

Freshwater mussel (Unionidae) timed-search surveys in Medway Creek: the search for *Ptychobranchnus* *fasciolaris* (Kidneyshell)

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 Road
Burlington, ON
L7S 1A1

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Ontario and Prairie Region
Fisheries and Oceans Canada
Great Lakes Laboratory for Fisheries and Aquatic Sciences
867 Lakeshore Road
Burlington, ON
L7S 1A1

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ABSTRACT

Gibson, M.P., McNichols-O'Rourke, K.A., and Morris, T.J. 2025. Freshwater mussel (Unionidae) timed-search surveys in Medway Creek: the search for *Ptychobranthus fasciolaris* (Kidneyshell). Can. Manuscr. Rep. Fish. Aquat. Sci. 3304: vii + 39 p. <https://doi.org/10.60825/chmq-cz47>

Ptychobranthus fasciolaris (Kidneyshell) is currently listed as Endangered under the federal *Species at Risk Act* (SARA). There have been numerous shell records of *P. fasciolaris* in the Thames River watershed but only 4 live individuals found. All live captures have occurred in Medway Creek, a tributary of the North Thames River. In 2024, Fisheries and Oceans Canada (DFO) sampled 15 sites throughout Medway Creek to assess the current unionid assemblage and determine if live *P. fasciolaris* remain. A semi-quantitative timed-search survey was conducted at each site by a three- to six-person team for a total 4.5 person-hour effort. A total of 1534 live mussels of 14 species, including two species at risk (SAR), *Cambarunio iris* (Rainbow) and *Lampsilis fasciola* (Wavyrayed Lampmussel), were observed. There were no live *P. fasciolaris* individuals found, but 3 whole shells and 2 valves were found at three sites. Medway Creek supports a moderately abundant and highly diverse mussel assemblage; however, there are several pollutant tolerant species and the relative abundance of these species has shifted over time. Sites with *P. fasciolaris* records (live or shell) have intact riparian buffers of >30 m and water temperature was significantly lower at sites with live SAR present compared to sites without live SAR. These data provide an understanding of the current mussel assemblage in Medway Creek and can be used to inform a potential reintroduction of *P. fasciolaris* to this system.

RÉSUMÉ

Gibson, M.P., McNichols-O'Rourke, K.A., and Morris, T.J. 2025. Freshwater mussel (Unionidae) timed-search surveys in Medway Creek: the search for *Ptychobranchnus fasciolaris* (Kidneyshell). Can. Manuscr. Rep. Fish. Aquat. Sci. 3304: vii + 39 p. <https://doi.org/10.60825/chmq-cz47>

Ptychobranchnus fasciolaris (*Ptychobranchnus réniforme*) est actuellement désigné comme une espèce en voie de disparition sous la Loi sur les espèces en péril (SARA). Il y a déjà de nombreux enregistrements de coquilles de *P. fasciolaris* dans le bassin de la rivière Thames, mais seulement 4 individus vivants ont été trouvés. Toutes les instances d'individus vivants proviennent de Medway Creek, un affluent de la rivière Thames Nord. En 2024, Pêches et Océans Canada a échantillonné 15 sites le long de Medway Creek afin d'évaluer l'assemblage des unionidés et de déterminer si des individus vivants de *P. fasciolaris* sont toujours présents. Une équipe de 3 à 6 personnes a effectué des échantillons semi-quantitative de 4,5 heures-personnes à chaque site. Au total, 1 534 moules appartenant à 14 espèces ont été observées, dont deux espèces en péril: *Cambarunio iris* (*Villosa iris*) et *Lampsilis fasciola* (*Lampsilis fasciola*). Aucun *P. fasciolaris* a été trouvé vivant, mais 3 coquilles entières et deux valves ont été recueillies sur 3 sites. Medway Creek abrite un assemblage de moules d'eau douce modérément abondant et très diversifié. Cependant, il y existe plusieurs espèces tolérantes à la pollution, et l'abondance relative de ces espèces a changé au fil du temps. Les sites où *P. fasciolaris* a été trouvé (soi vivant ou comme coquille) possèdent des bonnes zones tampon riveraine à plus de 30 mètres, et la température de l'eau y était plus froide là où les espèces en péril étaient présents comparer aux sites sans espèces en péril. Les données présentées ici fournissent une meilleure compréhension de l'état actuel des moules d'eau douce dans Medway Creek, et peuvent être utilisés pour informer une réintroduction potentiel de *P. fasciolaris* dans ce système.

INTRODUCTION

Freshwater mussels (Bivalvia: Unionidae) are one of the most at-risk taxa in the world (Haag 2012). Based on the International Union for Conservation of Nature (IUCN) criteria, an estimated 33% of the Unionidae are threatened with extinction (Böhm et al. 2021). Of the 55 native freshwater mussel species in Canada, 40% have been assessed as at-risk by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) (Government of Canada 2023), with additional species remaining as candidates for assessment. The major threats contributing to global decline include commercial harvest, habitat degradation by pollution, siltation, dam operations, and channelization, as well as the invasion of non-native species such as dreissenid mussels (*Dreissena polymorpha*, Zebra Mussel; *D. rostriformis bugensis*, Quagga Mussel) and *Neogobius melanostomus* (Round Goby) (Williams et al. 1992; Ricciardi et al. 1998; Dextrase and Mandrak 2006; Lopes-Lima et al. 2018; Clark et al. 2022). The Thames River in southern Ontario was the site of one of Canada's largest commercial mussel harvests during the mid-1900s (Hayes Morris and Morris 2024). Moreover, urbanization is known to have negative impacts on mussels at all life stages through wastewater effluents and urban run-off containing road salt and other toxins (Gillis et al. 2017; 2022; Salerno et al. 2020). Agriculture also has an impact on freshwater mussels by polluting waterbodies with high nutrient loads and pesticides (Augspurger et al. 2003, Bringolf et al. 2007). Both agricultural and urbanized landscapes have differing impacts on freshwater mussels (Newton et al. 2008; Haag 2012), and these differences may alter mussel assemblages as land-use changes around waterbodies (Morris and Corkum 1996; Morris 1996). Freshwater mussels have an essential role in aquatic ecosystems as ecosystem engineers (Haag 2012). They filter large volumes of water, promote nutrient cycling across pelagic and benthic food webs, and provide habitat for other organisms (Haag 2012). Researching these organisms is crucial for their protection and to ensure that people continue to benefit from their ecosystem services.

Southern Ontario is one of the regions identified in Canada with high conservation priority for freshwater biodiversity under multiple scenarios tested by Chu et al. (2015) and is well-known as a biodiversity hotspot for freshwater mussels (McNichols-O'Rourke et al. 2012). Within southern Ontario, the Sydenham and Thames rivers are known to be Canada's most diverse mussel rivers, each historically home to nearly two-thirds of all Canadian species (McNichols-O'Rourke et al. 2012). Medway Creek is a tributary in the Upper Thames River sub-basin draining 205 km² into the North Thames River immediately below Fanshawe Dam and Reservoir (UTRCA 2022) in London, Ontario. The land cover of the Medway Creek watershed is more than 75% agricultural with less than 10% urbanized; however, urbanization is expanding (UTRCA 2022). The creek divides just upstream of the Arva Mill Dam impoundment, creating two branches, referred to as the West Branch and East Branch in the present study. Significant development around Medway Creek can be seen through examination of historical aerial photos, which show that development around the creek, below Fanshawe Park Road, was well-established since at least 2003 (Google Earth Pro 2024a). Between

2003 and 2016, development around Medway Creek occurred from Fanshawe Park Road north to Sunningdale Road, with more recent development, from 2013 to 2020, occurring north of Sunningdale Road. Moreover, a new subdivision is proposed in the area west of the village of Arva, both north and south along Medway Road, which is on the east side of Medway Creek in the area just downstream of Arva Mill Dam (Municipality of Middlesex Centre 2024). The area upstream of Arva Mill Dam remains agricultural land (Google Earth Pro 2024b); however, it could be developed in the future. Although the majority of the area downstream from Medway Road is urbanized, it is notable that there remains a forested riparian buffer (Google Earth Pro 2024b). Forested riparian buffers shade the river, moderate water temperature, and provide erosion control and water quality protection (Broadmeadow and Nisbet 2004).

The first unionid records in Medway Creek were reported in 1935 and included the shells of four species (LGLUD 2025), one of which, *Cambarunio iris* (Rainbow), is currently listed as Special Concern on Schedule 1 of the *Species at Risk Act* (SARA) (Government of Canada 2023; Table 1). The first confirmed live live unionid records were reported in 1994-95, but sampling of Medway Creek did not occur frequently until the early 2000s (LGLUD 2025). *Cambarunio iris* was first confirmed live in Medway Creek in the mid-1990s (LGLUD 2025). *Lampsilis fasciola* (Wavyrayed Lampmussel), also listed as Special Concern on Schedule 1 of SARA (Government of Canada 2023), was found live in Medway Creek for the first time in 2004 during a survey by Fisheries and Oceans Canada (DFO) (Morris and Edwards 2007). Another species at risk recorded from in Medway Creek is *Ptychobranhus fasciolaris* (Kidneyshell), which was listed on Schedule 1 of SARA as Endangered in 2005 and the status was reconfirmed during the 2013 COSEWIC reassessment (Government of Canada 2023). This species was recorded for the first time in 2004, with two live individuals found at a single site by Upper Thames River Conservation Authority (UTRCA) (Table 1; LGLUD 2025). An additional two live *P. fasciolaris* individuals were found, one in 2006 and one in 2007, during a mussel relocation project (Mackie 2008). Both of which were initially found in one of the prescribed search areas associated with a pipeline crossing project and were subsequently recovered at the one-month, one-year, and two-year monitoring events (Mackie 2008; LGLUD 2025). No additional live *P. fasciolaris* individuals have been found in Medway Creek since, and because the individuals found by Mackie appeared aged, the Medway Creek *P. fasciolaris* population was presumed to be reproductively senescent at the time of the COSEWIC reassessment in 2013 (COSEWIC 2013). Twenty records have been found in other areas of the Thames River between 1894 and 2022; however, no live *P. fasciolaris* have been found outside Medway Creek. The Thames River population was classified as extirpated during the 2023 recovery potential assessment (Colm and Morris 2025).

The primary goals of the Medway Creek surveys outlined in this report were to assess the current status of the freshwater mussel assemblage of Medway Creek and to determine if live *P. fasciolaris* persist in the system.

METHODS

SITE SELECTION

Sites were selected based on several criteria that included: the presence of live *P. fasciolaris* during previous surveys; the presence of the preferred habitat type of the species; accessibility; a representative distribution of sites throughout Medway Creek; and, the known distribution of other SAR (*C. iris* and *L. fasciola*) within the watershed. A total of 15 sites were selected for sampling (Table 2, Figure 1). These sites included the two sites where *P. fasciolaris* were previously found live (MEM06 and MMC26) and eight sites below the Arva Mill Dam to the confluence with the North Thames River. These 10 sites were situated within the area believed to represent the historical distribution of *P. fasciolaris* within Medway Creek. An additional five sites were sampled in the headwaters of Medway Creek upstream of the dam to examine the distributions of *C. iris* and *L. fasciola*.

FRESHWATER MUSSEL SAMPLING

A timed-search survey was conducted by a three-to-six-person crew for a total of 4.5 person-hours (ph) at each of the 15 selected sites following the methods of Metcalfe-Smith et al. (2000). Crew members used Nuova Rade Aquascope underwater viewers to visually search for live freshwater mussels and shells. Occasionally, tactile searching and scoops were also used when habitat characteristics necessitated (i.e., low visibility, increased depth). Live individuals and shells of all mussel species found were collected in dive bags and processed at the end of the search time. Live mussels were identified to species, measured (maximum length in millimeters) using calipers, and sexed (if sexually dimorphic), before being returned to the substrate. All whole shells and valves were identified to species. In addition, whole shells and valves of species not found live at the site were enumerated, and shell condition was recorded (fresh or weathered). All whole shells and valves of *P. fasciolaris* were kept as vouchers. Digital vouchers were taken for each species found following Morris et al. (2022).

