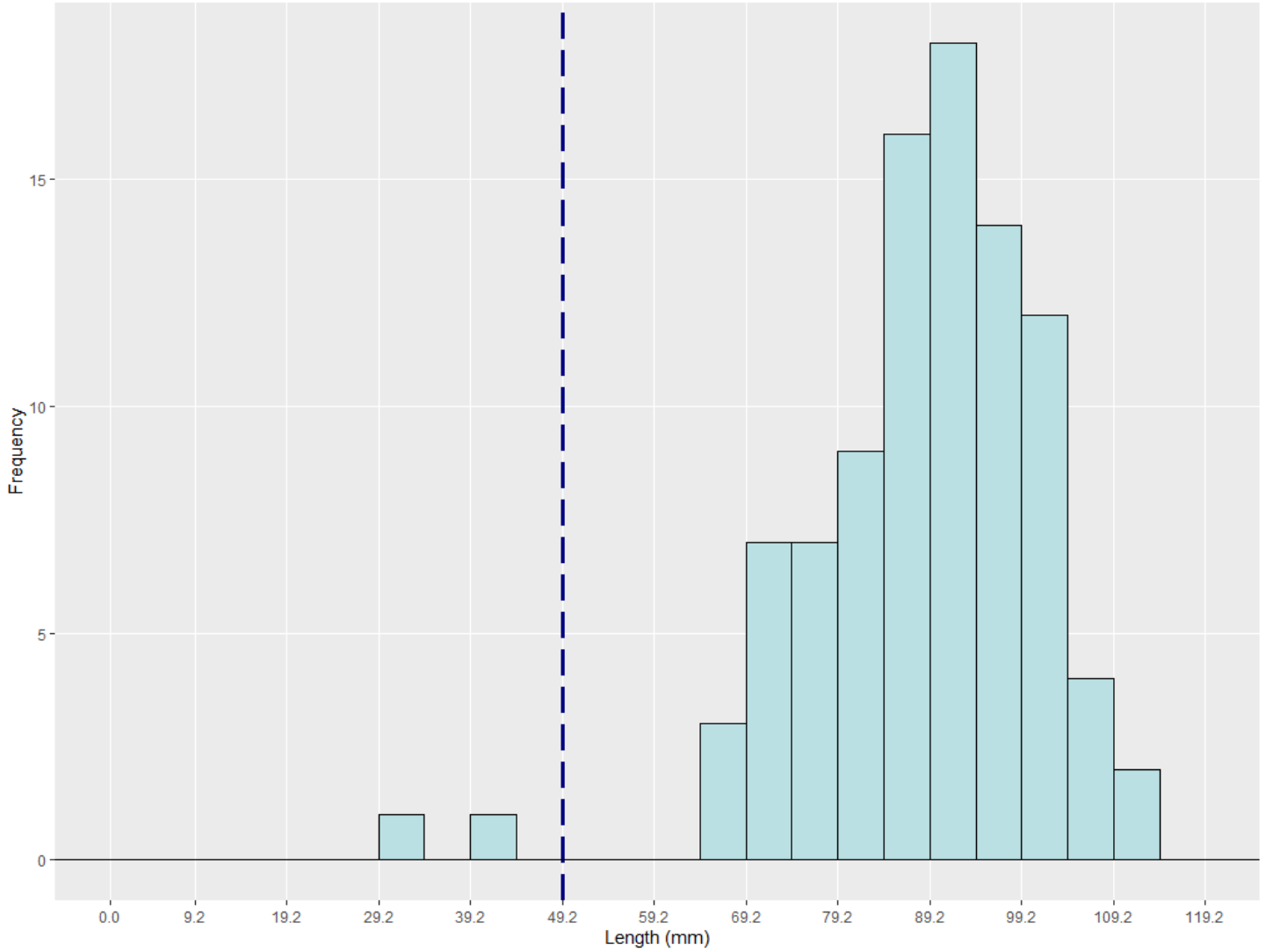


Figure 4. Length distribution of *Lasmigona costata* (Flutedshell) found in the Maitland River watershed (n = 94) during 2023 UMBO surveys. Line represents individuals classified as juveniles (< 49.2 mm) based on calculations and parameters from Haag and Rypel (2011) and Haag (2012).

