Tracking the recovery of freshwater mussels in the Saugeen River watershed: A comparison of long-term monitoring sampling events in 2011 and 2019

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Canada

2022

Canadian Manuscript Report of
Fisheries and Aquatic Sciences 3242

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Cat. No. Fs 97-4/3242E-PDF ISBN 978-0-660-42940-3 ISSN 1488-5387

Correct citation for this publication:

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. Can. Manuscr. Rep. Fish. Aquat. Sci. 3242: vi + 33 p.

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The Saugeen River watershed is located in the Lake Huron drainage with records of 14 unionid species including two species at risk (SAR). In order to monitor the unionid populations of the Saugeen River watershed, four index stations were established by Fisheries and Oceans Canada (DFO). A quantitative quadrat survey method was used to complete an initial survey of the index stations in 2011 and the first monitoring event in 2019. In the initial survey, 512 live individuals representing 6 species were found. In the first monitoring event, 819 live individuals representing 9 species were found. The overall watershed density did not change significantly over time and, similarly, no change was detected in the overall watershed species richness. The most abundant species in the watershed during both sampling events was *Eurynia dilatata* (Spike). No significant changes in the density of *E. dilatata* were observed at the watershed level; however, at the site level, one site showed a significant increase and one showed a significant decrease in density. A single SAR, *Cambarunio iris* (Rainbow), was also observed in both sampling events, and no significant changes to its watershed density was observed; however, one site showed a significant decrease in density. Continued monitoring of the Saugeen River watershed index stations will be critically important to track changes in the overall unionid community as well as the *C. iris* population.

RÉSUMÉ

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. Can. Manuscr. Rep. Fish. Aquat. Sci. 3242: vi + 33 p.

Le bassin hydrographique de la rivière Saugeen est situé dans le bassin hydrographique du lac Huron. On y a recensé quatorze espèces d'unionidés, dont deux espèces en péril. Afin de surveiller les populations d'unionidés du bassin hydrographique de la rivière Saugeen, quatre stations de pêche indicatrice ont été établies par Pêches et Océans Canada (MPO). Une méthode de relevé quantitatif par quadrats a été utilisée pour réaliser un relevé initial des stations en 2011, et une première activité de surveillance en 2019. Lors du relevé initial, 512 individus vivants représentant 6 espèces ont été trouvés. Lors de la première activité de surveillance, 819 individus vivants représentant 9 espèces ont été trouvés. La densité globale du bassin hydrographique n'a pas changé de manière importante au fil du temps, et aucun changement n'a été détecté dans la richesse globale des espèces du bassin hydrographique. L'espèce la plus abondante relevée dans le bassin hydrographique au cours des deux échantillonnages était *Eurynia dilatata* (elliptio pointu). Aucun changement important dans la densité d'*E. dilatata* n'a été observé au niveau du bassin hydrographique; cependant, on a remarqué une augmentation marquée de la densité à l'un des sites, et une diminution marquée à un autre. Une seule espèce en péril, soit *Cambarunio iris* (villeuse irisée), a été observée lors des deux échantillonnages, et aucun changement important de sa densité à l'échelle du bassin hydrographique n'a été observé; cependant, l'un des sites affichait une diminution importante de la densité. La surveillance continue des stations de pêche indicatrice du bassin hydrographique de la rivière Saugeen sera d'une importance capitale pour suivre les changements dans la communauté globale des unionidés, ainsi que dans la population de *C. iris*.

INTRODUCTION

Freshwater mussels are critically important components of the aquatic ecosystems in which they occur as they are natural environmental filters, provide physical and chemical habitat for algae and invertebrates, promote physical stability of the substrate, and facilitate the transfer of energy from aquatic to terrestrial environments (Haag 2012). In recent decades, this taxon has experienced global declines and is now considered one of the most imperilled in the world (Lopes-Lima et al. 2018). This trend of recent declines has also been seen nationally and has resulted in 35% of Canada's 55 native unionid species being considered at-risk (Ricciardi et al. 1998; Government of Canada 2021). Declines have been primarily driven by the invasion of dreissenid mussels [Zebra Mussel (*Dreissena polymorpha*), Quagga Mussel (*Dreissena rostriformis bugensis*)], habitat loss and degradation, and decreasing water quality (Ricciardi et al. 1998).

Ontario has the highest richness of unionid species in Canada, with 42 species occurring in the province [Metcalfe-Smith et al. 2005; Fisheries and Oceans Canada (DFO) unpublished data]. Of these, 15 species have been federally listed as Special Concern, Threatened, or Endangered under the *Species at Risk Act* (SARA) and one additional species has been assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as Threatened and is being considered for listing under SARA (Government of Canada 2021; Table 1). In order to meet recovery measures and objectives outlined in recovery strategies and management plans for Canada's SAR mussels (e.g., DFO 2018a; 2019), a need for a mussel monitoring program was identified. This program was first developed for the Sydenham River in 1999. The goal of this program was to "…collect precise and detailed baseline data on the distribution, abundance, demographics and habitat requirements of mussel populations…" and allow for the detection of changes in the health of mussel populations over time (Metcalfe-Smith et al. 2007). Since the initiation of this monitoring program, the design has been successfully implemented in six watersheds in southwestern Ontario including the Saugeen River (Baitz et al. 2008; Upsdell et al. 2012; Sheldon et al. 2020; DFO unpublished data).

Four index stations were established in the Saugeen River watershed by DFO in 2011 as part of the mussel monitoring program (Sheldon et al. 2020). At the time of establishment, an initial quantitative quadrat survey was completed at each index station with the objective of collecting baseline data to act as the foundation for the monitoring program in the Saugeen River watershed (Sheldon et al. 2020). In 2019, DFO returned to the index stations to complete the first monitoring event with the objective of providing a comparison to the baseline data collected during the initial survey in order to detect changes in the freshwater mussel populations, with a focus on SAR. The establishment of the index stations and the subsequent surveys aid in meeting short- and long-term recovery objectives identified in species-specific recovery strategies (DFO 2013; 2018a; 2018b; 2019).

METHODS

WATERSHED DESCRIPTION

The Saugeen River watershed is located within the Lake Huron drainage in southwestern Ontario and has a drainage area of 4,052 km^2, representing the third largest watershed in the province (Morris et al. 2007; Drinking Water Source Protection 2015). The main channel of the Saugeen River flows 192 km from Hanover, ON to its mouth at Lake Huron in the community of Southampton, ON [Saugeen Valley Conservation Authority (SVCA) 2018a; 2018b]. There are five major subwatersheds within the Saugeen River watershed: North Saugeen River, Rocky Saug

een River, Beatty Saugeen River, South Saugeen River, and Teeswater River (Morris et al. 2007). The North Saugeen River spans 52 km in length until draining into the main Saugeen River just downstream of Paisley, ON (SVCA 2018c; Ontario Steelheaders 2021). The Rocky Saugeen River runs 51 km through highly forested land before joining the South Saugeen River on the west edge of Hanover, ON (SVCA 2018d). The Beatty Saugeen River travels 46 km and drains into the South Saugeen River just west of Hanover, ON (SVCA 2018e). The South Saugeen River runs through mainly agricultural land for 97 km before joining the Saugeen River west of Hanover, ON (SVCA 2018f). The Teeswater River flows for 75 km through primarily agricultural land before joining the main Saugeen River in Paisley, ON (SVCA 2018g).

Fourteen species of unionid mussels have been observed alive or as shells in the Saugeen River watershed including two SAR (McNichols-O'Rourke et al. 2012). *Cambarunio iris* (Rainbow) is the only SAR to be detected both historically and currently in the watershed and is listed federally and provincially as Special Concern (Morris et al. 2007; McNichols-O'Rourke et al. 2012; COSEWIC 2015; Sheldon et al. 2020; Goguen et al. 2021; Table 1). *Truncilla donaciformis* (Fawnsfoot) is listed federally and provincially as Endangered (Table 1). The only record of *T. donaciformis* from the Saugeen River watershed was a single live individual found in 2005 in Muskrat Creek, a tributary of the Teeswater River in Teeswater, ON (COSEWIC 2008; Goguen et al. 2021). In 2019, DFO completed extensive surveys in Muskrat Creek and the Teeswater River in the area around the confluence of the two waterbodies and found no evidence of *T. donaciformis* concluding that there is not currently a viable population of *T. donaciformis* in Muskrat Creek or the Teeswater River (Goguen et al. 2021). Additionally, no evidence of *T. donaciformis* was found during the initial surveys at the Saugeen River watershed index stations (Sheldon et al. 2020).