Length frequency distributions were generated for all live species at risk (SAR) detected. 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 preliminary DFO unpublished data. Age data using shells from southwestern Ontario were utilized to determine juvenile cut-off lengths for each species by determining size at first maturity, calculated following the methods of Haag (2012). 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 when data are available.

Shannon Diversity Index (H') was calculated for each site using the following formula from Morin (1999), which was adapted from Shannon (1948):

$$H' = \sum_{i=1}^S -p_i \times \ln(p_i)$$

Where S is the total number of species present and p_i is the number of individuals of one species (i).

ENVIRONMENTAL DATA

Before conducting the survey at each site, air temperature and wind speed were recorded using a Kestrel 2000 Pocket Wind Meter, water velocity was measured using an OTT MF Pro flow meter, water clarity was assessed using a 0.60 m turbidity tube, and water temperature and chemistry were measured using an EXO2 Multiparameter YSI. Before starting the time for the timed-search, downstream coordinates were obtained using Google Maps on a cellular device, and at the end of the survey, the upstream coordinates were obtained. After the survey, a qualitative assessment of substrate composition was recorded as percentages of the total substrate at the site. Substrate sizes were modified from Stanfield (2010) and defined as: 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 organic material), and detritus (plant matter). In addition, stream morphology was assessed and recorded as percentages; these include riffle, run, and pool, defined in Fuller (2018), and flat (shallow to moderately deep, low water velocity, no turbulence). Stream shading (dense, partly open, open) and algal growth (absent; present, <50% coverage; and abundant, >50% coverage) were also recorded. River dimensions were measured using a Nikon Laser 1200S waterproof laser range finder, or with mapping tools in Google Maps upon return to the office and included the length of river reach searched and the minimum, maximum, and average river width at the site. The minimum and maximum depth searched was measured using a metre stick. Site photos were taken through the sampling events using a cellular device.

Average riparian buffer width for left and right banks were measured and calculated at sites where *P. fasciolaris* were either found live in previous surveys or were found as shells in the current study. The methods and definitions of intact and fragmented buffers were employed from Lu et al. (2024), with minor modifications. Ten stream widths were marked; they each spanned across the stream perpendicular to the edges of the stream and at 20 m intervals, progressing upstream from the downstream coordinates of the site. Riparian buffer widths were measured from the edge of the stream at the delineated stream width interval to where the buffer recedes to an anthropogenic land use (e.g., road, agricultural field, paved pathway) or to another point on the river in cases where the river meandered.

STATISTICAL ANALYSIS AND DATA VISUALIZATION

A Welch two-sample t-test was performed using R v4.2.2 software (R Core Team 2022) to determine significant differences in water metric data and unionid community

composition data (i.e., species abundance and richness) between sites with SAR and sites without SAR. Since temperature data is affected by month and time of day, a Welch two-sample t-test was performed between sites sampled in June and July, and between sites sampled in the morning and the afternoon, for water temperature and air temperature. Shapiro-Wilk tests were performed to test normality of each water metric parameter across all sites.

Figures and maps were created in R (R Core Team 2022; Posit Team 2023) using the ggplot2 package (Wickham 2016). Additional packages used for creating maps were cowplot (Wilke 2020), ggrepel (Slowikowski 2023), ggspatial (Dunnington 2022), maptiles (Giraud 2022), sf (Pebesma 2018), terra (Hijmans 2024), tidyterra (Hernangomez 2024), and tidyverse (Wickham et al. 2019). The viridis package was used for colour palettes (Garnier et al. 2024).

RESULTS

Freshwater mussel surveys were conducted from June 10th to 14th, 2024, and July 24th to 26th, 2024, at 15 sites throughout Medway Creek (Figure 1; Table 2). A total of 1534 live mussels were observed representing 14 species, including two SAR: *C. iris* and *L. fasciola* (Table 3). *Pyganodon grandis* (Giant Floater) was the dominant species at 9 of 15 sites (60%), found live at all sites, and the most abundant species across all sites with 531 individuals. The second most abundant species across all sites was *Eurynia dilatata* (Spike) with 387 individuals found at 10 of 15 (66.7%) sites. The third most abundant species was *Strophitus undulatus* (Creeper), which had 142 individuals found at 10 of 15 (66.7%) sites. Of the 15 sites sampled, 10 were located downstream of the Arva Mill Dam (Table 2), where a total of 1244 mussels were observed representing 12 species, including two SAR (Table 3). The remaining 5 sites were located upstream of the Arva Mill Dam, with two sites located on the East Branch and three on the West Branch (Table 2). A total of 290 mussels were observed in this area, representing eight species and including one SAR (Table 3). Upstream of the Arva Mills Dam, 74% of the individuals were found in the West Branch; the West Branch assemblage had eight species, and the East Branch had three.

No live *P. fasciolaris* was found at any of the 15 sites surveyed (Table 3). Whole shells and/or valves of *P. fasciolaris* were found at 3 of 15 sites (20.0%), with a total of 3 whole shells and 2 valves, all in weathered condition. The whole shells and valves of *P. fasciolaris* were not found at sites where live *P. fasciolaris* were previously detected; two of the sites were upstream and one site was downstream of the sites where they were previously found live. No *P. fasciolaris* shells were found in the reaches upstream of Arva Mill Dam.

Cambarunio iris was found at 5 of 15 (33.3%) sites and totalled 56 individuals (Table 3). The relative abundance of *C. iris* varied from 0.01 to 0.67 among sites. Of the 56 live individuals found across all sites, 13 (23.2%) were found downstream of the dam, 43 (76.8%) were found upstream of the dam on the West Branch, and no evidence of *C.*

iris was found at the East Branch sites. The majority of individuals (n = 41) were found at a single West Branch site, LSC-MDW-05. Lengths of all individuals found (n = 56) ranged from 22.4 to 77.1 mm, with a single individual (<0.02%) determined to be a juvenile (<22.7 mm, preliminary DFO unpublished data; Figure 2).

Lampsilis fasciola was found at 6 of 15 (40.0%) sites, with 27 live individuals found across all sites (Table 3). The relative abundance of *L. fasciola* ranged from 0.01 to 0.14. No live individuals or shells were found upstream of the Arva Mill Dam. Lengths (n = 27) ranged from 49.0 to 90.5 mm, with no individuals classified as juveniles (<35.8 mm, preliminary DFO unpublished data; Figure 3). *Cambarunio iris* and *L. fasciola* were found live together at 2 of 15 (13.3%) sites (Table 3).

Shannon Diversity Index (H') values range from 0.00 to 1.92, with a mean (\pm standard error) of 1.28 ± 0.144 (Table 3). The mean H' values for the East Branch, West Branch, and Mainstem are 0.34 ± 0.345 , 0.85 ± 0.132 , and 1.60 ± 0.0886 , respectively (Table 4). Length ranges from individuals collected across all sites for each species are provided in Table 4.

Buffer analysis at sites where *P. fasciolaris* were either found live in previous surveys or were found as shells in the current study revealed that all of these sites have intact buffers; the average buffer width was >30 m on both sides of the river (Table 5; Figure 4). Note that in occurrences where the river greatly meandered, the riparian buffer ended at another point on the river. Additionally, many sites had paved pathways that interrupted the buffer; however, forest habitat continued past these points. In some cases, the 10 stream widths at 20 m intervals did not extend to the upstream coordinates (Length of Reach >180 m), and in other cases, the intervals extended past the upstream coordinates (Length of Reach <180 m).

Survey data are summarized in Table 6. The length of reach surveyed ranged from 85 to 1232 m with a mean (\pm standard deviation) of 339.13 ± 305.89 m. Minimum width of reach searched ranged from 1 to 16 m with a mean of 10.17 ± 4.52 m. Maximum width of reach searched ranged from 9 to 35 m with a mean of 19.33 ± 6.32 m. Average width ranged from 4 to 20 m with a mean of 13.33 ± 4.27 m. Maximum depth searched ranged from 0.42 to 1.18 m with a mean of 0.85 ± 0.21 m. Average depth searched ranged from 0.25 to 1 m with a mean of 0.47 ± 0.18 m. Water clarity was consistently >0.60 m (the size of the Secchi Tube used), with the exception of one site, LSC-MDW-09, where water clarity was recorded as 0.442 m. Water velocity ranged from 0.053 to 2.45 m/s with a mean of 0.18 ± 0.10 m/s. Stream shading was 86.7% partly open, 13.3% open, and no sites were recorded as densely shaded. Algal growth was 73.3% abundant, 26.7% present, and no sites were recorded as having no algal growth. Air temperature ranged from 12.8 to 28.4°C with a mean of 20.1 ± 4.63 °C. Water temperature ranged from 15.2 to 21.9°C with a mean of 18.9 ± 2.28 °C. Conductivity ranged from 500 to 622.2 $\mu\text{s}/\text{cm}$ with a mean of 557.43 ± 36.74 $\mu\text{s}/\text{cm}$. Dissolved oxygen (%) ranged from 83.5 to 143% with a mean of 108.59 ± 18.14 %. Dissolved oxygen (mg/L) ranged from 8.1 to 12.62 mg/L with a mean of 10.07 ± 1.56 mg/L. The

pH values ranged from 7.9 to 8.35 with a mean of 8.17 ± 0.14 . Salinity ranged from 0.27 to 0.33 psu with a mean of 0.31 ± 0.02 psu. TDS ranged from 357.51 to 444 mg/L with a mean of 410.37 ± 27.44 mg/L. Turbidity ranged from 1.6 to 4.37 fnu with a mean of 2.71 ± 0.74 fnu.

Water temperature (mean \pm standard error) at sites where live SAR were present (n=9, 17.89 ± 0.74) was significantly lower than at sites where live SAR were absent (n=6, 20.47 ± 0.57) (t = 2.759; p = 0.016; Table 7; Figure 5). No other significant water quality parameter differences were observed between sites with and without SAR (Table 7). One site where live SAR were present, water velocity was not recorded.

There were no significant differences found between sites sampled in June (n=9) and sites sampled in July (n=6) for water temperature and air temperature (Table 8). There were no significant differences found between sites sampled in the morning (n=7) and sites sampled in the afternoon (n=8) for water temperature and air temperature (Table 9).

Sites with SAR detection were comprised of stream morphology averages (\pm standard error) of $12.5 \pm 2.99\%$ riffle, $5.6 \pm 1.75\%$ pool, $69.4 \pm 10.28\%$ run habitat; whereas sites without SAR detection, contained an average of $16.7 \pm 3.57\%$ riffle, $11.7 \pm 4.94\%$ pool, and $71.7 \pm 7.60\%$ run habitat (Table 7; Figure 6). Tests were not significantly different between sites where SAR were detected live and not detected live (Table 7). Stream morphology at one site where live SAR were present was not recorded. Substrate at sites with SAR detection had a mean of $6.0 \pm 1.39\%$ boulder, $26.8 \pm 2.93\%$ cobble, $33.0 \pm 1.89\%$ gravel, $21.7 \pm 2.01\%$ sand, $8.6 \pm 1.71\%$ silt, $3.1 \pm 1.06\%$ clay, $0.3 \pm 0.33\%$ muck, and $0.6 \pm 0.56\%$ detritus (Table 7; Figure 7). Sites without SAR contained mean substrate compositions of $9.0 \pm 4.47\%$ boulder, $23.3 \pm 5.43\%$ cobble, $32.5 \pm 5.88\%$ gravel, $22.5 \pm 4.03\%$ sand, $5.0 \pm 1.83\%$ silt, $6.5 \pm 2.09\%$ clay, $0.3 \pm 0.33\%$ muck, and $0.8 \pm 0.83\%$ detritus (Table 7; Figure 7). Tests were not significantly different between sites where SAR were detected live and not detected live (Table 7).