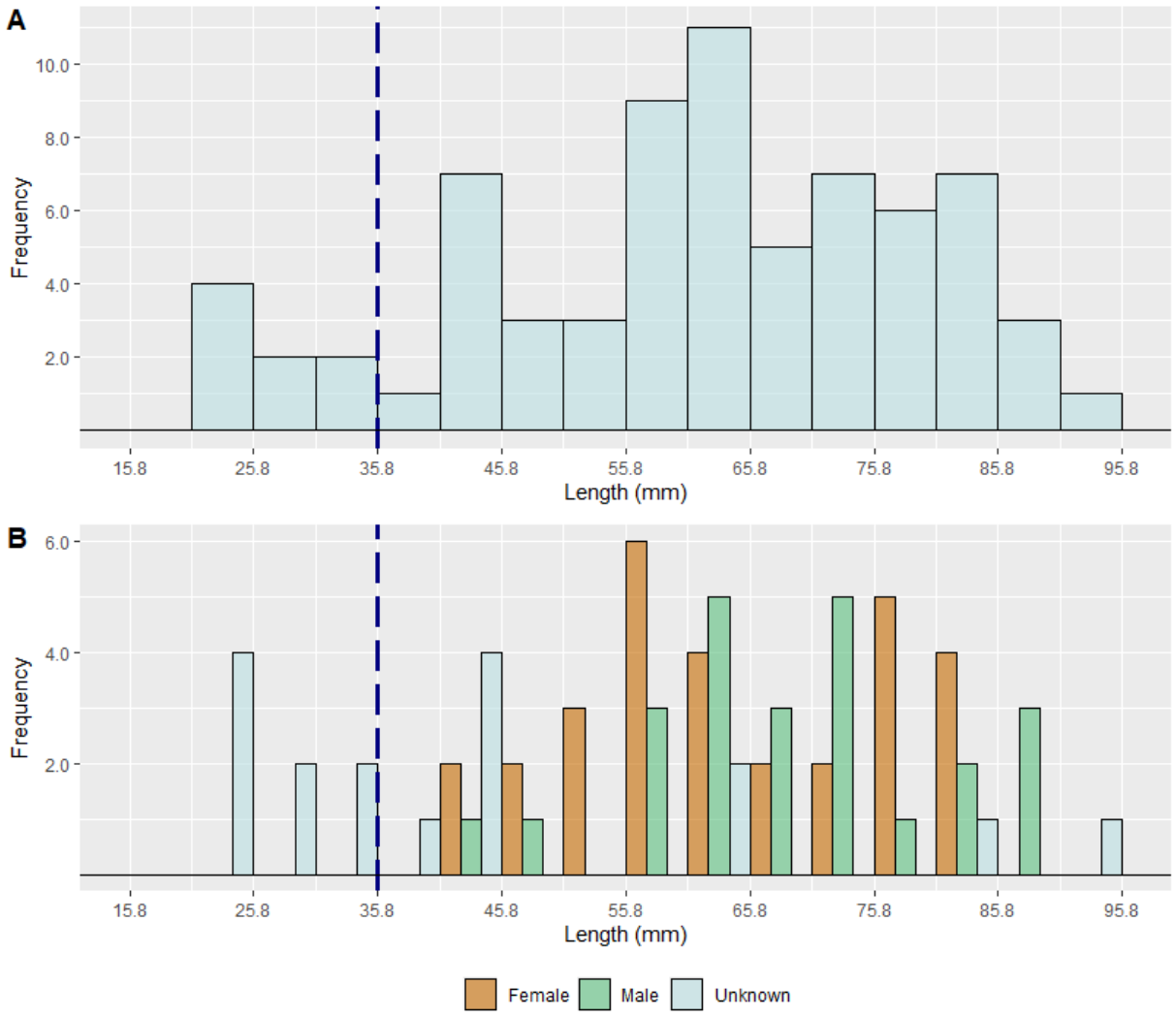


Figure 5. Length distribution of *Lampsilis fasciola* (Wavyrayed Lampmussel) found in the Maitland River watershed (n = 71) during 2023 UMBO surveys with (A) all individuals combined and (B) sexes separated. Unknown represents individuals that could not be sexed. Dashed line represents individuals classified as juveniles (< 35.8 mm, preliminary DFO unpublished data).