SITE SELECTION

Between 2006 and 2011, a total of 17 sites were sampled for freshwater mussels in the Saugeen River watershed using a semi-quantitative timed-search survey method (Morris et al. 2007; McNichols-O'Rourke et al. 2012). Based on mussel abundance, community species richness, and the occurrence of SAR observed during the semi-quantitative surveys, four sites were selected as index stations in the watershed

(Sheldon et al. 2020). These sites were located throughout the watershed with one site in each of the North Saugeen River, Beatty Saugeen River, Teeswater River, and Saugeen River (Table 2; Figure 1).

SAMPLING METHODS

The quantitative quadrat survey method used at each index station during the initial survey and the first monitoring event was modified from Metcalfe-Smith et al. (2007) and generally follows Sheldon et al. (2020), but is detailed here for clarity. A systematic sampling approach with three random starts was employed by a minimum three person crew. Generally, each site was divided into 25 blocks of 3 m x 5 m (15 m^2). Within each block, three quadrats (1 m^2) were excavated (Figure 2). The quadrats were selected randomly before the survey began and the same three quadrats were excavated in each block at a site. Each quadrat was searched, beginning at the downstream end of the quadrat using three different techniques: (1) visual scan with the naked eye; (2) visual search with a viewing box; and, (3) hand-excavation 10 to 15 cm into the substrate. After each method was used within a quadrat, all of the mussels found were identified, sexed visually (if sexually dimorphic), and measured (maximum length in millimeters) using Vernier calipers. As mussels were collected using each method, they were transferred to a mesh diver bag that remained in the water beside the quadrat. Substrate that was removed during excavation was placed outside the quadrat and when the quadrat was fully excavated, the substrate and any mussels found were returned to the 1 m^2 area from which they were collected. During the first monitoring event, a visual survey (<1 person hour) was completed in the initial survey area to confirm the presence of mussels. If there was no evidence of live mussels, the search area was extended until mussels were observed. Once mussels were consistently observed the first monitoring event took place in that area. Shells and valves of species that were not found alive at a site were identified, classified as fresh (i.e., tissue present, intact ligament, intact periostracum) or weathered, and enumerated during the first monitoring event. All of the shells and valves detected were weathered unless otherwise stated. While 75 quadrats across 25 blocks was the standard, one site (SG08) in the initial survey had 78 quadrats across 26 blocks (Table 3).

Environmental data were also collected at each site. Before the survey began, water chemistry metrics including water temperature (°C), conductivity (µs/cm), total dissolved solids (mg/L), salinity (psu), dissolved oxygen (%, mg/L), pH, and turbidity (FNU) were measured using an EXO2 Multiparameter YSI sonde at the site on the first day of sampling. The YSI measurements were only collected during the first monitoring event. Before excavation began, water velocity (m/s; Swoffer flow meter), depth (m; meter stick), and water clarity (m; 0.60 m turbidity tube) were measured within each quadrat. The following data were collected in each quadrat through visual estimation after excavation was complete: substrate composition (%), degree of siltation (low, medium, high), degree of algal growth (low, medium, high), stream shading (open, partly open, dense), and presence or absence of aquatic macrophytes. Substrate

composition was estimated using the definitions from Stanfield (2010): boulder (>250 mm in diameter), cobble (65 – 250 mm), gravel (2 – 65 mm), sand (<2 mm), and "other" material (mud, muck, silt, and detritus). The estimation of siltation was based on the amount of silt disturbed into the water column while excavating the quadrat. The estimation of low, medium, or high siltation was subjective and differed between sites in order to capture variation within a site. The estimation of algal growth was categorized as low if <20% of surface substrate was covered in algae, medium if 20 – 50% coverage, and high if >50% coverage. Shading was estimated as open if no vegetation cover was directly above the quadrat, partly open if <50% vegetation cover, and dense if >50% vegetation cover. Any amount of aquatic macrophytes observed within a quadrat was recorded as present. The data visually estimated after excavation were collected to provide a general understanding of the site characteristics and were not meant to provide a quantitative measure. Only the environmental data that are relevant to this report will be presented (Appendix A).

DATA ANALYSIS

To account for the large number of individual statistical tests performed throughout this report as detailed below, the Bonferroni Correction was applied to the alpha values for each group of tests. The alpha values were adjusted using the following equation of the Bonferroni Correction:

$$[1] \qquad \alpha_{crit} = \frac{\alpha}{k}$$

where alpha is 0.05 (the single test significance level) and k is the number of comparisons in a group of tests (McDonald 2009). The groups of tests were separated as follows: 1) density of all species with 5 independent t-tests, 2) species richness with 5 independent t-tests, and 3) density of individual species with 4 – 5 t-tests per species. Critical alpha values reported in the text and tables reflect the Bonferroni corrected values.

Watershed Comparisons

Mean watershed density was compared between the initial survey and the first monitoring event. First, the block density was calculated for each block across all index stations in the initial survey and first monitoring event using the following equation from Thompson (2012):

$$[2] \qquad D_{block} = \frac{\tau}{A}$$

where τ is the total abundance of unionids in the block and A is the total area sampled in the block (i.e., number of 1 m^2 quadrats excavated). Mean watershed density was calculated using the following equation:

$$[3] \qquad D_W = \frac{\sum D_{block_i}}{n}$$

where $\sum D_{block_i}$ is the summation of the block densities across the watershed within a sampling event and n is the total number of blocks. Standard error of the mean watershed density was calculated by dividing the standard deviation by the square root of the number of samples (McDonald 2009); the number of samples refers to the number of blocks surveyed within the watershed in the sampling event. A two-sample t-test assuming unequal variances ($\alpha_{crit} = 0.010$) was completed in Microsoft Excel 2016 using all of the block densities from the initial survey (n = 101) and all of the block densities from the first monitoring event (n = 100).

Sorenson's Coefficient (CC), which calculates the similarity between two communities, was used to compare the watershed mussel community between the initial survey and first monitoring event using the following equation:

$$[4] \qquad \mathrm{CC} = \left(\frac{2*\mathrm{C}}{\mathrm{S}_1+\mathrm{S}_2}\right)$$

where C represents the total number of species that were found in both communities being compared (initial survey and first monitoring event), S_1 represents the number of species in community one (initial survey), and S_2 represents the number of species in community two (first monitoring event) following the protocol of Sokal and Sneath (1963). Resulting values range from 0 to 1 where 0 represents complete community dissimilarity and 1 represents complete community overlap.

Mean watershed species richness was compared between the initial survey and the first monitoring event. First, the block species richness was calculated for each block in the initial survey and the first monitoring event using the following equation:

$$[5] \qquad SR_{block} = \frac{number\ of\ species}{A}$$

where the numerator is the total number of species in the block and A is the total area sampled in the block (i.e., number of 1 m^2 quadrats excavated). Mean watershed species richness was calculated using the following equation:

$$[6] \qquad SR_W = \frac{\sum SR_{block_i}}{n}$$

where $\sum SR_{block_i}$ is the summation of the block species richness values within the sampling event and n is the total number of blocks. Standard error of the mean watershed species richness was calculated as detailed for mean watershed density (McDonald 2009). A two-sample t-test assuming unequal variances ($\alpha_{crit} = 0.010$) was completed in Microsoft Excel 2016 using all of the block species richness from the initial survey (n = 101) and all of the block species richness from the first monitoring event (n = 100).

Site-level Comparisons

Using all unionid species, mean site density and mean site species richness were compared between the initial survey and the first monitoring event for all four index stations to investigate site level changes over time. To compare mean site density, the block density (D_{block}) was calculated for each block at a site using Equation [2]. Mean site density was calculated using the following equation:

$$[7] \qquad D_{site} = \frac{\sum D_{block_i}}{n}$$

where $\sum D_{block_i}$ is the summation of the block densities within a site and n is the total number of blocks surveyed at the site. Standard error of the mean site density was calculated as detailed above where the number of samples refers to the number of blocks surveyed within the site (McDonald 2009). A two-sample t-test assuming unequal variances ($\alpha_{crit} = 0.010$) was completed for each index station in Microsoft Excel 2016 using the block densities from the initial survey and the block densities from the first monitoring event at a site.

To compare mean site species richness, the block species richness (SR_{block}) was calculated for each block at a site using Equation [4]. Mean site species richness was calculated using the following equation:

$$[8] \qquad SR_{site} = \frac{\sum SR_{block_i}}{n}$$

where $\sum SR_{block_i}$ is the summation of the block species richness values within a site and n is the total number of blocks surveyed at the site. Standard error of the mean site species richness was calculated as detailed for mean site density (McDonald 2009). A two-sample t-test assuming unequal variances ($\alpha_{crit} = 0.010$) was completed for each index station in Microsoft Excel 2016 using the block species richness values from the initial survey and the block species richness values from the first monitoring event at a site.