DISCUSSION

Medway Creek sites display a moderately abundant and highly diverse mussel assemblage compared to other similarly surveyed sites in southern Ontario (LGLUD 2025). In the Lower Great Lakes Unionid Database (LGLUD), DFO's database for freshwater mussel research in Canada, an average of 85.0 individuals and 6.6 species are found in all 4.5 ph timed-searches conducted by DFO since 2015. In the present study, 40% of Medway Creek sites found more than the average number of individual mussels and 53.3% found more than the average number of species.

There is higher mean diversity and abundance in the mainstem of Medway Creek despite being adjacent to urbanized land. The site with the highest abundance was MMC26 with 377 live individuals, and the most speciose site was LSC-MDW-10 with 12 species. LSC-MDW-10 also had the highest diversity, the third highest total abundance, and was one of only two sites that had live individuals of both *C. iris* and *L. fasciola*.

This site was dominated by *E. dilatata*, but as indicated by its high diversity index value, it had a relatively evenly distributed mussel assemblage. The site where live *P. fasciolaris* was first discovered in Medway Creek (MEM06) had the second highest abundance with 309 individuals and was the second most speciose with 10 species; however, it had a moderate diversity due to its lack of evenness and dominance of *P. grandis*. MMC26 was also dominated by *P. grandis* but had the second highest diversity. These three sites are all in the mainstem, below Arva Mill Dam. LSC-MDW-10 is in the area that was developed before 2003 (Google Earth Pro 2024a). MMC26 and MEM06 are both in the area that has undergone development since 2003. The riparian buffer width of the sites where *P. fasciolaris* were found alive in previous surveys or as shells in the current study are all considered intact (buffers >30 m), as recommended under established guidelines to protect aquatic habitat in Canada (Environment Canada 2013). Sites with intact buffers are associated with higher habitat quality for freshwater mussels (Lu et al. 2024).

Reaches upstream of the Arva Mill Dam have lower abundance and diversity overall. The site with the lowest abundance, species richness, and diversity was LSC-MDW-06. This site is in the East Branch, adjacent to agricultural fields, with very little tree cover, and very little buffer between the creek and the agricultural fields (Google Earth Pro 2024b). One site on the upper west branch, m11, sampled by DFO in 2021, had high abundance (311 individuals), relatively low species richness (5 species), and relatively low diversity (0.574 H' value) (LGLUD 2025). This may indicate that more sampling should occur throughout the upper reaches of the West and East branches to permit a better understanding of the mussel assemblages and their distribution throughout the upper part of the system.

The 2024 surveys indicate that *P. fasciolaris* is likely extirpated from Medway Creek, as suggested by Colm and Morris (2025). No live individuals were found, and although whole shells and valves were observed, all were of weathered condition. The last live individuals were found in 2008 and considered reproductively senescent at that time (COSEWIC 2013). The historically occupied reach of *P. fasciolaris* in Medway Creek, based on all shells and live records, is the area from Sunningdale Road to ~1 km downstream of Fanshawe Park Road.

There has never been live *P. fasciolaris* collected from anywhere in the Thames River watershed outside of Medway Creek. However, there have been numerous shell collections over the last 130 years (LGLUD 2025). Not including Medway Creek, there are 14 records comprising 18 shells and valves of varying reported condition: 6 fresh, whole shells; 1 fresh valve; 3 weathered, whole shells; and 8 weathered valves. The Upper Thames River watershed, exclusive of Medway Creek, contains a single record: a weathered valve collected in the South Thames River in 2017, in the eastern part of London. There are 13 records from the Lower Thames River comprising 17 shells and valves. They span from just north of Muncey to the centre of Chatham (near the confluence with McGregor Creek). Therefore, the historical occupied reach of *P.*

fasciolaris in the Thames River watershed, excluding Medway Creek, is a ~186 km stretch from the South Thames River to Chatham on the Lower Thames River, near the confluence with McGregor Creek (Figure 8).

The Thames River is a well-studied Canadian mussel river. In recent years, a total of 293 sampling events occurred from 2009 to 2024 in the Thames River watershed (McNichols-O'Rourke et al. 2012; Currier et al. 2018; Sheldon et al. 2020; Coghlan et al. 2021; Gillis et al. 2022; LeBaron et al. 2023; Gibson et al. 2023; Goguen et al. 2023; LGLUD 2025; Porto-Hannes et al. 2025). This includes 22 quadrat surveys conducted at 16 index stations, from Fisheries and Oceans Canada's Unionid Monitoring and Biodiversity Observation Network (UMBO), with a total of 1620 m² effort (Sheldon et al. 2020; LGLUD 2025). There were 116 timed-search surveys totaling 659.75 ph in search effort (McNichols-O'Rourke et al. 2012; Gillis et al. 2022; Gibson et al. 2023; Goguen et al. 2023; LGLUD 2025; Porto-Hannes et al. 2025). Quadrat excavation from known relocations yielded an additional 3215 m² of effort from 16 records. Brail surveys included 20 sampling events, 1-5 transects each (LeBaron et al. 2023). An additional 6 sampling events were eDNA collections (Currier et al. 2018; Coghlan et al. 2021; LGLUD 2025). The remaining 113 records did not have a specified effort; these represent non-timed visual surveys, shell/midden surveys, graduate student work, work involving multiple sampling methods, training events, incidentals, and other non-measurable sampling activities. Therefore, with the amount of search effort conducted in the Thames River, it is highly likely that *P. fasciolaris* is extirpated from the entire watershed.

The only species other than *P. fasciolaris* not found, that has been previously found live in Medway Creek, is *Lampsilis siliquoidea* (Fatmucket). No shells of this species were found in the current study. There is only one record of *L. siliquoidea* in Medway Creek: 1 live individual and 4 shells, collected in 1994 (LGLUD 2025, Table 1). No live individuals or shells were found since, suggesting that the species is no longer present.

Sites with more than one survey within the suspected *P. fasciolaris* historical reach were used to compare changes in species relative abundances (Appendix A). Relative abundances were calculated for each species at semi-quantitative and quantitative surveys, conducted in the known occupied reach of *P. fasciolaris* in Medway Creek and recorded in LGLUD (Appendix A; LGLUD 2025). The proportional change in relative abundances between the 2004-2010 surveys and the 2024 surveys was calculated for each species. The proportional change in the average relative abundance for *P. grandis* is positive (+0.316), and the proportional change for *Fusconaia flava* (Wabash Pigtoe), *P. fasciolaris*, and *Lasmigona costata* (Flutedshell) are negative (-0.166, -0.115, and -0.110, respectively). All other species had a proportional rate between -0.100 and +0.100, representing little to no change. It is notable that *L. complanata*, with a proportional change of 0.015, was not found during previous sampling in the area. The positive proportional change for *P. grandis* and the negative proportional changes for *F. flava* and *L. costata* (in addition to *P. fasciolaris*) suggest that the *P. grandis* population

is growing, whereas *F. flava* and *L. costata* populations are diminishing. However, all three species, as well as *L. complanata*, are considered to be tolerant to pollution and other disturbances (Morris 1996; Metcalfe-Smith et al. 2003; Haag 2012). More research into the interactions of these tolerant species could help determine the exact cause of the shifts in the Medway Creek assemblage.

Since freshwater mussels are obligate parasites, they require a host, usually a fish, to complete their lifecycle (Haag 2012). Therefore, it is important to know their host fish species and understand their distributions. With the known information on host fish for *P. fasciolaris*, *C. iris*, and *L. fasciola* (Zale and Neves 1982; Watters and O'Dee 1997; O'Dee and Watters 2000; McNichols and Mackie 2003; McNichols et al. 2005; Watters et al. 2005; McNichols 2007; McNichols et al. 2011, Gibson et al. 2015), and UTRCA electrofishing data (UTRCA unpublished data), we can determine where host fishes for each species are present in Medway Creek. Four of the five *P. fasciolaris* host fishes were detected in Medway Creek in the last 10 years. The four species are *Percina maculata* (Blackside Darter), *Etheostoma flabellare* (Fantail Darter), *Etheostoma nigrum* (Johnny Darter), and *Culaea inconstans* (Brook Stickleback) - Iowa Darter (*Etheostoma exile*) was not observed. Two (*E. flabellare* and *E. nigrum*) of the four species were detected within the suspected *P. fasciolaris* historical reach in 2024. A fourth host (*P. maculata*) was detected in recent sampling (<10 years) in tributaries and the upper reaches of the Medway Creek watershed. Six host species for *C. iris* (O'Dee and Watters 2000) found in Canada were detected in Medway Creek in 2024, on the West Branch, East Branch, and the mainstem (*Ethostoma blennioides* (Greenside Darter), *Ethostoma caeruleum* (Rainbow Darter), *Lepomis cyanellus* (Green Sunfish), *Micropterus dolomieu* (Smallmouth Bass), *Micropterus nigricans* (Largemouth Bass), and *Perca flavescens* (Yellow Perch)). Three of the four *Lampsilis fasciola* host species (*M. dolomieu*, *M. nigricans*, *Pimephales notatus* (Bluntnose Minnow)) are present in Medway Creek - *Cottus bairdii* (Mottled Sculpin) was not collected. *Micropterus dolomieu* (Smallmouth Bass), the primary host for *L. fasciola* (McNichols et al. 2011), was found on the mainstem of Medway Creek in 2024 and has not been detected in the upper reaches since 2014; this could explain why *L. fasciola* is not found in this area. However, there are two other hosts for *L. fasciola* found in the upper reaches. The dam likely remains a barrier to fish movement, which would prevent the possibility of *L. fasciola* colonizing the upper reaches of Medway Creek.

Cambarunio iris occurs above the Arva Mill Dam while *L. fasciola* does not, and they were only found together at 2 of the 15 sites sampled. Moreover, it is important to note that the low number of *C. iris* and *L. fasciola* juveniles detected during this study is not necessarily an indication of low recruitment, as timed-searches are not as effective as quadrat excavation at recovering smaller individuals (Vaughn et al. 1997). However, the detection of a single juvenile *C. iris* is a positive sign that there is some recruitment for that species.

The lower water temperatures found at sites where SAR are present may be a result of forested riparian buffers that are intact. Although the buffer analysis was limited to the four sites in the suspected historical reach of *P. fasciolaris*, this area coincides with sites where live SAR are present. This information supports the idea that this habitat is high-quality for SAR mussels.

Medway Creek has a moderately abundant and highly diverse mussel assemblage, with two SAR: *C. iris* and *L. fasciola*. *Ptychobranchnus fasciolaris* is likely extirpated from the entire Thames River watershed, with the last recorded live collection occurring in 2008. Although there are abundant numbers of tolerant mussel species, and evidence of shifts in these tolerant species, the mussel assemblage within Medway Creek has remained consistent over time. In addition, there appear to be positive habitat characteristics that currently support SAR, such as the presence of large, forested buffers and lower water temperature. Medway Creek is a potential candidate for a reintroduction site for *P. fasciolaris* in the Thames River watershed.

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Table 1. Mussel species found live (Y) and as shells (SH) in the Medway Creek watershed during surveys recorded in the Lower Great Lakes Unionid Database (LGLUD). 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 2024) as of December 2024. Nomenclature here and throughout follows MolluscaBase eds. (2024) for scientific names and Williams et al. (2017) for common names.