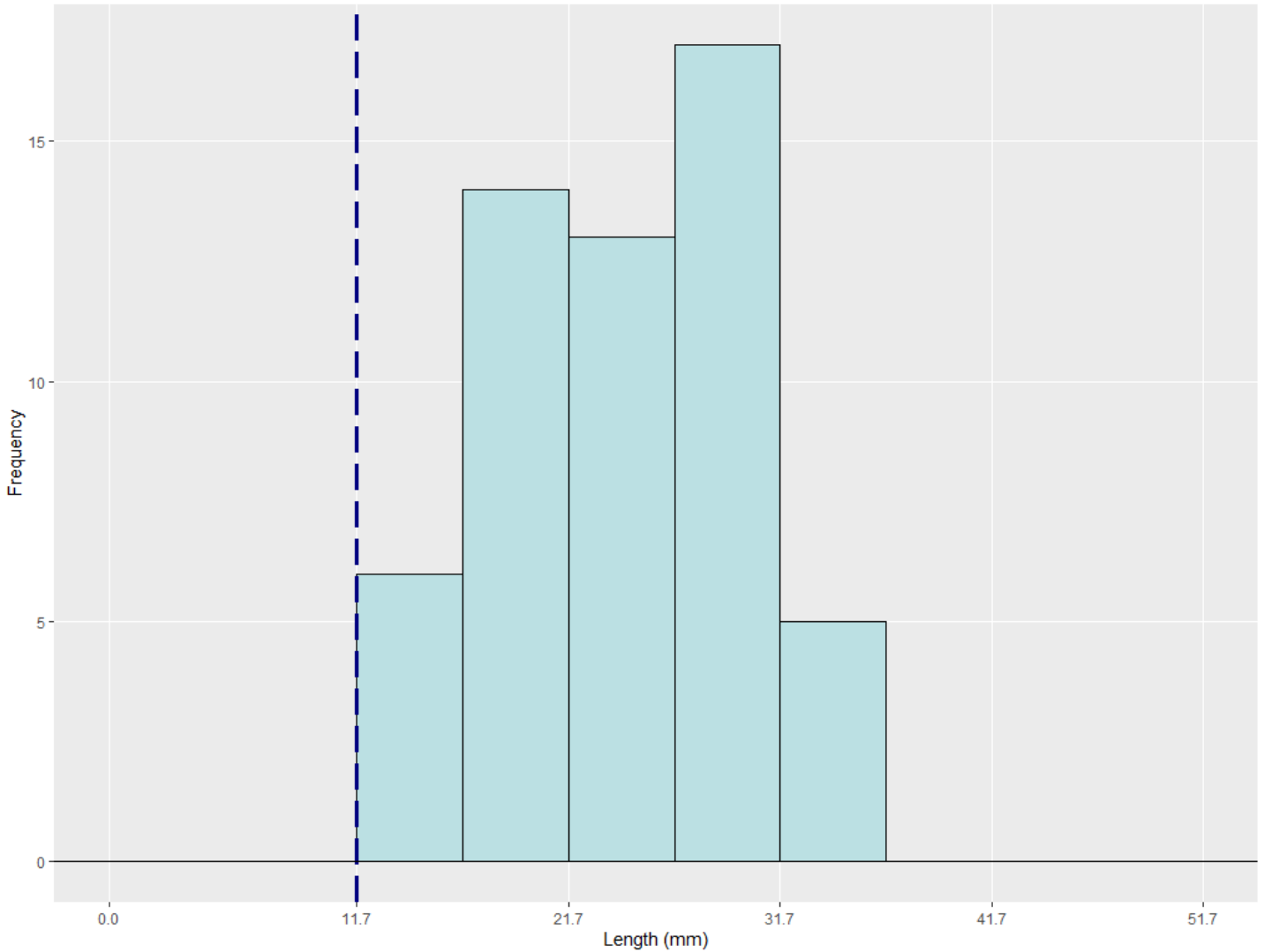


Figure 6. Length distribution of *Alasmidonta viridis* (Slippershell) found in the Maitland River watershed (n = 55) during 2023 UMBO surveys. Line represents individuals classified as juveniles (< 11.7 mm) based on calculations and parameters from Haag and Rypel (2011) and Haag (2012).

APPENDICES

Appendix A. Summary of results of molecular analyses from Hanner Lab at University of Guelph (Hanner pers. comm. 2024). Dashes represent individuals that could not be identified from the specified method. Visual Identification represents individuals identified in the field or from digital vouchers. Digital vouchers provided following table.

Site Code	Voucher ID	Length (mm)	Sex	Genetic Voucher Type	Genetic Result from Hanner Lab	Visual Identification
MR-09	LS-04	85.4	M	Swab	-	*Lampsilis* sp.*
	LS-05	94.4	M	Swab	-	*Lampsilis* sp.*
MR-14	AV-00	15.7	U	Whole-Animal	*Alasimidonta viridis*	*Alasimidonta viridis*
	CI-02	72	M	Swab	*Cambarunio iris*	*Cambarunio iris*
	LC-01	81.5	M	Swab	-	*Lampsilis* sp.*
	LS-01	113.2	M	Swab	-	*Lampsilis* sp.*
	LS-02	101	U	Swab	*Lampsilis cardium*	-
	LC-02	103	M	Swab	-	*Lampsilis* sp.*
MR-16	OL-01	113.1	U	Swab	-	*Ortmanniana ligamentina*†
	LS-03	104.7	M	Swab	-	*Lampsilis* sp.*

*Could not be confirmed genetically, identified to genus