Species-level Comparisons

Species-level comparisons were completed for two species: (1) the most abundant species in the watershed, *Eurynia dilatata* (Spike); and, (2) the only SAR hat occurs in the watershed, *C. iris*. Mean watershed density and mean site density were compared between the initial survey and the first monitoring event for each of these species. Mean watershed density was calculated using Equation [2] and Equation [3] and mean site density was calculated using Equation [2] and Equation [7] as detailed above using the abundance and density for each species. Standard error was calculated as detailed above with the number of samples referring to the number of blocks surveyed in the watershed and/or the site including blocks where the species was not detected (McDonald 2009). For mean watershed density, a two-sample t-test assuming unequal variances (*C. iris*: $\alpha_{crit} = 0.013$; *E. dilatata*: $\alpha_{crit} = 0.010$) was

completed in Microsoft Excel 2016 using all of the species block densities from the initial survey and the first monitoring event. For mean site density, a two-sample t-test assuming unequal variances (*C. iris*: $\alpha_{crit} = 0.013$; *E. dilatata*: $\alpha_{crit} = 0.010$) was completed in Microsoft Excel 2016 using all of the species block densities from the initial survey and all of the block densities from the first monitoring event at a site. A separate t-test was completed for each index station.

Population Size Structure

Length frequency distribution graphs were generated for *E*. *dilatata* and *C*. *iris*. Within a sampling event, length data for a species was combined across all four index stations. Length frequency distributions were generated using 10 mm size classes beginning at 0 mm and ending at the length of the largest individual observed during the sampling event. The first size class was adjusted to ensure that the following classes could be clearly separated into juveniles and adults (i.e., if the cut-off for juvenile length was 25 mm, the first class was 0 – 5 mm so the subsequent 10 mm classes would not include a class with both juveniles and adults). A Shapiro-Wilks test of normality was completed in RStudio Version 1.4.1106 (RStudio Team 2021) to analyze the normality of the size distributions using the following equation (Shapiro and Wilk 1965; Royston 1982):

$$[9] \qquad W = \frac{\left(\sum_{i=1}^{n} a_i x_{(i)}\right)^2}{\sum_{i=1}^{n} (x_i - \overline{x})^2}$$

where a_i are constants generated from the covariances, variances, and means of the sample (size n) from a normally distributed sample, $x_{(i)}$ is an individual data point value, and $\overline{x}$ is the sample mean.

A normal Shapiro-Wilks test result is not always indicative of a healthy unionid population. Annual recruitment has been observed to range greatly in unionid populations from years with no apparent recruitment to years where 50% of a population was represented by recruits (Haag 2012). As such, a healthy reproducing population may not follow a normal length distribution due to variation in recruitment rates overtime. The Shapiro-Wilks test provided quantitative insight into overall trends within a population (e.g., if skewed towards older or younger individuals) and was used to identify changes in the population size structure over time.

Proportion of Juveniles

The proportion of individuals considered to be juveniles was calculated separately for *E*. *dilatata* and *C*. *iris* to investigate the status of recruitment both within a population and within each sampling event. The quadrat survey method, with excavation 10 – 15 cm into the substrate, is effective at detecting juveniles (Metcalfe-Smith et al. 2007; Reid and Morris 2017). Juveniles were classified as individuals under a specified length cut-off determined differently between species depending on data availability. If no species specific cut-off could be found in the literature, the cut-off of 25

mm in length was used which represents individuals recruited into the population within the last 2 – 3 years (Haag and Warren 2007). This general cut-off was applied to *E. dilatata*. The 25 mm cut-off was also used for *C. iris* as the age of maturity for this species in Ohio was determined to be three years which falls into the 2 – 3 year range encompassed by this cut-off (Haag and Warren 2007; Watters et al. 2009; COSEWIC 2015). As Ohio is in close geographic proximity to the Saugeen River watershed and shares a similar climate, the age at maturity should not vary greatly between the populations.

RESULTS

WATERSHED COMPARISONS

All four index stations were successfully re-sampled in 2019 during the first monitoring event. Three of the sites remained in the same location as that of the initial survey; however, SG04a was moved approximately 200 m downstream from the initial survey location. The location was shifted within the site as no mussels were found during a preliminary visual search at the location of the initial survey. A large number of mussels were observed downstream within the site; therefore, the decision was made to shift the location of the first monitoring event survey to an area with a greater abundance of mussels within the site.

A total of 1,331 unionids were detected with 61.53% (819 individuals) found during the first monitoring event in 2019 compared to 38.47% (512 individuals) in the initial survey in 2011 (Table 4; Appendix B). Mean watershed density in the initial survey was 1.69 ± 0.26 mussels/m^2 and in the first monitoring event was 2.73 ± 0.45 mussels/m^2. This increase did not represent a significant change over time after correction for multiple tests ($t_{1,160}$=-1.986; p=0.050). Strong community overlap was observed between the two sampling events (CC = 0.80) despite three additional common species being found only during the first monitoring event: *Lasmigona compressa* (Creek Heelsplitter), *L. costata* (Flutedshell), and *Strophitus undulatus* (Creeper). Despite an absolute increase in the number of species observed in the first monitoring event, there was no change ($t_{1,196}$ = 1.319; p = 0.189) in mean sampling event species richness from the initial survey at 0.42 ± 0.03 species/m^2 to the first monitoring event at 0.37 ± 0.02 species/m^2.

SITE-LEVEL COMPARISONS

Changes in mean density and mean species richness were also compared between sampling events at the site level. A significant increase in mean site density was detected at SG04a in the Beatty Saugeen River and was likely caused by the change in location of the search area within the site (Table 5). A significant decrease in both mean site density and mean site species richness was detected at SG11 in the Teeswater River (Table 5, 6). There was no consistent pattern in changes to mean site

density or mean site species richness throughout the Saugeen River watershed between the two sampling events.

SPECIES-LEVEL COMPARISONS

Eurynia dilatata was the most abundant species in both sampling events, accounting for 86.33% of individuals in the initial survey and 93.89% of individuals in the first monitoring event (Table 4). *Eurynia dilatata* was also the most widespread species, being found at all four index stations in both sampling events (Table 4). Mean watershed density for *E. dilatata* showed no significant change ($t_{1,156}$ = -2.176; p = 0.031) from the initial survey at 1.45 ± 0.25 mussels/m^2 to the first monitoring event at 2.56 ± 0.44 mussels/m^2. A significant increase in average site density of *E. dilatata* was detected at SG04a while a significant decrease was detected at SG11 (Table 7). The length frequency distribution of observed *E. dilatata* was non-normal and left-skewed towards larger individuals in 2011 (W = 0.833; p = 0.026; Figure 3) and 2019 (W = 0.762; p = 0.003; Figure 3). The proportion of juvenile *E. dilatata* observed increased from 0.45% in the initial survey to 0.65% in the first monitoring event; however, the proportion of juveniles observed was very low in both sampling events (Figure 3).

Cambarunio iris was the only SAR detected live or as shells/valves in either sampling event (Table 4). A total of 80 live *C. iris* were detected, but the relative abundance decreased between sampling events with 57.50% (46 individuals) found during the initial survey and 42.50% (34 individuals) found during the first monitoring event. *Cambarunio iris* was detected alive at 50% (2/4) of sites in both sampling events; however, it was only detected alive in both sampling events at SGR-SGR-05 in the North Saugeen River. In the initial survey, *C. iris* was detected live at SG11 in the Teeswater River as well as SGR-SGR-05. In the first monitoring event, live *C. iris* was detected at SG08 in the Saugeen River as well as SGR-SGR-05. Over 250 shells and valves of *C. iris* were found at SG11 in the first monitoring event, but no live individuals were detected in 2019 after live individuals had been found at the site in the 2011 initial survey. Mean watershed density for *C. iris* did not change significantly ($t_{1,199}$ = 0.867; p = 0.387) over the two sampling events. When average site density was compared between the three index stations at which *C. iris* were detected, SG11 showed a significant decrease from the initial survey to the first monitoring event (Table 8). The length frequency distribution of observed *C*. *iris* was non-normal and left-skewed towards larger individuals in both 2011 (W = 0.776; p = 0.023; Figure 4) and 2019 (W = 0.767; p = 0.013; Figure 4). The proportion of juveniles observed decreased between sampling events with 6.52% of individuals representing recent recruits in the initial survey and no juveniles observed in the first monitoring event (Figure 4).