Sub-Family	Tribe	Scientific Name	Common Name	1935	1994-1995	2001-2010	2011-2023	DFO 2024	COSEWIC Assessment	SARA (Federal)	ESA (Provincial)
Unioninae	Anodontini	<i>Alasmidonta marginata</i>	Elktoe	-	Y	Y	-	Y			
Unioninae	Anodontini	<i>Alasmidonta viridis</i>	Slippershell	SH	-	-	Y	Y			
Unioninae	Anodontini	<i>Anodontoides ferussacianus</i>	Cylindrical Papershell	-	-	Y	-	Y			
Ambleminae	Lampsilini	<i>Cambarunio iris</i> *†	Rainbow	SH	Y	Y	Y	Y	Special Concern	Special Concern	Special Concern
Ambleminae	Pleurobemini	<i>Eurynia dilatata</i>	Spike	SH	Y	Y	Y	Y			
Ambleminae	Pleurobemini	<i>Fusconaia flava</i>	Wabash Pigtoe	-	SH	Y	Y	Y			
Ambleminae	Lampsilini	<i>Lampsilis cardium</i> *	Plain Pocketbook	-	Y	Y	-	Y			
Ambleminae	Lampsilini	<i>Lampsilis fasciola</i> *	Wavyrayed Lampmussel	-	-	Y	Y	Y	Special Concern	Special Concern	Threatened
Ambleminae	Lampsilini	<i>Lampsilis siliquoidea</i> *	Fatmucket	-	Y	-	-	-			
Unioninae	Anodontini	<i>Lasmigona complanata</i>	White Heelsplitter	-	-	-	-	Y			
Unioninae	Anodontini	<i>Lasmigona compressa</i>	Creek Heelsplitter	-	Y	Y	Y	Y			
Unioninae	Anodontini	<i>Lasmigona costata</i>	Flutedshell	SH	Y	Y	Y	Y			
Ambleminae	Lampsilini	<i>Ortmanniana ligamentina</i>	Mucket	-	SH	Y	Y	Y			
Ambleminae	Lampsilini	<i>Ptychobranchnus fasciolaris</i>	Kidneyshell	-	-	Y	-	SH	Endangered	Endangered	Endangered
Unioninae	Anodontini	<i>Pyganodon grandis</i>	Giant Floater	-	Y	Y	Y	Y			
Unioninae	Anodontini	<i>Strophitus undulatus</i>	Creeper	-	Y	Y	Y	Y			
Live Species Richness				-	9	13	10	14			

*Sexually dimorphic species

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

Table 2. Site locations and sample dates for sites sampled in the Medway Creek watershed in 2024. Highlighted sites include *Ptychobranchnus fasciolaris* (Kidneyshell) detection in previous surveys. Sites without public access marked with an asterisk. Sites are displayed in order of upstream to downstream, with the east branch before the west branch, followed by the mainstem. Time of Day is as follows: Early Morning is prior to 10:30, Late Morning is from 10:31-12:00, Early Afternoon is from 12:01-14:30, and Late Afternoon is 14:31 and on. Dotted line represents the Arva Mill Dam.

Site Code	Branch	Upstream Coordinates	Downstream Coordinates	Date	Time of Day
LSC-MDW-06	East branch	43.121574, -81.306963	43.1142017, -81.2991758	25-Jul-2024	Early Morning
LSC-MDW-14	East branch	43.074452, -81.273428	43.072691, -81.276644	24-Jul-2024	Early Afternoon
LSC-MDW-15	West branch	43.088461, -81.306871	43.0843277, -81.3070216	25-Jul-2024	Early Afternoon
LSC-MDW-05	West branch	43.0776133, -81.2977961	43.0762392, -81.2968309	24-Jul-2024	Late Morning
LSC-MDW-16	West branch	43.0707752, -81.2913397	43.0662799, -81.2882441	26-Jul-2024	Early Morning
LSC-MDW-09*	Mainstem	43.0512152, -81.2994269	43.051140, -81.300804	24-Jul-2024	Early Morning
MMC17	Mainstem	43.0320671, -81.3030875	43.031029, -81.302485	10-Jun-2024	Late Afternoon
MMC14	Mainstem	43.0286457, -81.3052440	43.0285411, -81.306840	10-Jun-2024	Early Afternoon
MMC26	Mainstem	43.0281364, -81.3077797	43.026204, -81.307987	11-Jun-2024	Early Morning
MEM06	Mainstem	43.0235986, -81.3064597	43.0231783, -81.3053231	11-Jun-2024	Late Afternoon
LSC-MDW-10	Mainstem	43.0177292, -81.3048611	43.016541, -81.305670	12-Jun-2024	Early Morning
LSC-MDW-11	Mainstem	43.0140417, -81.3026191	43.0130793, -81.3002450	12-Jun-2024	Early Afternoon
LSC-MDW-12	Mainstem	43.009811, -81.296728	43.009831, -81.293284	12-Jun-2024	Late Afternoon
LSC-MDW-13	Mainstem	43.00561, -81.29137	43.007256, -81.290192	13-Jun-2024	Early Afternoon
TR-37	Mainstem	43.012501, -81.271234	43.012307, -81.26919	13-Jun-2024	Early Morning

Table 3. Species detected at sites in the Medway Creek watershed. Total abundance, live species richness, Shannon Diversity Index (H'), *Cambarunio iris* (Rainbow) and *Lampsilis fasciola* (Wavyrayed Lamppussel) relative abundance are calculated for each site. Species at risk are highlighted. S# represents species found as whole shells and the number of shells found. V# represents species found as valves (one half of a full shell) and the number of valves found. Y represents Yes, N represents No. Dotted line delineates the dam.

Branch	East Branch		West Branch			Mainstem										Total
Site code	LSC-MDW-06	LSC-MDW-14	LSC-MDW-15	LSC-MDW-05	LSC-MDW-16	LSC-MDW-09	MMC17	MMC14	MMC26	MEM06	LSC-MDW-10	LSC-MDW-11	LSC-MDW-12	LSC-MDW-13	TR-37	
Suspected <i>P. fasciolaris</i> Historical Reach (Y/N)	N	N	N	N	N	N	Y	Y	Y	Y	N	N	N	N	N	
Live SAR Presence (Y/N)	N	N	N	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	N	
<i>Alasmidonta marginata</i>	0	0	0	0	0	0	1	0	1	0	1	0	0	1	0	4
<i>Anodontoides ferussacianus</i>	3V	3	5	1V	0	0	0	0	0	0	0	0	0	0	0	8
<i>Alasmidonta viridis</i>	0	0	3	0	0	0	0	1V	0	1V	0	0	0	0	0	3
<i>Cambarunio iris</i>	0	0	0	41	2	2V	2V	1S	0	7	5	1	0	0	0	56
<i>Euryntia dilatata</i>	0	0	48	60	1S, 1V	1V	13	14	77	55	59	58	2	0	1	387
<i>Fusconaia flava</i>	0	0	0	1S	0	2S, 12V	6	3	44	16	29	11	1	0	1	111
<i>Lampsilis cardium</i>	0	0	0	0	0	1V	7	6	11	12	7	4	5	2	2	56
<i>Lampsilis fasciola</i>	0	0	0	0	0	1S	8	3	8	2	3	0	3	0	0	27
<i>Lasmigona complanata</i>	0	0	0	0	0	0	0	0	2	4	6	2	1	0	0	15
<i>Lasmigona compressa</i>	0	0	1	0	0	2V	0	0	0	0	1	0	0	0	0	2
<i>Lasmigona costata</i>	0	0	1	0	0	6	1	1	28	12	12	2	1	0	1	65
<i>Ortmanniana ligamentina</i>	0	0	0	0	0	3	3	5	24	16	51	23	1	0	1	127
<i>Ptychobranthus fasciolaris</i>	0	0	0	0	0	0	2S, 1V	1V	0	0	0	1S	0	0	0	3S, 2V
<i>Pyganodon grandis</i>	2	33	49	4	1	10	19	28	113	170	49	36	14	1	2	531
<i>Strophitus undulatus</i>	0	36	1	0	0	2	1	2	69	15	7	7	2	0	0	142
Total Abundance	2	72	108	105	3	21	59	62	377	309	230	144	30	4	8	1534
Live Species Richness	1	3	7	3	2	4	9	8	10	10	12	9	9	3	6	14
H'	0.00	0.69	1.09	0.81	0.64	1.21	1.81	1.59	1.84	1.52	1.92	1.60	1.70	1.04	1.73	1.28
<i>C. iris</i> Relative Abundance	N/A	N/A	N/A	0.39	0.67	N/A	N/A	N/A	N/A	0.02	0.02	0.01	N/A	N/A	N/A	0.04

Branch	East Branch		West Branch			Mainstem										
Site code	LSC-MDW-06	LSC-MDW-14	LSC-MDW-15	LSC-MDW-05	LSC-MDW-16	LSC-MDW-09	MMC17	MMC14	MMC26	MEM06	LSC-MDW-10	LSC-MDW-11	LSC-MDW-12	LSC-MDW-13	TR-37	
Suspected <i>P. fasciolaris</i> Historical Reach (Y/N)	N	N	N	N	N	N	Y	Y	Y	Y	N	N	N	N	N	
Live SAR Presence (Y/N)	N	N	N	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	N	Total
<i>L. fasciola</i> Relative Abundance	N/A	N/A	N/A	N/A	N/A	N/A	0.14	0.05	0.02	0.01	0.01	N/A	0.10	N/A	N/A	0.02

Table 4. Species detected in each branch of the Medway Creek watershed. Total abundance, live species richness, average Shannon-Winer Diversity Index (H'), and dominant species are calculated for each branch. Species at risk are highlighted. S# represents species found as complete shells and the number of shells found. V# represents species found as valves (one half of a full shell) and the number of valves found.

Branch	East Branch	West Branch	Mainstem	Total	Length Range (mm)
<i>Alasmidonta marginata</i>	0	0	4	4	45.8 – 76.2
<i>Anodontoides ferussacianus</i>	3	5	0	8	42.2 – 70.2
<i>Alasmidonta viridis</i>	0	3	2V	3	24.4 – 32.6
<i>Cambarunio iris</i>	0	43	13	56	22.4 – 77.1
<i>Eurynia dilatata</i>	0	108	279	387	44.7 – 122
<i>Fusconaia flava</i>	0	1S	111	111	30.7 – 110
<i>Lampsilis cardium</i>	0	0	56	56	85.4 – 147
<i>Lampsilis fasciola</i>	0	0	27	27	49 – 90.5
<i>Lasmigona complanata</i>	0	0	15	15	80.5 – 135.4
<i>Lasmigona compressa</i>	0	1	1	2	71.6 – 74.3
<i>Lasmigona costata</i>	0	1	64	65	64.3 – 116
<i>Ortmanniana ligamentina</i>	0	0	127	127	66.7 – 190
<i>Ptychobranchus fasciolaris</i>	0	0	3S, 2V	3S, 2V	-
<i>Pyganodon grandis</i>	35	54	442	531	31.7 – 128.8
<i>Strophitus undulatus</i>	36	1	105	142	37.1 – 94.8
Total Abundance	74	216	1244	1534	
Live Species Richness	3	8	12	14	
Average H'	0.34	0.85	1.60	1.28	
Dominant Species	<i>Strophitus undulatus</i>	<i>Eurynia dilatata</i>	<i>Pyganodon grandis</i>	<i>Pyganodon grandis</i>	

Table 5. Buffer analysis results for sites where *P. fasciolaris* were found live in previous surveys or as shells in the current study. All are >30 m; therefore, buffers are considered intact.

Site Code	MMC17	MMC14	MMC26	MEM06	LSC-MDW-11
Average Width of Left Bank (m)	57.39	40.19	45.11	49.76	190.7
Average Width of Right Bank (m)	87.83	176.9	220.4	99.36	202.4

Table 6. Environmental data collected at each site in the Medway Creek watershed.