†Could not be confirmed genetically, identified to species from digital vouchers

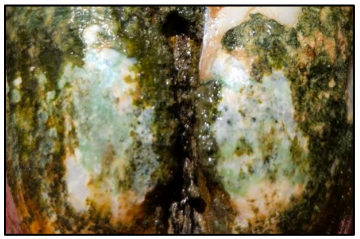
Voucher ID	Lateral View of Left Valve	Wide Dorsal View	Dorsal Close-Up View
LS-04			
LS-05			

Voucher ID	Lateral View of Left Valve	Wide Dorsal View	Dorsal Close-Up View
AV-00			
CI-02			

Voucher ID	Lateral View of Left Valve	Wide Dorsal View	Dorsal Close-Up View
LC-01			
LS-01		-	-

Voucher ID	Lateral View of Left Valve	Wide Dorsal View	Dorsal Close-Up View
LS-02			

Voucher ID	Lateral View of Left Valve	Wide Dorsal View	Dorsal Close-Up View
LC-02			

Voucher ID	Lateral View of Left Valve	Wide Dorsal View	Dorsal Close-Up View
OL-01			

Voucher ID	Lateral View of Left Valve	Wide Dorsal View	Dorsal Close-Up View
LS-03			

Appendix B. Site specific information on species abundance (number of mussels), relative abundance (%), density (mussel/m^2), and occurrence (% of individuals across quadrats). Species at risk (SAR) are highlighted in grey. S(#) represents species found as complete shells (i.e., two valves) and the number of shells found. V(#) represents species found as valves (i.e., one half of a complete shell) and the number of valves found. Sites are presented in western to eastern order by their sub-watershed and in downstream to upstream order within each sub-watershed.