DISCUSSION

The status of the Saugeen River watershed unionid community appears relatively unchanged between the two sampling events; however, future surveys should be continued as long-term monitoring is necessary to understand the status of the unionid community. There was a slight increasing trend in watershed unionid density, which is positive; however, these differences were not significant and not consistently observed at the site level. The significant increase in mean site density observed at one index station (SG04a) was likely driven by the change in the location of the monitoring area within the site and may not be reflective of a change in density caused by an increase in the mussel community between sampling events. *Eurynia dilatata* represented the overwhelming majority of individuals detected in both sampling events and it experienced a significant increase in density over time; however, this increase was also likely a result of relocating the monitoring area at SG04a slightly downstream. The significant changes and insignificant trends seen in the *E. dilatata* population were mirrored in the full unionid community across the watershed, indicating that the changes in *E. dilatata* are driving the observed watershed-level changes. The previous surveys in the Saugeen River watershed completed by Morris et al. (2007) and McNichols-O'Rourke et al. (2012) also found the unionid community to be dominated by *E. dilatata* with 67% and over 90% relative abundance, respectively. *Eurynia dilatata* has been numerically dominant in the watershed over the period of formal surveys and while it is difficult to assess what is happening with most other species due to low abundance, it is important to recognize that changes in *E. dilatata* may reflect changes in the total unionid community because of this numerical dominance.

SAR CONCLUSIONS

The results of the first two sampling events in the long-term monitoring program of the Saugeen River watershed suggest that the *C. iris* population has remained stable over time. While the only significant change detected in *C. iris* was a decrease at one site, the watershed density did not change over time and the two other sites at which *C. iris* were detected had a non-significant trend of increased density indicating stability in the population overall between sampling events. Reid and Morris (2017) raised some concern about the ability of the 1 m^2 systematic quadrat sampling protocol with 20% site coverage (i.e., excavating three quadrats per block) to reliably detect a SAR as most are rare (i.e., density <0.10 m^2). *Cambarunio iris* was found at a mean site density greater than 0.10 m^2 at three of the four surveys at which it was detected; therefore, *C. iris* would not be considered rare overall in the watershed and the method used was likely able to accurately detect any changes in density between the sampling events.

Although the observed densities suggest *C. iris* has remained stable over time, some observations made in our surveys raise concerns. The number of shells observed at the Teeswater River site (SG11) during the first monitoring event is troubling and should be investigated as it could indicate high mortality in the Teeswater River subpopulation. Live *C. iris* have been detected in the Teeswater River (Morris et al.

2007; McNichols-O'Rourke et al. 2012; Sheldon et al. 2020; Goguen et al. 2021), but previous surveys have also detected a high volume of *C. iris* shells/valves in the waterbody. Morris et al. (2007) and Goguen et al. (2021) both found high numbers of *C. iris* shells/valves, relative to the shells of other species, at sites in the Teeswater River ~40 km upstream of the SG11 index station. This could indicate that there was a previous mass mortality event that occurred before 2006 and the shells/valves detected in recent surveys are merely evidence of that event or there could be ongoing high levels of mortality in the Teeswater River. Continued monitoring of the Teeswater River *C. iris* population will be critical to detecting further indication of high mortality rates.

The decrease in the proportion of observed juveniles over time, with zero juveniles detected in the first monitoring event, suggests the Saugeen River watershed *C. iris* population may be on the verge of a decline and future monitoring will be important. Morris et al. (2007) and McNichols et al. (2012) found evidence of recent reproduction and recruitment in *C. iris* in the Saugeen River watershed. While juveniles are generally difficult to detect and could have been missed during the sampling events, quadrat excavation is known to be effective for the detection of juveniles so it is likely that if *C. iris* juveniles were present at an index station they would have been detected during the survey (Metcalfe-Smith et al. 2007; Haag 2012; Reid and Morris 2017). Annual recruitment varies considerably between unionid species with some species reproducing and recruiting new individuals into the population every year while other species follow a more episodic reproductive schedule and have years with little to no recruitment (Haag 2012). *Cambarunio iris* is classified as following an opportunistic life history strategy which is characterized by a short life span, early maturation, high fecundity, and generally moderate to large body size (Haag 2012). Opportunistic species typically exhibit a strategy of high but variable recruitment over time with high proportions of recruits in some years and no detectable recruits in other years (Haag 2012). The first monitoring event could have occurred following a period in which the *C. iris* population naturally had low recruitment and this may not indicate a decrease in population health from the initial survey.

Successful recruitment could also be impacted by host availability in the watershed. In conjunction with the first monitoring event in 2019, fish community assessments were completed at each index station (Gáspárdy et al. 2021). Of the nine known host species for *C. iris*, three host species *Micropterus dolomieu* (Smallmouth Bass), *Luxilus chrysocephalus* (Striped Shiner), and *Etheostoma caeruleum* (Rainbow Darter) were detected across the index stations during the fish community assessments. Host species were found at three of the four index stations with none found at SGR-SGR-05 in the North Saugeen River. Interestingly, this was the only site at which *C. iris* were found live in both sampling events. The presence of *C. iris* host species in the watershed and at the majority of the index stations suggests host availability is not likely contributing to a reduction in recruitment.

CONCLUSIONS

The completion of the first monitoring event provided valuable insight into the status and trends of the unionid community and provided some notable conclusions and considerations:

- community density changes were driven by *E. dilatata* and may not represent the true patterns for all species;
- density of the *C. iris* population has remained stable over time, but failure to detect juveniles during the 2019 sampling along with absence of adults and abundance of spent shells at SG11 in 2019 may be indicative of future declines;
- consideration should be given to increasing the effort in future surveys in order to strengthen the reliability of rare species detections and population estimates;
- and the addition of more index stations in the watershed would aid in understanding the overall mussel community as well as *C*. *iris*, a species of special concern.

Continued monitoring of the Saugeen River watershed index stations will be critically important to track changes in the overall unionid community as well as the *C. iris* population.

ACKNOWLEDGEMENTS

Thank you to Vanessa Minke-Martin, Samantha Esteves, Jason Read, Maria Dolan, Brooklyn Foucault, Samuel Turner, Kali Zammit, and Saugeen Valley Conservation Authority staff for their assistance with field work and/or data organization. Thank you to Julia Colm for creating the GIS map. We also thank Dave Andrews and Julia Colm of Fisheries and Oceans Canada for reviewing this manuscript. Financial support for this project was provided by the Fisheries and Oceans Canada's Species at Risk program.

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Table 1. Species at risk in Ontario and their current COSEWIC assessment (Government of Canada 2021), federal *Species at Risk Act* listing (Government of Canada 2021), and provincial *Endangered Species Act* listing (OMNRF 2021) as of November 2021. UC indicates species that are under consideration for SARA listing. The historical (H) and current (C) occurrence of each SAR in the Saugeen River watershed is indicated as summarized in McNichols-O'Rourke et al. (2012). Species found live in the watershed are indicated by Y and species known only as shells/valves in the watershed as indicated by SH. Nomenclature here and throughout follows MolluscaBase eds. (2021).

Scientific Name	Common Name	COSEWIC	SARA (Federal)	ESA (Provincial)	H	C
Cambarunio iris[1]	Rainbow	Special Concern	Special Concern	Special Concern	Y	Y
Cyclonaias tuberculata	Purple Wartyback	Threatened	UC	UC	-	-
Epioblasma rangiana	Northern Riffleshell	Endangered	Endangered	Endangered	-	-
Epioblasma triquetra	Snuffbox	Endangered	Endangered	Endangered	-	-
Lampsilis fasciola	Wavyrayed Lampmussel	Special Concern	Special Concern	Threatened	-	-
Obliquaria reflexa	Threehorn Wartyback	Threatened	Threatened	Threatened	-	-
Obovaria olivaria	Hickorynut	Endangered	Endangered	Endangered	-	-
Obovaria subrotunda	Round Hickorynut	Endangered	Endangered	Endangered	-	-
Paetulunio fabalis[2]	Rayed Bean	Endangered	Endangered	Endangered	-	-
Pleurobema sintoxia	Round Pigtoe	Endangered	Endangered	Endangered	-	-
Ptychobranchus fasciolaris	Kidneyshell	Endangered	Endangered	Endangered	-	-
Quadrula quadrula	Mapleleaf	Special Concern[4]	Special Concern[4]	Special Concern	-	-
Sagittunio nasutus[3]	Eastern Pondmussel	Special Concern	Special Concern	Special Concern	-	-
Simpsonaias ambigua	Salamander Mussel	Endangered	Endangered	Endangered	-	-
Toxolasma parvum	Lilliput	Endangered	Endangered	Threatened	-	-
Truncilla donaciformis	Fawnsfoot	Endangered	Endangered	Endangered	-	Y

Species currently listed under SARA and formerly known as:
[1]*Villosa iris*
[2]*Villosa fabalis*
[3]*Ligumia nasuta*
[4]Great Lakes - Upper St. Lawrence population

Table 2. Site specific details for the index stations surveyed in the Saugeen River watershed (Figure 1). Sites are presented in upstream to downstream order. Some sites have been previously reported under a different site code; where applicable, the original site code and relevant report is provided. The coordinates provided are from the first monitoring event in 2019.