Site Details	Site Code	LSC-MDW-06	LSC-MDW-14	LSC-MDW-15	LSC-MDW-05
	Branch	East Branch	East Branch	West Branch	West Branch
Search Area Measurements	Length of Reach (m)	1232	380	583	174
	Min Width of Reach (m)	1	3	9	11
	Max Width of Reach (m)	9	20	18	15
	Avg Width (m)	4	9	12	13
	Max Depth Searched (m)	0.42	0.8	0.95	0.95
	Avg Depth Searched (m)	0.27	0.42	0.4	0.5
Water Clarity (m)		>0.60	>0.60	>0.60	>0.60
Water Velocity (m/s)		0.082	0.283	0.053	0.122
Stream Shading		Partly Open	Partly Open	Partly Open	Partly Open
Algal Growth		Abundant	Present	Abundant	Abundant
Air Temperature (°C)		18.3	23.5	19.8	22.9
YSI Measurements	Water Temperature (°C)	18.23	21.342	20.24	21.17
	Conductivity (µS/cm)	529	580	500	516
	ODO (%)	98.8	97.3	110.5	101.8
	ODO (mg/L)	9.32	8.6	9.99	9.04
	pH	7.9	8.04	8.03	8.1
	Salinity (psu)	0.3	0.3	0.27	0.27
	TDS (mg/L)	394.95	405.63	357.51	361.59
	Turbidity (FNU)	2.7	1.76	1.6	1.75
Stream Morphology	Riffle (%)	10	15	10	0
	Pool (%)	0	10	5	0
	Run (%)	90	75	85	0
	Flat (%)	0	0	0	0
Substrate Composition	Bedrock (%)	0	0	0	0
	Boulder (%)	0	0	10	0
	Rubble (%)	10	5	40	40
	Gravel (%)	50	45	20	30
	Sand (%)	20	40	20	20
	Silt (%)	5	0	5	0
	Clay (%)	15	5	5	7
	Muck (%)	0	0	0	3
Detritus (%)	0	5	0	0	

Table 6. (Continued) Environmental data collected at each site in the Medway Creek watershed.

Site Details	Site Code	LSC-MDW-16	LSC-MDW-09	MMC17	MMC14
	Branch	West Branch	Mainstem	Mainstem	Mainstem
Search Area Measurements	Length of Reach (m)	726	114	165	135
	Min Width of Reach (m)	5	12.5	7	16
	Max Width of Reach (m)	14	19.5	12.5	23
	Avg Width (m)	8	16	9	16
	Max Depth Searched (m)	0.85	0.85	1.1	1.18
	Avg Depth Searched (m)	0.43	0.48	0.5	1
Water Clarity (m)		>0.60	0.44	>0.60	>0.60
Water Velocity (m/s)		-	0.25	0.166	0.189
Stream Shading		Partly Open	Open	Partly Open	Partly Open
Algal Growth		Present	Abundant	Present	Present
Air Temperature (°C)		15	22	16.8	13
YSI Measurements	Water Temperature (°C)	16.62	21.47	17.025	15.99
	Conductivity (µS/cm)	514	537	563.5	550.8
	ODO (%)	83.5	104.3	125.2	106.1
	ODO (mg/L)	8.1	9.25	12.07	10.45
	pH	7.96	8.2	8.31	8.25
	Salinity (psu)	0.3	0.28	0.33	0.33
	TDS (mg/L)	397.77	374.08	432	432
	Turbidity (FNU)	3.1	4.37	3.04	3.33
Stream Morphology	Riffle (%)	20	10	5	25
	Pool (%)	10	10	5	0
	Run (%)	70	80	90	75
	Flat (%)	0	0	0	0
Substrate Composition	Bedrock (%)	0	0	0	0
	Boulder (%)	5	0	2	5
	Rubble (%)	30	30	30	25
	Gravel (%)	40	40	40	30
	Sand (%)	10	25	15	24
	Silt (%)	0	0	12	15
	Clay (%)	10	3	1	1
	Muck (%)	0	2	0	0
Detritus (%)	5	0	0	0	

Table 6. (Continued) Environmental data collected at each site in the Medway Creek watershed.

Site Details	Site Code	MMC26	MEM06	LSC-MDW-10	LSC-MDW-11
	Branch	Mainstem	Mainstem	Mainstem	Mainstem
Search Area Measurements	Length of Reach (m)	263	85	159	222
	Min Width of Reach (m)	10	11	10	14
	Max Width of Reach (m)	18	15	35	24
	Avg Width (m)	15	13	15	16
	Max Depth Searched (m)	-	-	0.85	0.8
	Avg Depth Searched (m)	-	-	0.48	0.45
Water Clarity (m)		>0.60	>0.60	>0.60	>0.60
Water Velocity (m/s)		0.142	0.069	0.061	0.094
Stream Shading		Partly Open	Partly Open	Partly Open	Partly Open
Algal Growth		Abundant	Abundant	Abundant	Abundant
Air Temperature (°C)		12.8	22	16.4	22.6
YSI Measurements	Water Temperature (°C)	15.2	17.93	16.7	18.9
	Conductivity (µS/cm)	537.2	563.5	558	572.6
	ODO (%)	90.6	131	85.8	126.3
	ODO (mg/L)	9.07	12.4	8.32	11.73
	pH	8.18	8.35	8.12	8.29
	Salinity (psu)	0.32	0.32	0.32	0.32
	TDS (mg/L)	429	423	431	421
	Turbidity (FNU)	2.82	2.82	3.61	2.6
Stream Morphology	Riffle (%)	10	0	10	10
	Pool (%)	0	0	10	10
	Run (%)	90	0	80	80
	Flat (%)	0	0	0	0
Substrate Composition	Bedrock (%)	0	0	0	0
	Boulder (%)	10	8	14	5
	Rubble (%)	18	20	20	18
	Gravel (%)	35	35	30	35
	Sand (%)	25	25	25	30
	Silt (%)	10	10	10	10
	Clay (%)	2	2	1	2
	Muck (%)	0	0	0	0
Detritus (%)	0	0	0	0	

Table 6. (Continued) Environmental data collected at each site in the Medway Creek watershed.

Site Details	Site Code	LSC-MDW-12	LSC-MDW-13	TR-37
	Branch	Mainstem	Mainstem	Mainstem
Search Area Measurements	Length of Reach (m)	283	397	169
	Min Width of Reach (m)	13	14	16
	Max Width of Reach (m)	25	18	24
	Avg Width (m)	20	16	18
	Max Depth Searched (m)	0.8	1	0.5
	Avg Depth Searched (m)	0.45	0.47	0.25
Water Clarity (m)		>0.60	>0.60	>0.60
Water Velocity (m/s)		0.33	0.29	0.32
Stream Shading		Open	Partly Open	Partly Open
Algal Growth		Abundant	Abundant	Abundant
Air Temperature (°C)		26.1	28.4	22.4
YSI Measurements	Water Temperature (°C)	21.5	21.931	19.599
	Conductivity (µS/cm)	606	622.2	611.7
	ODO (%)	143	128.6	96
	ODO (mg/L)	12.62	11.24	8.79
	pH	8.35	8.3	8.19
	Salinity (psu)	0.32	0.32	0.33
	TDS (mg/L)	422	430	444
	Turbidity (FNU)	2.33	2.29	2.58
Stream Morphology	Riffle (%)	20	25	30
	Pool (%)	10	35	10
	Run (%)	70	40	60
	Flat (%)	0	0	0
Substrate Composition	Bedrock (%)	0	0	0
	Boulder (%)	5	25	19
	Rubble (%)	40	30	25
	Gravel (%)	22	15	25
	Sand (%)	21	10	20
	Silt (%)	10	10	10
	Clay (%)	2	10	1
	Muck (%)	0	0	0
Detritus (%)	0	0	0	

Table 7. Relevant environmental data (mean \pm SE) and results from a Welch's two-sample t-test used to detect differences between environmental data at sites with live species at risk (SAR) absent (n=6) and sites with live SAR present (n=9).

Parameter	Live SAR Absent		Live SAR Present		Test Statistic		
	Mean	SE	Mean	SE	df	t	p-value
Water velocity (m/s)	0.21	0.05	0.15	0.03	9.1	1.160	0.276
Air temperature (°C)	22.4	1.43	18.6	1.73	12.85	1.7524	0.1035
Water temperature (°C)	20.47	0.57	17.89	0.74	13.0	2.759	0.016 *
Conductivity (μ S/cm)	563.32	19.97	553.51	9.56	7.3	0.443	0.671
ODO (%)	105.92	5.04	110.37	7.24	12.8	-0.505	0.622
ODO (mg/L)	9.53	0.39	10.42	0.61	12.6	-1.227	0.242
pH	8.11	0.06	8.21	0.04	10.1	-1.380	0.197
Salinity (psu)	0.30	0.01	0.31	0.01	9.4	-1.288	0.229
TDS (mg/L)	401.03	13.37	416.60	7.73	8.3	-1.008	0.342
Turbidity (FNU)	2.55	0.41	2.82	0.18	7.1	-0.611	0.560
Riffle (%)	16.7	3.57	12.5	2.99	11.0	1.194	0.258
Pool (%)	11.7	4.94	5.6	1.75	6.2	1.278	0.247
Run (%)	71.7	7.60	69.4	10.28	12.5	0.708	0.492
Boulder (%)	9.0	4.47	6.0	1.39	6.0	0.640	0.546
Cobble (%)	23.3	5.43	26.8	2.93	7.9	-0.558	0.592
Gravel (%)	32.5	5.88	33.0	1.89	6.0	-0.081	0.938
Sand (%)	22.5	4.03	21.7	2.01	7.5	0.185	0.858
Silt (%)	5.0	1.83	8.6	1.71	11.9	-1.422	0.181
Clay (%)	6.5	2.09	3.1	1.06	7.6	1.444	0.189
Muck (%)	0.3	0.33	0.3	0.33	12.3	0.000	1.000
Detritus (%)	0.8	0.83	0.6	0.56	9.3	0.277	0.788

*Significant difference.

Table 8. Relevant environmental data (mean \pm SE) and results from a Welch's two-sample t-test used to detect differences between water temperature and air temperature data at sites sampled in June (n=9) and sites sampled in July (n=6).

Parameter	June		July		Test Statistic		
	Mean	SE	Mean	SE	df	t	p-value
Air temperature (°C)	20.1	1.86	20.3	1.32	12.868	0.085	0.9333
Water temperature (°C)	18.3	0.79	19.8	0.81	12.1	1.358	0.1993

Table 9. Relevant environmental data (mean \pm SE) and results from a Welch's two-sample t-test used to detect differences between water temperature and air temperature data at sites sampled in the morning (n=7) and sites sampled in the afternoon (n=8).