Site MR-01

Species	Abundance (No. of mussels)	Relative Abundance (%)	Density (mussels/m^2)	Occurrence (% of quadrats)
Alasmidonta marginata	7	12.28	0.09	8.00
Alasmidonta viridis	22	38.60	0.29	24.00
Cambarunio iris	9	15.79	0.12	10.67
Lampsilis cardium	6	10.53	0.08	8.00
Lampsilis fasciola	6	10.53	0.08	8.00
Lampsilis siliquoidea	0	0.00	0.00	0.00
Lampsilis sp.	0	0.00	0.00	0.00
Lasmigona compressa	0	0.00	0.00	0.00
Lasmigona costata	7	12.28	0.09	9.33
Ortmanniana ligamentina	0	0.00	0.00	0.00
Pyganodon grandis	0	0.00	0.00	0.00
Strophitus undulatus	0	0.00	0.00	0.00

Site MR-09

Species	Abundance (No. of mussels)	Relative Abundance (%)	Density (mussels/m^2)	Occurrence (% of quadrats)
Alasmidonta marginata	2	0.72	0.03	2.67
Alasmidonta viridis	21	7.61	0.28	20.00
Cambarunio iris	210	76.09	2.80	73.33
Lampsilis cardium	8	2.90	0.11	10.67
Lampsilis fasciola	26	9.42	0.35	29.33
Lampsilis siliquoidea	0	0.00	0.00	0.00
Lampsilis sp.	3	1.09	0.04	4.00
Lasmigona compressa	2	0.72	0.03	1.33
Lasmigona costata	3	1.09	0.04	4.00
Ortmanniana ligamentina	1	0.36	0.01	1.33
Pyganodon grandis	S(1)	0.00	0.00	0.00
Strophitus undulatus	V(1)	0.00	0.00	0.00

Appendix B. Continued.

Site MR-16				
Species	**Abundance (No. of mussels)**	**Relative Abundance (%)**	**Density (mussels/m²)**	**Occurrence (% of quadrats)**
Alasmidonta marginata	3	2.91	0.04	4.00
Alasmidonta viridis	1	0.97	0.01	1.33
Cambarunio iris	65	63.11	0.87	49.33
Lampsilis cardium	8	7.77	0.11	8.00
Lampsilis fasciola	11	10.68	0.15	12.00
Lampsilis siliquoidea	1	0.97	0.01	1.33
Lampsilis sp.	1	0.97	0.01	1.33
Lasmigona compressa	0	0.00	0.00	0.00
Lasmigona costata	12	11.65	0.16	10.67
Ortmanniana ligamentina	1	0.97	0.01	1.33
Pyganodon grandis	0	0.00	0.00	0.00
Strophitus undulatus	0	0.00	0.00	0.00

Site MR-14				
Species	**Abundance (No. of mussels)**	**Relative Abundance (%)**	**Density (mussels/m²)**	**Occurrence (% of quadrats)**
Alasmidonta marginata	12	7.69	0.16	13.33
Alasmidonta viridis	4	2.56	0.05	4.00
Cambarunio iris	19	12.18	0.25	20.00
Lampsilis cardium	23	14.74	0.31	28.00
Lampsilis fasciola	24	15.38	0.32	25.33
Lampsilis siliquoidea	0	0.00	0.00	0.00
Lampsilis sp.	3	1.92	0.04	4.00
Lasmigona compressa	1	0.64	0.01	1.33
Lasmigona costata	68	43.59	0.91	56.00
Ortmanniana ligamentina	1	0.64	0.01	1.33
Pyganodon grandis	0	0.00	0.00	0.00
Strophitus undulatus	1	0.64	0.01	1.33

Appendix B. Continued.