Site Code (Original Site Code)	Drainage	Waterbody	Latitude	Longitude	Initial Survey	First Monitoring Event
SG04a (SG04[1], SG4[2])	Lake Huron	Beatty Saugeen River	44.11714	-80.94364	27-Jul-11	22-Jul-19
SG08 (SG8[2])	Lake Huron	Saugeen River	44.22752	-81.16566	25-Jul-11	08-Jul-19
SGR-SGR-05 (DM11[1])	Lake Huron	North Saugeen River	44.30453	-81.21513	29-Jun-11	02-Jul-19
SG11[1]	Lake Huron	Teeswater River	44.27482	-81.27623	27-Jun-11	06-Aug-19

[1]Original site code and survey from McNichols-O'Rourke et al. (2012).
[2]Original site code and survey from Morris et al. (2007).

Table 3. Search effort and survey results from the initial survey and first monitoring event at each index station in the Saugeen River watershed. Sites are presented in upstream to downstream order.

	SG04a Beatty Saugeen River		SG08 Saugeen River		SGR-SGR-05 North Saugeen River		SG11 Teeswater River	
Date surveyed	27-Jul-11	22-Jul-19	25-Jul-11	08-Jul-19	29-Jun-11	02-Jul-19	27-Jun-11	06-Aug-19
# of blocks	25	25	26	25	25	25	25	25
# of quadrats	75	75	78	75	75	75	75	75
Total live abundance	30	250	122	346	212	189	148	34
Total live species richness	1	4	4	7	3	4	4	3
Mean unionid density (m^2)	0.40 ± 0.06	3.33 ± 0.73	1.56 ± 0.88	4.61 ± 1.54	2.83 ± 0.38	2.52 ± 0.30	1.97 ± 0.25	0.45 ± 0.11
Mean species richness (m^2)	0.24 ± 0.03	0.35 ± 0.04	0.28 ± 0.05	0.36 ± 0.04	0.64 ± 0.05	0.56 ± 0.06	0.51 ± 0.05	0.21 ± 0.04

Table 4. Total abundance, relative abundance, and frequency of occurrence of mussels observed at index stations in the Saugeen River watershed during the initial survey in 2011 and the first monitoring event in 2019. Species at risk are highlighted. S(#) indicates a species observed as complete shells and the number of shells found. V(#) indicates a species observed as a valve (one half of a full shell) and the number of valves found. Sites are presented in upstream to downstream order.

		2011							2019						
Scientific Name	**Common Name**	**SG04a**	**SG08**	**SGR-SGR-05**	**SG11**	**Totals**	**Relative Abundance (%)**	**Frequency of Occurrence (%)**	**SG04a**	**SG08**	**SGR-SGR-05**	**SG11**	**Totals**	**Relative Abundance (%)**	**Frequency of Occurrence (%)**
Alasmidonta marginata	Elktoe	-	-	13	2	**15**	**2.93**	**50.00**	1	V(16)	4	V(3)	**5**	**0.61**	**50.00**
Alasmidonta viridis	Slippershell	-	1	-	1	**2**	**0.39**	**50.00**	1	1	V(5)	1	**3**	**0.37**	**75.00**
Cambarunio iris	Rainbow	-	-	29	17	**46**	**8.98**	**50.00**	V(1)	3	31	S(28);V(253)	**34**	**4.15**	**50.00**
Eurynia dilatata	Spike	30	114	170	128	**442**	**86.33**	**100.00**	246	338	153	32	**769**	**93.89**	**100.00**
Lampsilis cardium	Plain Pocketbook	-	5	-	-	**5**	**0.98**	**25.00**	-	1	S(2);V(2)	V(4)	**1**	**0.12**	**25.00**
Lampsilis siliquoidea	Fatmucket	-	2	-	-	**2**	**0.39**	**25.00**	-	1	-	-	**1**	**0.12**	**25.00**
Lasmigona compressa	Creek Heelsplitter	-	-	-	-	**-**	**-**	**-**	2	V(1)	V(2)	-	**2**	**0.24**	**25.00**
Lasmigona costata	Flutedshell	-	-	-	-	**-**	**-**	**-**	-	1	1	1	**3**	**0.37**	**75.00**
Strophitus undulatus	Creeper	-	-	-	-	**-**	**-**	**-**	-	1	S(1)	-	**1**	**0.12**	**25.00**
Total Abundance		30	122	212	148	**512**			250	346	189	34	**819**		
Live Species Richness		1	4	3	4	**6**			4	7	4	3	**9**		
Total Species Richness		1	4	3	4	**6**			5	9	8	6	**9**		

Table 5. Density of mussels observed at the index stations in the Saugeen River watershed during the initial survey in 2011 and the first monitoring event in 2019. SE represents standard error. DF represents degrees of freedom. Sites are presented in upstream to downstream order. Significant differences at a given site over time (α_{crit}<0.010) are highlighted with significant increases highlighted in green and significant decreases highlighted in red.

	Density (#/m^2)							
Block	**SG04a**		**SG08**		**SGR-SGR-05**		**SG11**	
	2011	**2019**	**2011**	**2019**	**2011**	**2019**	**2011**	**2019**
1	0.67	6.00	0.67	9.33	0.67	2.00	2.33	1.33
2	0.67	13.67	0.33	8.00	1.67	5.33	1.33	0.33
3	0.67	5.00	0.33	2.33	5.33	1.00	0.00	0.00
4	1.00	9.33	1.00	1.00	4.33	0.00	0.67	0.00
5	0.33	6.33	0.33	13.33	2.67	3.00	1.00	0.33
6	0.33	0.33	0.00	25.33	3.33	1.33	0.67	0.33
7	0.00	3.33	0.00	19.00	4.33	4.00	1.67	0.00
8	0.67	3.00	0.00	24.33	3.33	0.67	1.33	1.00
9	0.67	3.33	0.00	2.33	2.33	1.67	1.00	0.67
10	0.67	0.33	0.00	1.00	6.00	3.33	3.00	0.00
11	0.33	0.33	1.33	0.33	1.00	3.33	1.33	0.67
12	0.00	0.33	0.00	0.33	1.00	1.00	0.67	0.33
13	0.67	2.33	0.00	0.33	2.00	1.33	2.00	0.33
14	0.00	0.00	23.00	0.33	6.33	5.00	2.00	1.67
15	0.33	0.67	2.00	0.00	1.00	2.00	1.00	0.67
16	0.00	0.33	0.67	0.33	0.33	0.67	3.33	0.33
17	0.00	0.33	3.67	0.00	2.67	2.33	2.00	0.67
18	1.00	8.33	2.33	0.00	0.67	4.00	1.33	0.00
19	0.33	1.33	2.33	0.33	1.67	1.67	3.33	0.00
20	0.00	7.33	1.33	1.00	0.67	2.33	5.33	1.67
21	0.33	5.33	0.00	0.33	5.00	3.67	1.67	0.00
22	0.33	5.67	0.67	1.00	4.67	2.67	3.67	0.00
23	0.00	0.33	0.00	0.67	0.33	5.33	2.67	0.00
24	0.33	0.00	0.33	2.00	5.00	3.33	3.67	1.00
25	0.67	0.00	0.33	2.33	4.33	2.00	2.33	0.00
26	-	-	0.00	-	-	-	-	-
Mean	**0.40**	**3.33**	**1.56**	**4.61**	**2.83**	**2.52**	**1.97**	**0.45**
SE	**0.06**	**0.73**	**0.88**	**1.54**	**0.38**	**0.30**	**0.25**	**0.11**
α		**<0.001**		**0.093**		**0.531**		**<0.001**
DF		**24**		**38**		**45**		**33**
t		**-4.000**		**-1.723**		**0.631**		**5.688**

Table 6. Species richness of mussels observed at the index stations in the Saugeen River watershed during the initial survey in 2011 and the first monitoring event in 2019. SE represents standard error. DF represents degrees of freedom. Sites are presented in upstream to downstream order. Significant differences at a given site over time (α_{crit}<0.010) are highlighted with significant increases highlighted in green and significant decreases highlighted in red.