Parameter	Morning		Afternoon		Test Statistic		
	Mean	SE	Mean	SE	df	t	p-value
Air temperature (°C)	18.5	1.51	21.5	1.75	12.9	1.290	0.2198
Water temperature (°C)	18.4	0.91	19.4	0.79	12.4	0.771	0.4549

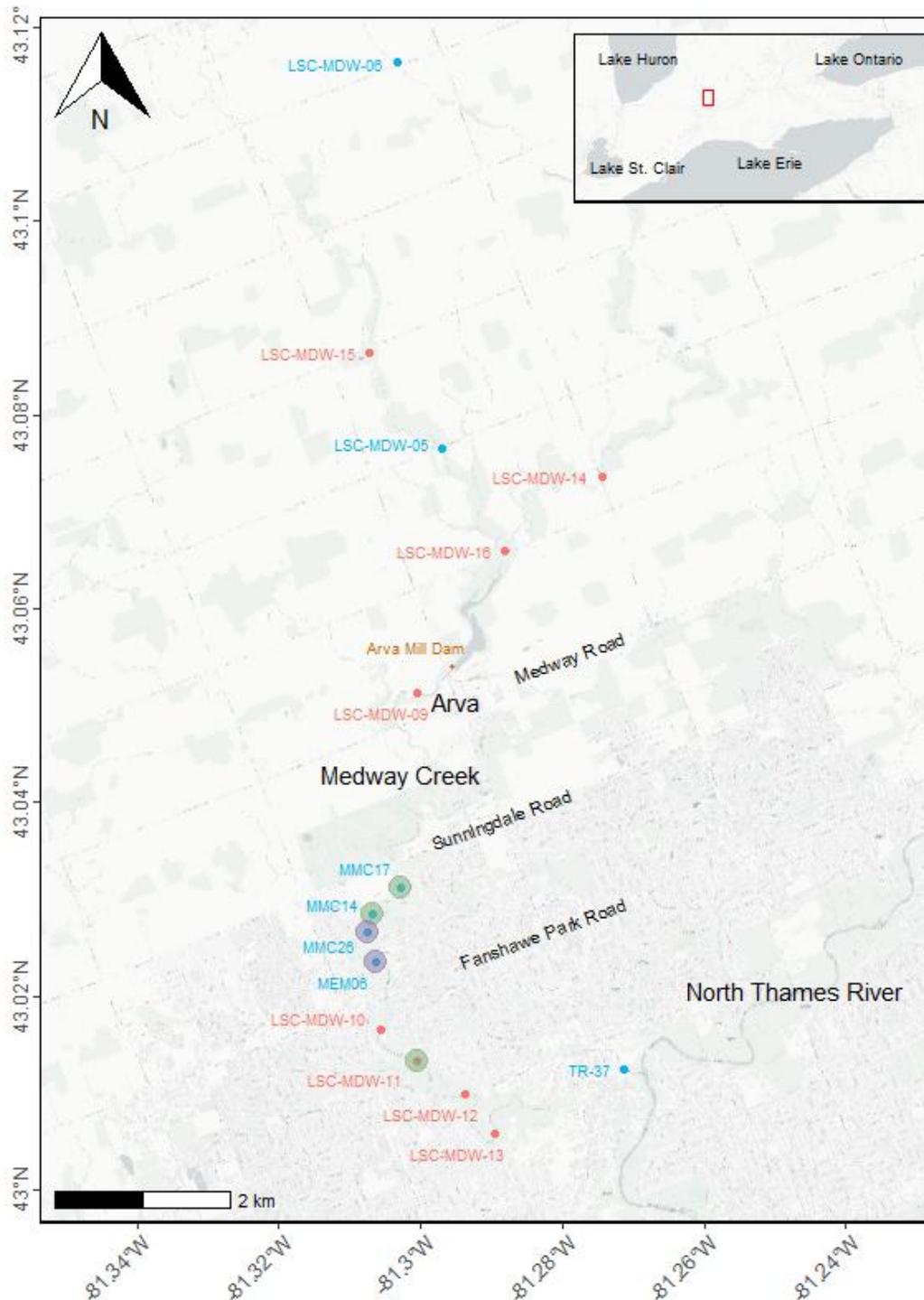


Figure 1. Sites sampled in the Medway Creek watershed in 2024. Sites where previous timed-search surveys were completed are shown in blue, new sites surveyed in the present study are shown in pink, and important structures are shown in orange. *Ptychobranchus fasciolaris* (Kidneyshell) shells found during 2024 surveys are displayed with a green circle. Sites where live *P. fasciolaris* were found during previous surveys are displayed with a purple circle. Map tiles by CartoDB, under CC BY 3.0. Data by OpenStreetMap, under ODbL.

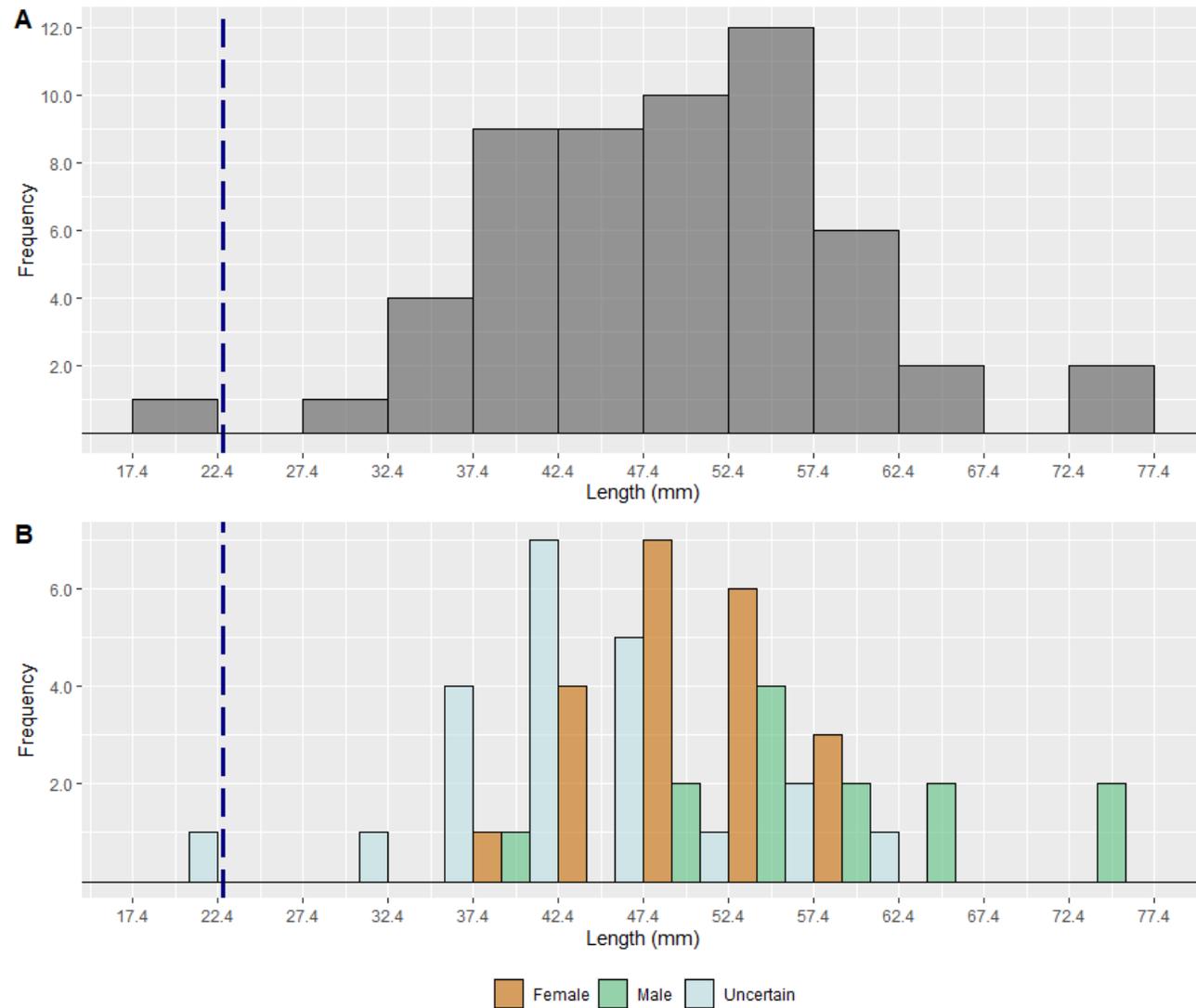


Figure 2. Length frequency of *Cambarunio iris* (Rainbow, n = 56) found at 5 of 15 (33.3%) sites in the Medway Creek watershed with (A) all individuals combined and (B) sexes separated. Uncertain represents individuals that could not be sexed. Dashed line represents individuals classified as juveniles (<22.7 mm, preliminary DFO unpublished data).

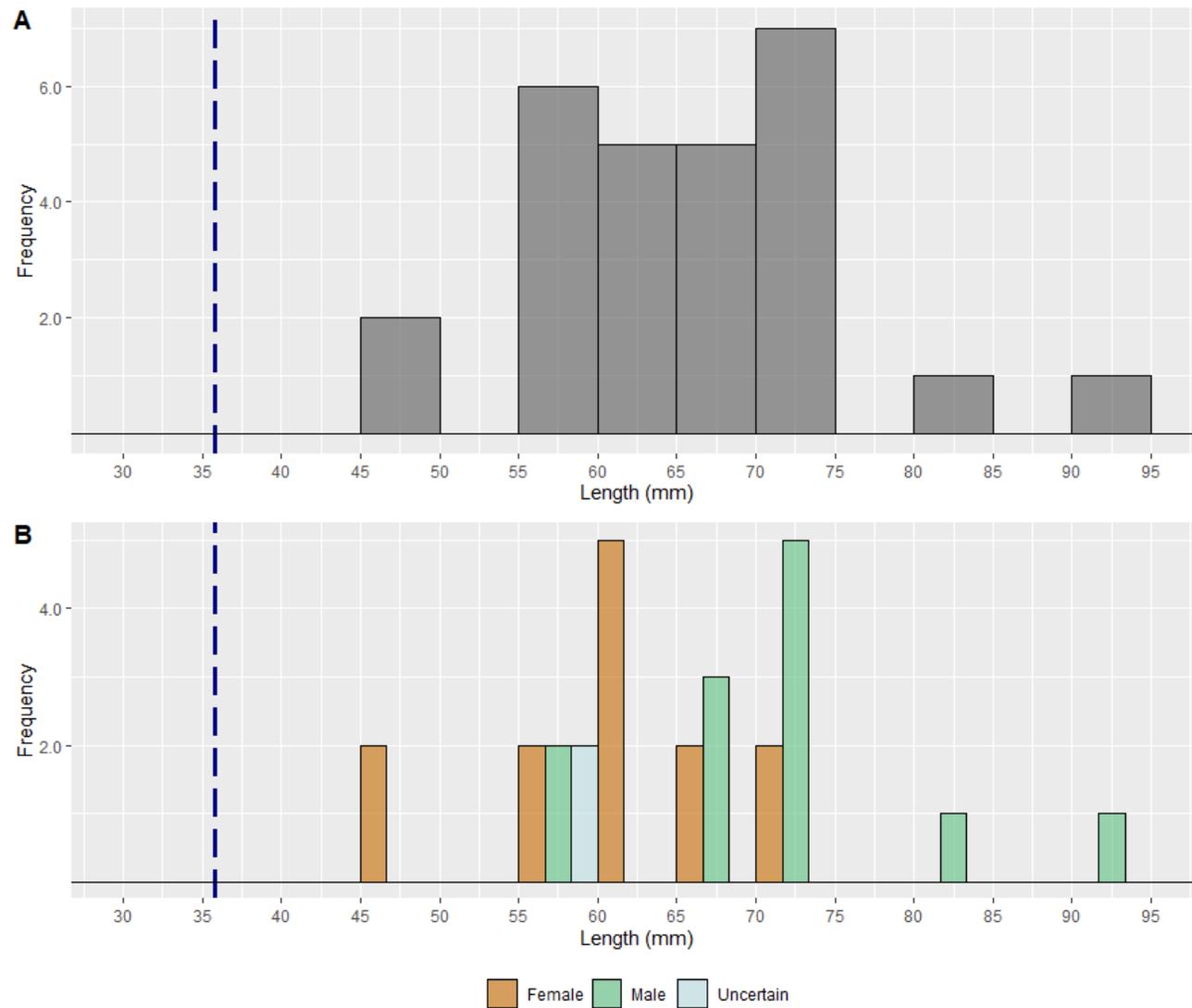


Figure 3. Length frequency of *Lampsilis fasciola* (Wavyrayed Lampmussel, n = 27) found at 6 of 15 (40.0%) sites in the Medway Creek watershed with (A) all individuals combined and (B) sexes separated. Uncertain represents individuals that could not be sexed. Dashed line represents individuals classified as juveniles (<35.8 mm, preliminary DFO unpublished data).