Site MR-02				
Species	**Abundance (No. of mussels)**	**Relative Abundance (%)**	**Density (mussels/m^2)**	**Occurrence (% of quadrats)**
Alasmidonta marginata	1	3.85	0.01	1.33
Alasmidonta viridis	5	19.23	0.07	5.33
Cambarunio iris	13	50.00	0.17	14.67
Lampsilis cardium	2	7.69	0.03	2.67
Lampsilis fasciola	4	15.38	0.05	5.33
Lampsilis siliquoidea	0	0.00	0.00	0.00
Lampsilis sp.	0	0.00	0.00	0.00
Lasmigona compressa	0	0.00	0.00	0.00
Lasmigona costata	1	3.85	0.01	1.33
Ortmanniana ligamentina	0	0.00	0.00	0.00
Pyganodon grandis	(V)1	0.00	0.00	0.00
Strophitus undulatus	0	0.00	0.00	0.00

Site MR-21				
Species	**Abundance (No. of mussels)**	**Relative Abundance (%)**	**Density (mussels/m^2)**	**Occurrence (% of quadrats)**
Alasmidonta marginata	(V)1	0.00	0.00	0.00
Alasmidonta viridis	2	8.33	0.03	2.67
Cambarunio iris	19	79.17	0.25	21.33
Lampsilis cardium	(V)3	0.00	0.00	0.00
Lampsilis fasciola	0	0.00	0.00	0.00
Lampsilis siliquoidea	0	0.00	0.00	0.00
Lampsilis sp.	0	0.00	0.00	0.00
Lasmigona compressa	(V)1	0.00	0.00	0.00
Lasmigona costata	3	12.50	0.04	4.00
Ortmanniana ligamentina	0	0.00	0.00	0.00
Pyganodon grandis	(V)2	0.00	0.00	0.00
Strophitus undulatus	0	0.00	0.00	0.00

Appendix C. Calculation of *Lasmigona costata* (Flutedshell) length at age-at-first-maturity (i.e., juvenile cut-off).

Using the equation modified from Haag (2012):

(1) $$t_{mat} = 0.690K^{-1.031} - 1$$

And the von Bertalanffy growth equation modified from Haag and Rypel (2011):

(2) $$L_{mat} = L_{inf}(1 - e^{-K(t_{mat} - t_0)})$$

where t_{mat} is the age-at-first-maturity, K is the growth constant, L_{mat} is the length (mm) at age-at-first-maturity, L_{inf} is the length (mm) at time infinity, and t_0 is the time at which length = 0.

Using equation (1), age-at-first-maturity is 1.88.

Using the value for age-at-first-maturity in equation (2) and the defined parameters from Table 1 of Haag and Rypel (2011)*:

$$L_{inf} = 133.9$$

$$K = 0.250$$

$$t_0 = 0.049$$

Length at age-at-first-maturity for *Lasmigona costata* is 49.2 mm.

*Note: these parameters are from populations in Licking River, Kentucky, USA.

Appendix D. Calculation of *Alasmidonta viridis* (Slippershell) length at age-at-first-maturity (i.e., juvenile cut-off).

Using the equation modified from Haag (2012):

(1) $$t_{mat} = 0.690K^{-1.031} - 1$$

And the von Bertalanffy growth equation modified from Haag and Rypel (2011):

(2) $$L_{mat} = L_{inf}(1 - e^{-K(t_{mat} - t_0)})$$

where t_{mat} is the age-at-first-maturity, K is the growth constant, L_{mat} is the length (mm) at age-at-first-maturity, L_{inf} is the length (mm) at time infinity, and t_0 is the time at which length = 0.

Using equation (1), age-at-first-maturity is 0.70.

Using the value for age-at-first-maturity in equation (2) and the defined parameters from Table 1 of Haag and Rypel (2011)*:

$$L_{inf} = 34.6$$

$$K = 0.417$$

$$t_0 = -0.290$$

Length at age-at-first-maturity for *Alasmidonta viridis* is 11.7 mm.

*Note: these parameters are from populations in Brushy Creek, Kentucky, USA.