	Species Richness (#/m^2)							
Block	**SG04a**		**SG08**		**SGR-SGR-05**		**SG11**	
	2011	**2019**	**2011**	**2019**	**2011**	**2019**	**2011**	**2019**
1	0.33	0.33	0.67	0.33	0.33	0.67	0.33	0.33
2	0.33	0.67	0.33	0.33	0.67	1.00	0.33	0.33
3	0.33	0.33	0.33	0.33	0.67	0.33	0.00	0.00
4	0.33	0.67	1.00	0.33	0.67	0.00	0.67	0.00
5	0.33	0.67	0.33	0.67	0.67	0.67	0.33	0.33
6	0.33	0.33	0.00	0.67	1.00	0.33	0.33	0.33
7	0.00	0.33	0.00	0.33	0.67	0.67	0.67	0.00
8	0.33	0.33	0.00	0.67	0.67	0.33	0.33	0.33
9	0.33	0.33	0.00	0.33	1.00	0.33	0.33	0.33
10	0.33	0.33	0.00	0.67	1.00	0.67	1.00	0.00
11	0.33	0.33	0.67	0.33	0.33	1.00	0.33	0.33
12	0.00	0.33	0.00	0.33	0.67	0.33	0.33	0.33
13	0.33	0.33	0.00	0.33	0.67	0.67	0.67	0.33
14	0.00	0.00	0.67	0.33	0.67	0.67	0.33	0.33
15	0.33	0.33	0.33	0.00	1.00	0.33	0.33	0.33
16	0.00	0.33	0.33	0.33	0.33	0.33	0.67	0.33
17	0.00	0.33	0.67	0.00	0.67	0.67	0.67	0.33
18	0.33	0.33	0.33	0.00	0.33	0.33	0.67	0.00
19	0.33	0.33	0.33	0.33	0.67	0.33	0.67	0.00
20	0.00	0.67	0.33	0.33	0.33	0.67	0.67	0.67
21	0.33	0.33	0.00	0.33	0.67	0.67	0.33	0.00
22	0.33	0.33	0.33	0.33	0.67	0.67	1.00	0.00
23	0.00	0.33	0.00	0.33	0.33	0.67	0.67	0.00
24	0.33	0.00	0.33	0.67	1.00	1.33	0.67	0.33
25	0.33	0.00	0.33	0.33	0.67	0.33	0.33	0.00
26	-	-	0.00	-	-	-	-	-
Mean	**0.24**	**0.35**	**0.28**	**0.36**	**0.65**	**0.56**	**0.51**	**0.21**
SE	**0.03**	**0.04**	**0.05**	**0.04**	**0.05**	**0.06**	**0.05**	**0.04**
α		**0.028**		**0.248**		**0.205**		**<0.001**
DF		**47**		**44**		**46**		**46**
t		**-2.263**		**-1.172**		**1.287**		**4.820**

Table 7. Density of the most abundant common species (*Eurynia dilatata,* Spike) observed in the Saugeen River watershed during the initial survey in 2011 and the first monitoring event in 2019. SE represents standard error. DF represents degrees of freedom. Sites are presented in upstream to downstream order. Significant differences at a given site over time ($\boldsymbol{\alpha_{crit}}$<0.010) are highlighted with significant increases highlighted in green and significant decreases highlighted in red.

	***Eurynia dilatata* (#/m^2)**							
Block	**SG04a**		**SG08**		**SGR-SGR-05**		**SG11**	
	2011	**2019**	**2011**	**2019**	**2011**	**2019**	**2011**	**2019**
1	0.67	6.00	0.33	9.33	0.67	1.67	2.33	1.33
2	0.67	13.33	0.33	8.00	1.00	4.33	1.33	0.33
3	0.67	5.00	0.00	2.33	4.67	1.00	0.00	0.00
4	1.00	9.00	0.33	1.00	3.00	0.00	0.33	0.00
5	0.33	6.00	0.33	13.00	2.00	2.33	1.00	0.33
6	0.33	0.33	0.00	24.33	2.00	1.33	0.67	0.33
7	0.00	3.33	0.00	19.00	4.00	2.67	1.33	0.00
8	0.67	3.00	0.00	24.00	3.00	0.67	1.33	1.00
9	0.67	3.33	0.00	2.33	1.67	1.67	1.00	0.67
10	0.67	0.33	0.00	0.67	4.33	3.00	2.33	0.00
11	0.33	0.33	1.00	0.33	1.00	1.67	1.33	0.67
12	0.00	0.33	0.00	0.33	0.67	1.00	0.67	0.33
13	0.67	2.33	0.00	0.33	1.67	1.00	1.67	0.33
14	0.00	0.00	22.67	0.33	5.67	4.33	2.00	1.67
15	0.33	0.67	2.00	0.00	0.33	2.00	1.00	0.67
16	0.00	0.33	0.67	0.00	0.33	0.67	2.67	0.00
17	0.00	0.33	3.33	0.00	2.00	1.33	1.67	0.67
18	1.00	8.33	2.33	0.00	0.67	4.00	1.00	0.00
19	0.33	1.33	2.33	0.33	1.00	1.67	2.67	0.00
20	0.00	7.00	1.33	1.00	0.67	1.67	4.00	1.33
21	0.33	5.33	0.00	0.33	3.67	2.33	1.67	0.00
22	0.33	5.67	0.67	1.00	4.33	2.33	3.00	0.00
23	0.00	0.33	0.00	0.67	0.33	4.33	2.00	0.00
24	0.33	0.00	0.00	1.67	4.00	2.00	3.33	1.00
25	0.67	0.00	0.33	2.33	4.00	2.00	2.33	0.00
26	-	-	0.00	-	-	-	-	-
Mean	0.40	3.28	1.46	4.51	2.27	2.04	1.71	0.43
SE	0.06	0.71	0.87	1.51	0.33	0.24	0.19	0.10
α		**<0.001**		**0.088**		**0.280**		**<0.001**
DF		**24**		**38**		**44**		**36**
t		**-4.020**		**-1.750**		**0.558**		**5.882**

Table 8. Density of the single SAR (*Cambarunio iris*, Rainbow) observed in the Saugeen River watershed during the initial survey in 2011 and the first monitoring event in 2019. SE represents standard error. DF represents degrees of freedom. Sites are presented in upstream to downstream order. Significant differences at a given site over time (α_{crit}<0.013) are highlighted with significant increases highlighted in green and significant decreases highlighted in red.

	***Cambarunio iris* (#/m²)**							
Block	**SG04a**		**SG08**		**SGR-SGR-05**		**SG11**	
	2011	**2019**	**2011**	**2019**	**2011**	**2019**	**2011**	**2019**
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.67	0.67	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	1.33	0.00	0.33	0.00
5	0.00	0.00	0.00	0.00	0.67	0.67	0.00	0.00
6	0.00	0.00	0.00	1.00	0.67	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.33	1.33	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	1.33	0.33	0.33	0.00
11	0.00	0.00	0.00	0.00	0.00	1.33	0.00	0.00
12	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.33	0.33	0.00
14	0.00	0.00	0.00	0.00	0.67	0.67	0.00	0.00
15	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00
17	0.00	0.00	0.00	0.00	0.67	1.00	0.33	0.00
18	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00
19	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00
20	0.00	0.00	0.00	0.00	0.00	0.67	1.33	0.00
21	0.00	0.00	0.00	0.00	1.33	1.33	0.00	0.00
22	0.00	0.00	0.00	0.00	0.00	0.33	0.33	0.00
23	0.00	0.00	0.00	0.00	0.00	1.00	0.67	0.00
24	0.00	0.00	0.00	0.00	0.67	0.67	0.33	0.00
25	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00
26	-	-	0.00	-	-	-	-	-
Mean	0.00	0.00	0.00	0.04	0.39	0.41	0.23	0.00
SE	0.00	0.00	0.00	0.04	0.09	0.10	0.07	0.00
α		**-**		**0.327**		**0.841**		**0.002**
DF		**-**		**24**		**48**		**24**
t		**-**		**-1.000**		**-0.202**		**3.440**