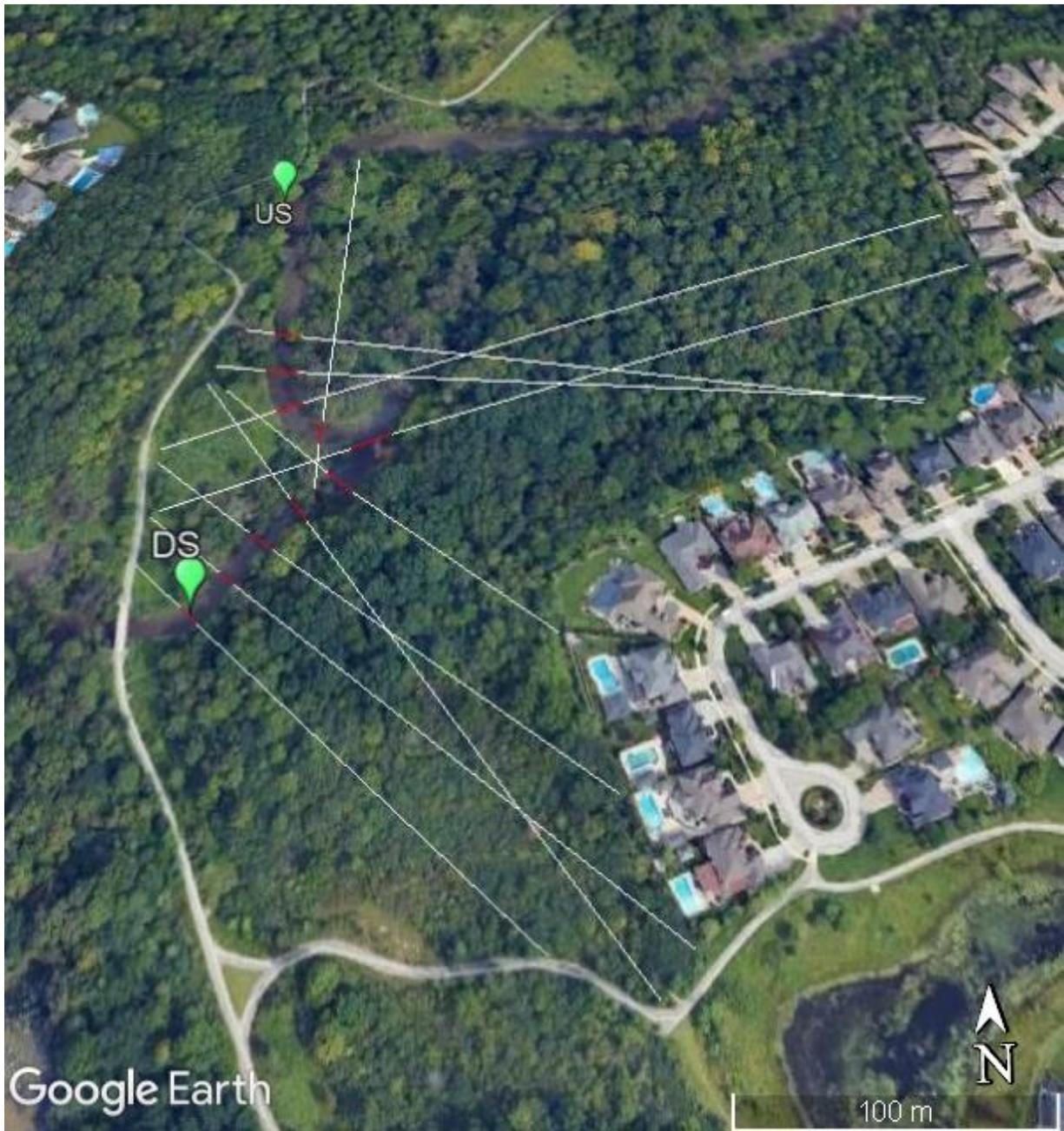


Figure 4. Riparian Buffer Analysis of MMC26. Methods following Lu et al. (2024) were repeated for all sites where *P. fasciolaris* was found live in previous surveys or as shells in the current study. Stream widths in red taken at 20 m intervals progressing upstream from the downstream coordinates of the site. Riparian buffer widths in white, extending from the edge of the stream at the stream width measurement to where the buffer recedes to an anthropogenic land use. Upstream (US) and downstream (DS) points of site indicated with green marker. Image and measurements taken using Google Earth Pro, satellite images from August 13th, 2023.

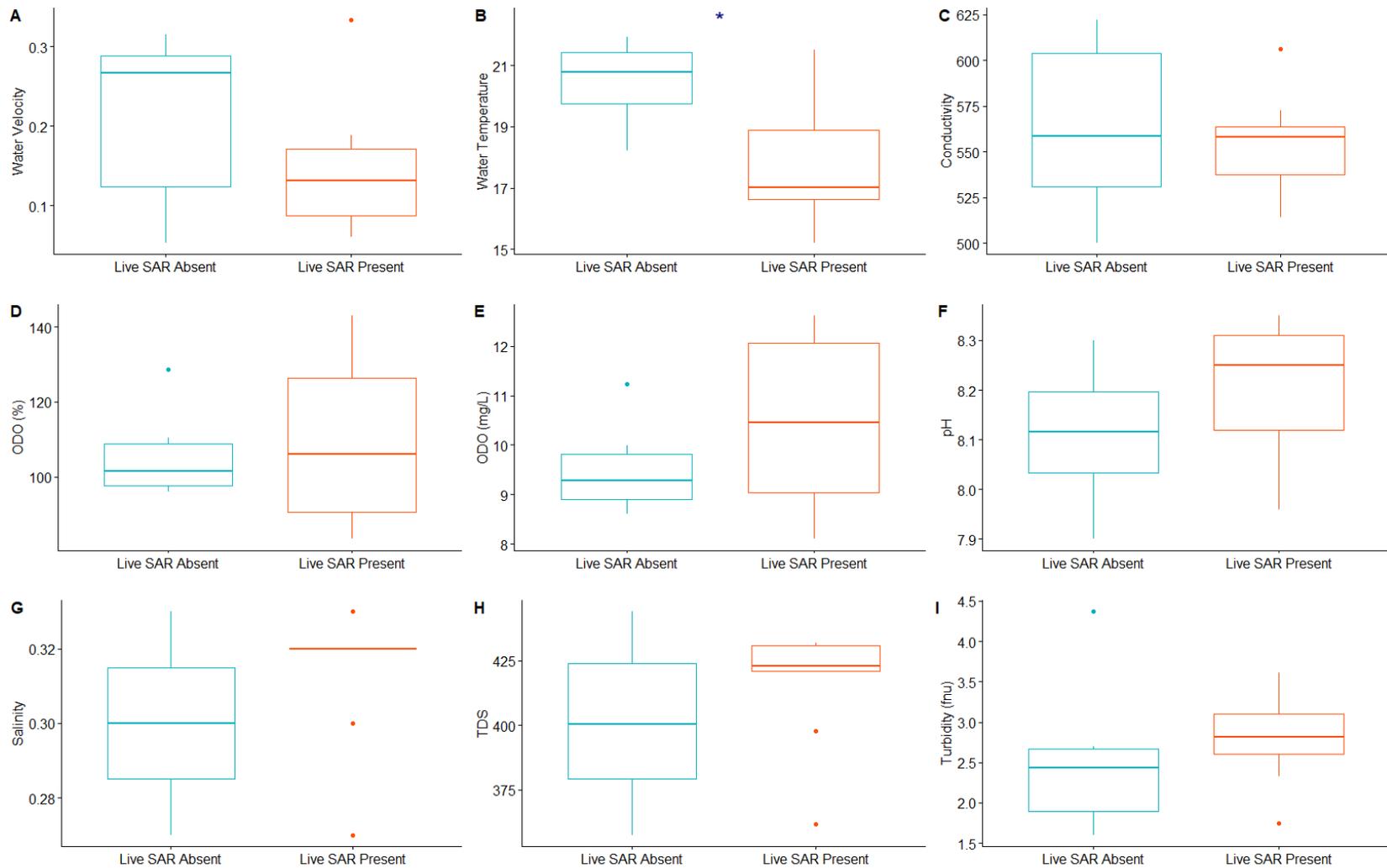
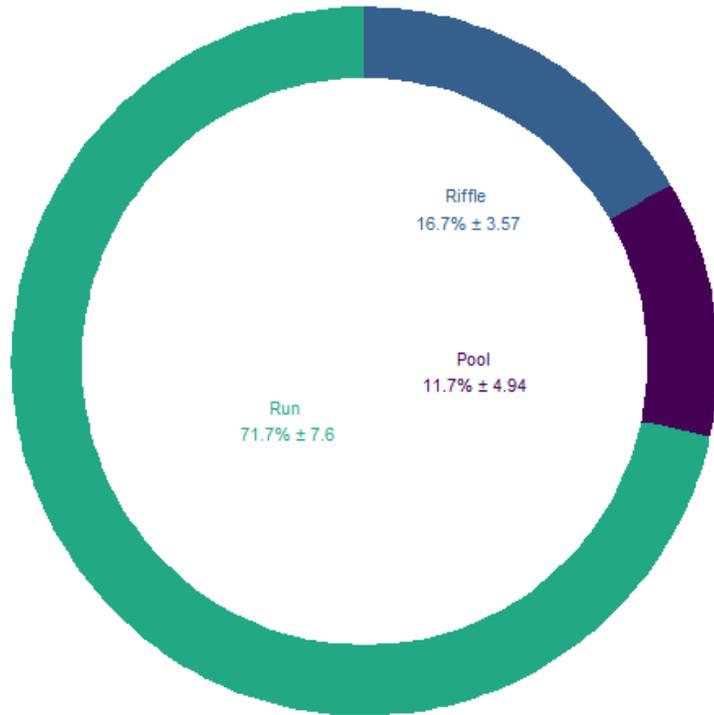


Figure 5. Comparative boxplots displaying the following environmental characteristics between sites where live species at risk (SAR) are absent (n=6) and where live SAR are present (n=9): A) Water Velocity, B) Water Temperature, C) Conductivity, D) ODO (%), E) ODO mg/L, F) pH, G) Salinity, H) TDS, and I) Turbidity. Significant t-test indicated with an asterisk.

A



B

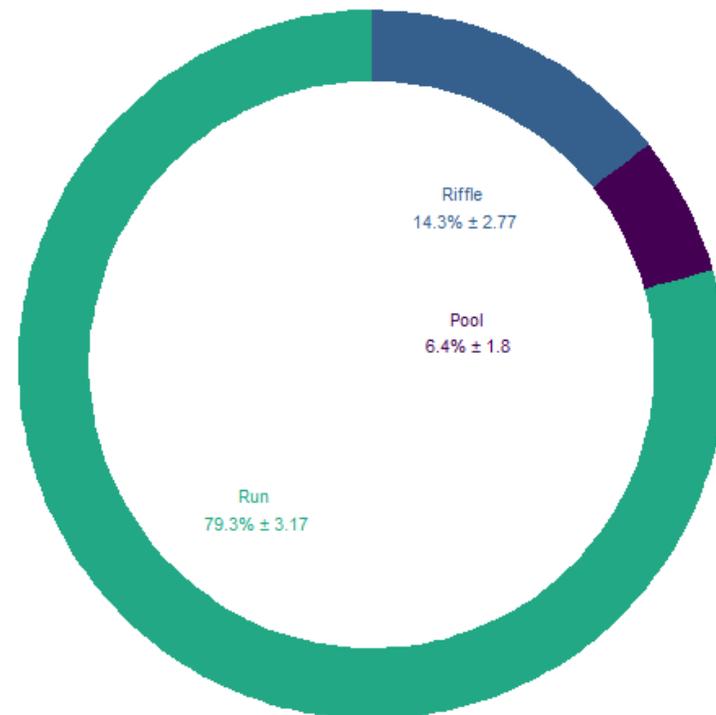


Figure 6. Mean percentage of riffle, pool, and run stream morphology for A) sites without live species at risk (SAR) and B) sites with live SAR in the Medway Creek watershed, 2024.

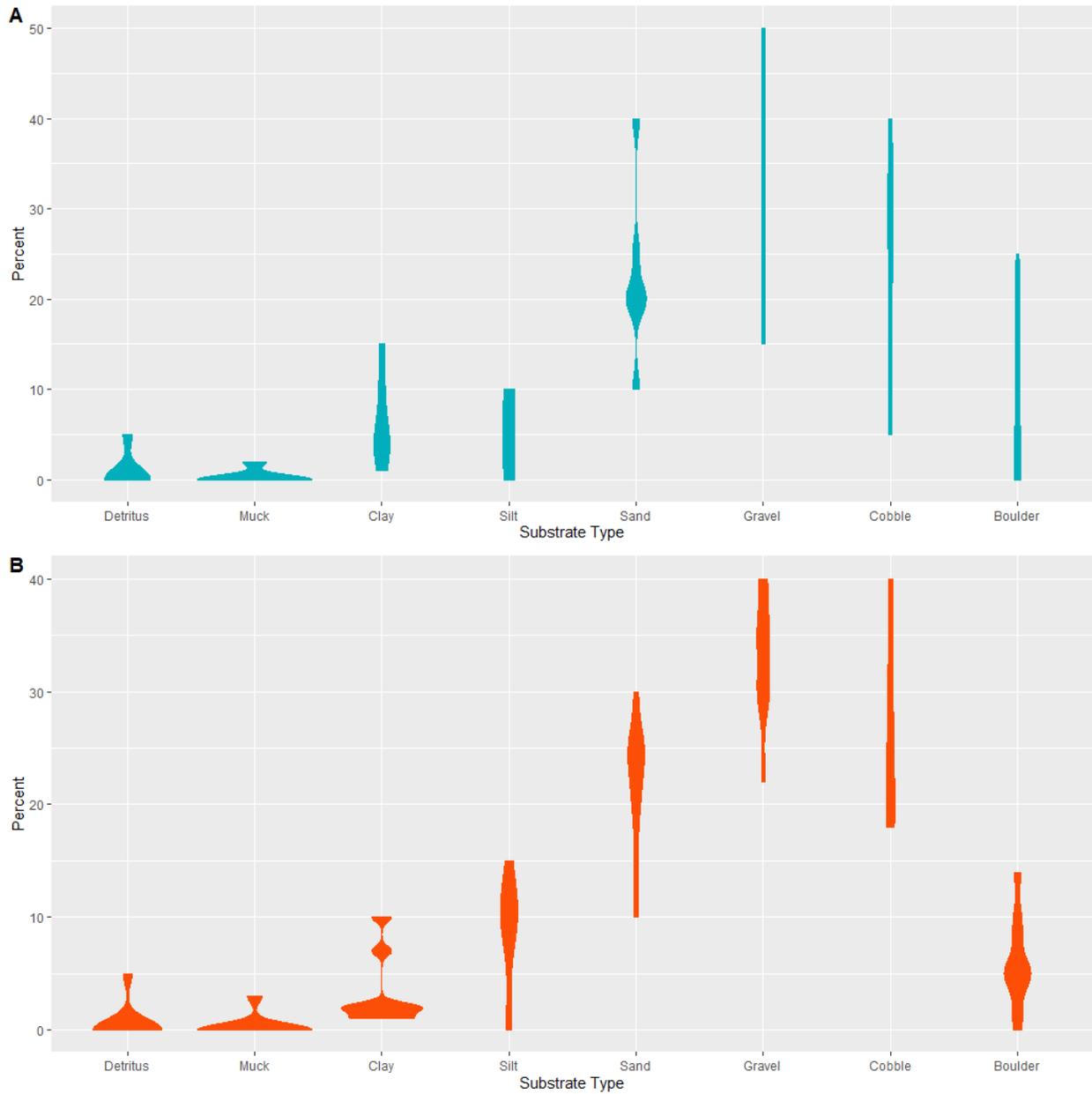


Figure 7. Mean percentage of substrate for A) sites where live species at risk (SAR) are absent and B) sites where live SAR are present in the Medway Creek watershed, 2024.

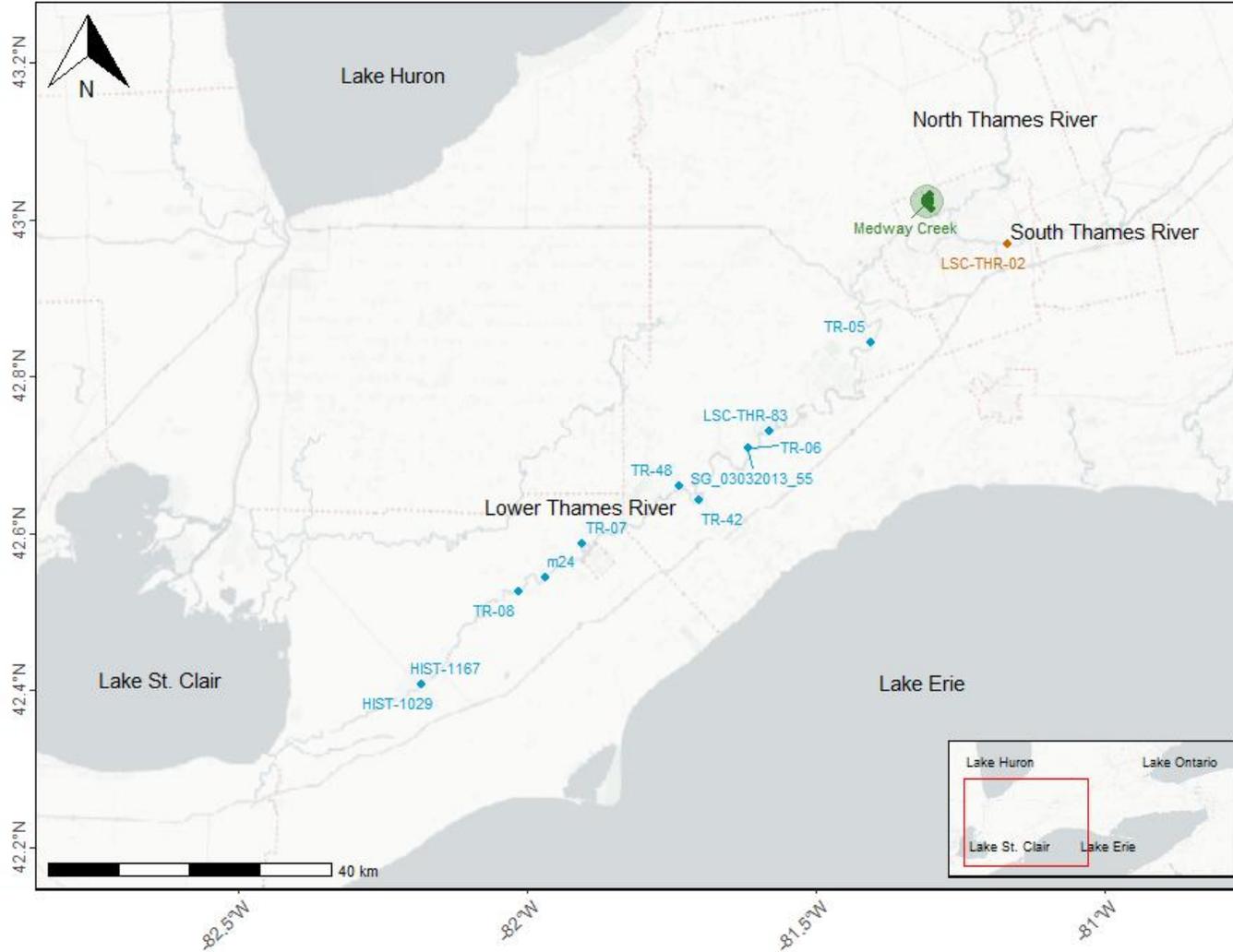


Figure 8. Historical locations where *Ptychobranchus fasciolaris* (Kidneyshell) records have been recorded in the Thames River watershed. Medway Creek records, labelled in green, include both live and shell records. All other records in the Thames River are shell records. Lower Thames River records are shown in blue and the single South Thames River record is shown in orange.

Appendix A. Relative abundances for each species found at sites within the suspected *Ptychobranchnus fasciolaris* (Kidneyshell) distribution. Surveys included were semi-quantitative and quantitative surveys from the Lower Great Lakes Unionid Database (2025). Relocation data only includes the original data from the Prescribed Search Areas (PSA). The most positive proportional (α) change is highlighted blue and the most negative is highlighted orange. Species at risk are highlighted grey. Sampling Types are represented as follows: Timed-search = TS, Relocation = R. Unknown Effort represented by U.

Site/R Project	MEM06	TR-52	Fanshawe Park Road Phase IIA	Fox Hollow	Fanshawe Park Road Phase IIB	MMC17	MMC14	MMC26	MEM06	LSC-MDW-10	LSC-MDW-11	Average Relative Abundances per Timeframe		α Change
Year	2004	2005	2006	2007	2010	2024	2024	2024	2024	2024	2024	2004-2010	2024	
Collectors	UTRCA	DFO	WSA	WSA	WSA	DFO	DFO	DFO	DFO	DFO	DFO			
Sampling Type	TS	TS	R	R	R	TS	TS	TS	TS	TS	TS			
Effort	U	4.5 ph	720 m2	729 m2	1830 m2	4.5 ph	4.5 ph	4.5 ph	4.5 ph	4.5 ph	4.5 ph			
<i>Alasmidonta marginata</i>	0.11	0.01	0.01	0.04	0.02	0.02		0.003		0.004		0.039	0.008	-0.031
<i>Cambarunio iris</i>	0.11			0.02					0.02	0.02	0.01	0.064	0.017	-0.047
<i>Euryنيا dilatata</i>	0.11	0.36	0.44	0.39	0.21	0.22	0.23	0.20	0.18	0.26	0.40	0.302	0.248	-0.054
<i>Fusconaia flava</i>	0.11	0.11	0.26	0.19	0.59	0.10	0.05	0.12	0.05	0.13	0.08	0.253	0.087	-0.166
<i>Lampsilis cardium</i>			0.03	0.02	0.01	0.12	0.10	0.03	0.04	0.03	0.03	0.019	0.057	0.038
<i>Lampsilis fasciola</i>			0.01	0.02	0.04	0.14	0.05	0.02	0.01	0.01		0.021	0.045	0.024
<i>Lasmigona complanata</i>								0.01	0.01	0.03	0.01	0.000	0.015	0.015
<i>Lasmigona compressa</i>	0.11		0.01							0.004		0.059	0.004	-0.055
<i>Lasmigona costata</i>	0.11	0.34	0.08	0.14	0.06	0.02	0.02	0.07	0.04	0.05	0.01	0.146	0.035	-0.110
<i>Ortmanniana ligamentina</i>		0.14	0.11	0.07	0.02	0.05	0.08	0.06	0.05	0.22	0.16	0.083	0.105	0.021
<i>Ptychobranchnus fasciolaris</i>	0.22		0.01									0.115	0.000	-0.115
<i>Pyganodon grandis</i>			0.01	0.07	0.01	0.32	0.45	0.30	0.55	0.21	0.25	0.032	0.348	0.316
<i>Strophitus undulatus</i>	0.11	0.04	0.04	0.05	0.04	0.02	0.03	0.18	0.05	0.03	0.05	0.055	0.060	0.0045
Total Abundance	9	80	141	26	417	59	62	377	309	230	144	134.6	196.8	
Species Richness	8	6	11	10	9	9	8	10	10	12	9	13	13	