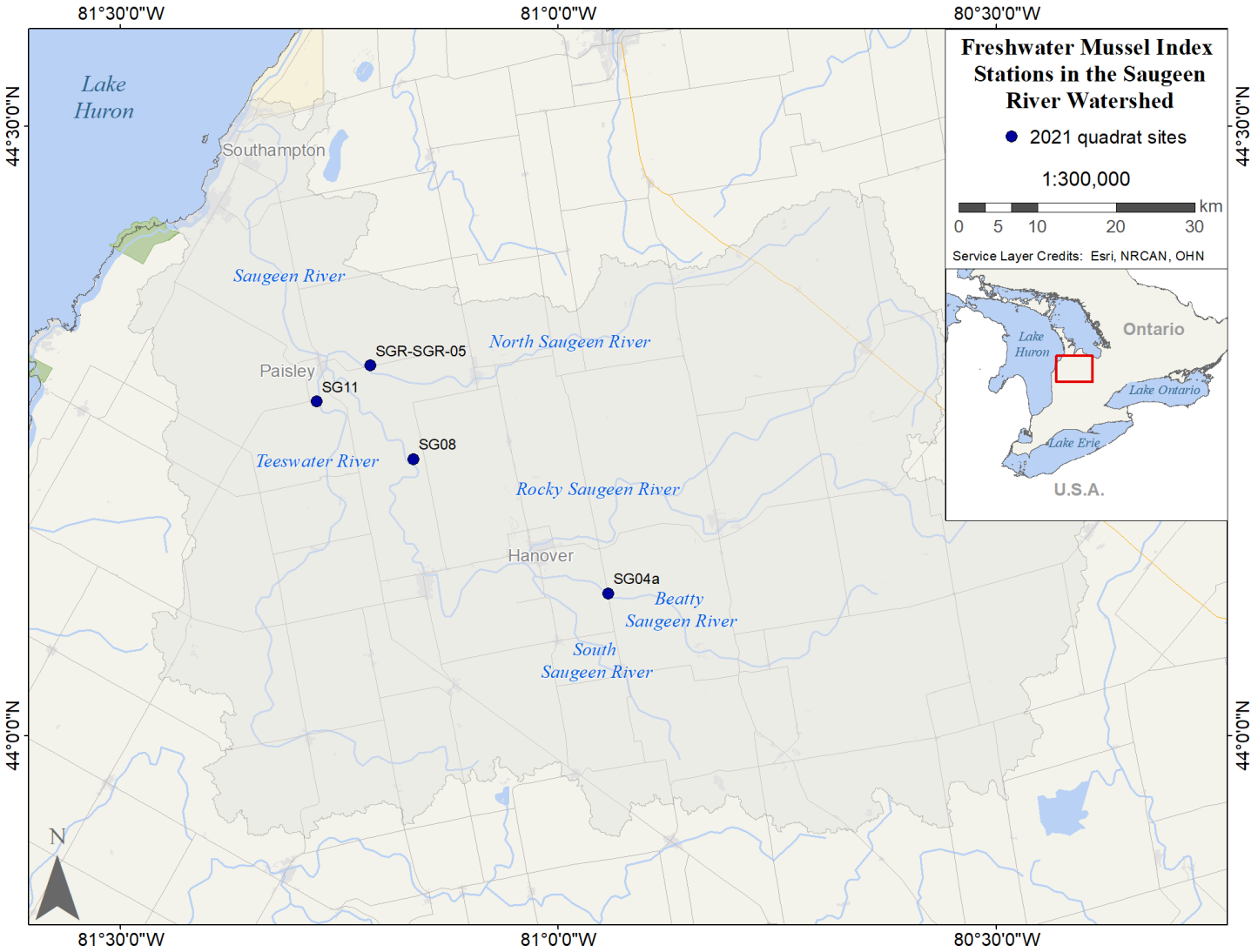

Figure 1. Location of four index stations in the Saugeen River watershed in Ontario.

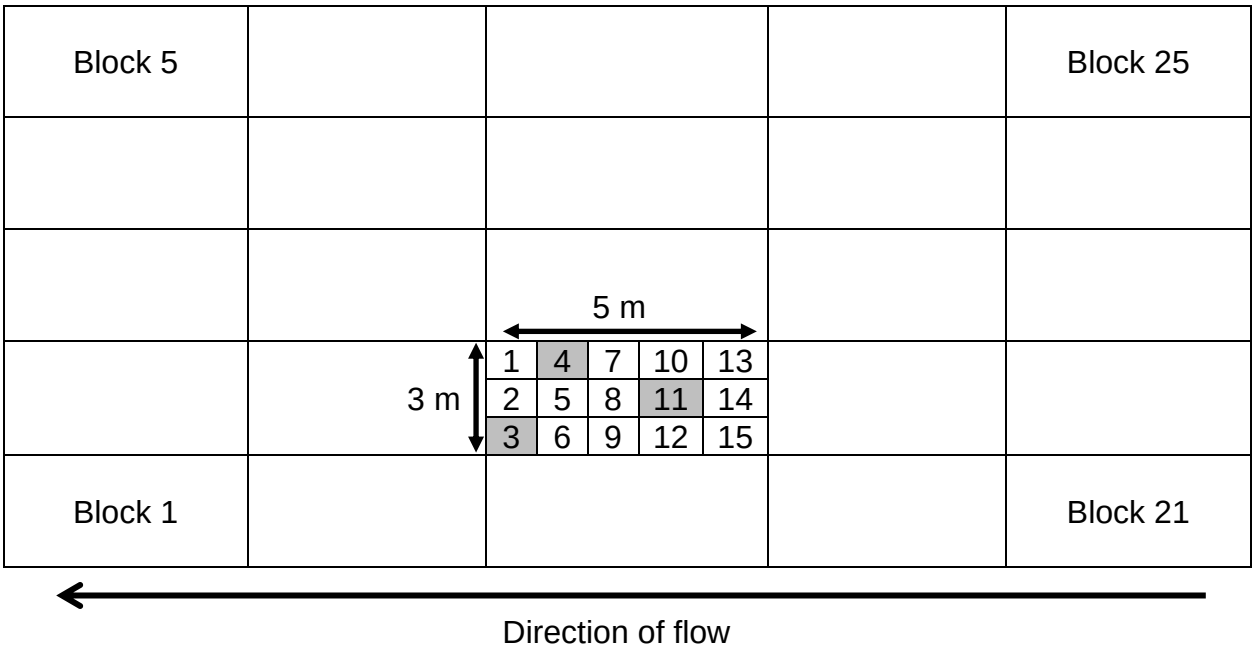

Figure 2. Systematic sampling design (Metcalfe-Smith et al. 2007) implemented at all index stations during the initial survey and first monitoring event. The shaded boxes mark the location of the randomly selected quadrats that would be sampled in each block at a site.

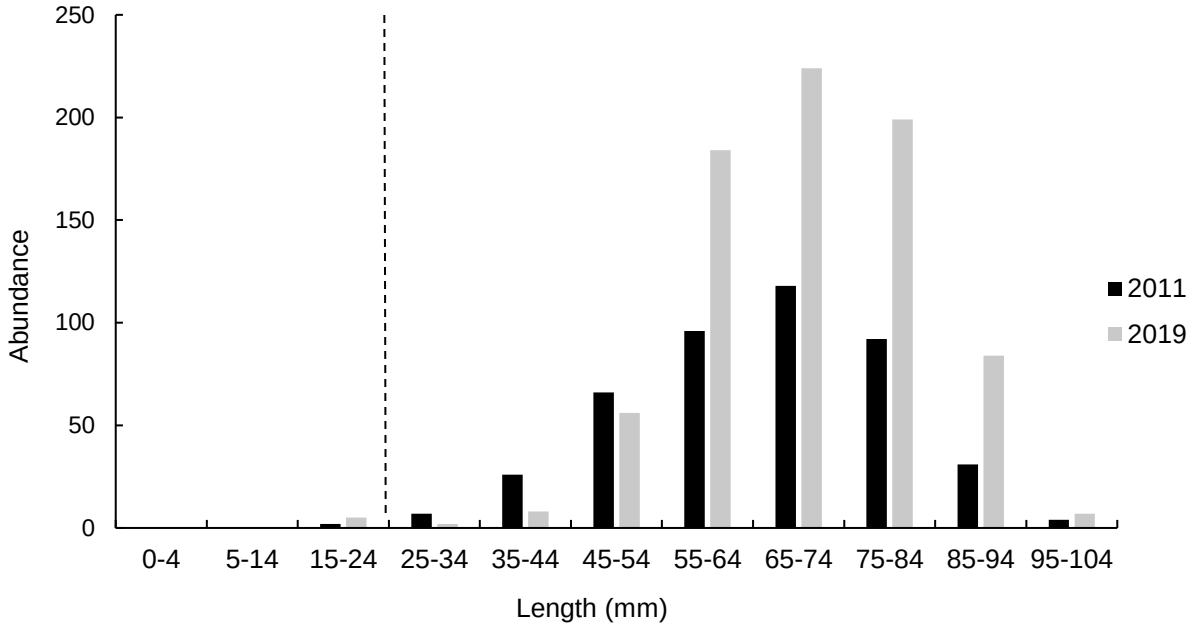

Figure 3. Length frequency distribution for *Eurynia dilatata* (n = 1,211) collected from the Saugeen River watershed in the initial survey in 2011 (n = 442) and the first monitoring event in 2019 (n = 769). The dashed vertical line represents the separation of juveniles (<5.0 mm) and adults (≥25.0 mm).

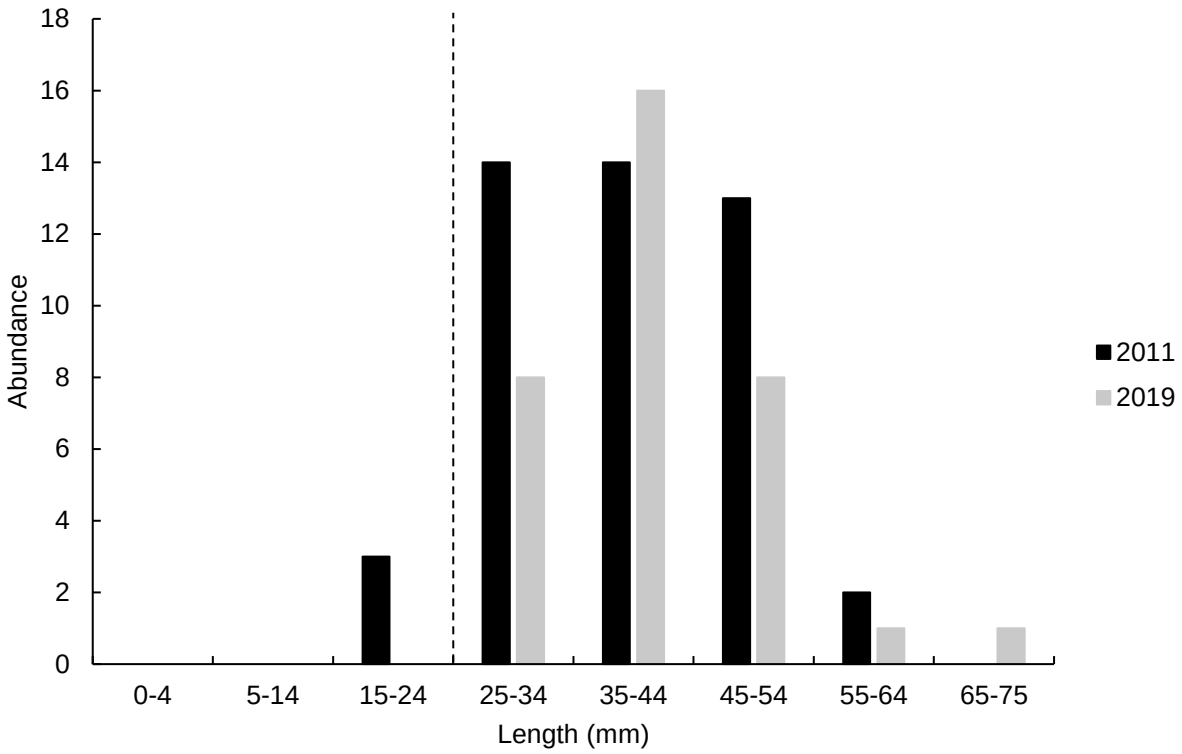

Figure 4. Length frequency distribution for *Cambarunio iris* (n = 80) collected from the Saugeen River watershed in the initial survey in 2011 (n = 46) and the first monitoring event in 2019 (n = 34). The dashed vertical line represents the separation of juveniles (<25.0 mm) and adults (≥25.0 mm).

Appendix A. Relevant environmental data (mean ± standard error) collected during the initial survey and first monitoring event at each index station in the Saugeen River watershed. Sites are presented in upstream to downstream order.

		SG04a Beatty Saugeen River		SG08 Saugeen River		SGR-SGR-05 North Saugeen River		SG11 Teeswater River	
		2011	2019	2011	2019	2011	2019	2011	2019
	Depth (m)	0.37 ± 0.01	0.43 ± 0.02	0.33 ± 0.01	0.33 ± 0.01	0.29 ± 0.01	0.29 ± 0.01	0.47 ± 0.01	0.34 ± 0.01
	Velocity (m/s)	0.25 ± 0.01	0.11 ± 0.01	0.32 ± 0.02	0.32 ± 0.02	0.56 ± 0.02	0.58 ± 0.01	0.35 ± 0.01	0.19 ± 0.01
	Water Clarity (m)	-	>0.60 ± 0.00	-	>0.60 ± 0.00	-	0.28 ± 0.79	-	0.44 ± 0.35
YSI Measurements	**Water Temperature (°C)**	-	23.54	-	22.72	-	22.74	-	25.07
	Conductivity (µs/cm)	-	524.00	-	603.00	-	428.90	-	506.00
	Total Dissolved Solids (mg/L)	-	350.16	-	409.90	-	291.52	-	328.54
	Salinity (psu)	-	0.26	-	0.31	-	0.22	-	0.24
	Dissolved Oxygen %	-	110.00	-	103.10	-	96.50	-	112.50
	Dissolved Oxygen (mg/L)	-	9.33	-	8.88	-	8.31	-	9.27
	pH	-	8.55	-	8.35	-	8.34	-	8.56
	Turbidity (FNU)	-	2.40	-	5.15	-	17.30	-	9.98
Substrate composition	**Bedrock (%)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Boulder (%)	12.08 ± 1.90	13.27 ± 1.67	0.00 ± 0.00	17.36 ± 2.84	15.15 ± 1.56	10.99 ± 1.38	16.38 ± 1.70	15.68 ± 1.40
	Rubble (%)	45.69 ± 1.79	35.57 ± 2.08	1.41 ± 0.47	12.80 ± 1.25	39.63 ± 1.72	34.05 ± 1.52	47.77 ± 2.21	36.42 ± 1.65
	Gravel (%)	26.11 ± 1.26	23.76 ± 1.58	22.44 ± 2.60	44.97 ± 2.47	23.36 ± 1.10	37.68 ± 1.30	21.31 ± 1.46	27.36 ± 1.50
	Sand (%)	13.26 ± 0.79	18.67 ± 1.36	43.85 ± 2.87	24.73 ± 2.01	16.42 ± 1.39	16.72 ± 1.49	12.77 ± 1.12	18.24 ± 1.24
	Silt (%)	2.01 ± 0.60	2.53 ± 0.80	14.81 ± 1.55	0.00 ± 0.00	4.63 ± 0.90	0.00 ± 0.00	1.38 ± 0.43	1.01 ± 0.36
	Clay (%)	0.14 ± 0.14	0.07 ± 0.07	0.06 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.15	0.00 ± 0.00
	Muck (%)	0.00 ± 0.00	2.87 ± 1.22	15.58 ± 2.22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.95 ± 0.59
	Marl (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Detritus (%)	0.69 ± 0.35	3.27 ± 1.22	1.86 ± 0.65	0.13 ± 0.09	0.82 ± 0.31	0.56 ± 0.22	0.23 ± 0.13	0.34 ± 0.15

Appendix B. Composition of the unionid community at each site in the initial survey (2011) and the first monitoring event (2019). Species at risk are highlighted. S(#) indicates a species observed as complete shells and the number of shells found. V(#) indicates a species observed as a valve (one half of a full shell) and the number of valves found. Sites are presented in upstream to downstream order.

	SG04a Beatty Saugeen River							
	2011				2019			
Species	**Abundance**	**Relative Abundance (%)**	**Density (mussels/m^2)**	**Occurrence (% of quadrats)**	**Abundance**	**Relative Abundance (%)**	**Density (mussels/m^2)**	**Occurrence (% of quadrats)**
Alasmidonta marginata	-	-	-	-	1	0.40	0.01	1.33
Alasmidonta viridis	-	-	-	-	1	0.40	0.01	1.33
Cambarunio iris	-	-	-	-	V(1)	-	-	-
Eurynia dilatata	30	100.00	0.40	28.00	246	98.40	3.28	62.67
Lasmigona compressa	-	-	-	-	2	0.80	0.03	2.67
Total abundance	**30**				**250**			
Total live richness	**1**				**4**			
Total species richness	**1**				**5**			

SG08 Main Saugeen River								
	2011				2019			
Species	**Abundance**	**Relative Abundance (%)**	**Density (mussels/m^2)**	**Occurrence (% of quadrats)**	**Abundance**	**Relative Abundance (%)**	**Density (mussels/m^2)**	**Occurrence (% of quadrats)**
Alasmidonta marginata	-	-	-	-	V(16)	-	-	-
Alasmidonta viridis	1	0.82	0.01	1.28	1	0.29	0.01	1.33
Cambarunio iris	-	-	-	-	3	0.87	0.04	2.67
Eurynia dilatata	114	93.44	1.46	34.62	338	97.69	4.51	57.33
Lampsilis cardium	5	4.10	0.06	6.41	1	0.29	0.01	1.33
Lampsilis siliquoidea	2	1.64	0.03	2.56	1	0.29	0.01	1.33
Lasmigona compressa	-	-	-	-	V(1)	-	-	-
Lasmigona costata	-	-	-	-	1	0.29	0.01	1.33
Strophitus undulatus	-	-	-	-	1	0.29	0.01	1.33
Total abundance	**122**				**346**			
Total live richness	**4**				**7**			
Total species richness	**4**				**9**			

	SGR-SGR-05 North Saugeen River							
	2011				2019			
Species	**Abundance**	**Relative Abundance (%)**	**Density (mussels/m^2)**	**Occurrence (% of quadrats)**	**Abundance**	**Relative Abundance (%)**	**Density (mussels/m^2)**	**Occurrence (% of quadrats)**
Alasmidonta marginata	13	6.13	0.17	16.00	4	2.12	0.05	5.33
Alasmidonta viridis	-	-	-	-	V(5)	-	-	-
Cambarunio iris	29	13.68	0.39	26.67	31	16.40	0.41	25.33
Eurynia dilatata	170	80.19	2.27	81.33	153	80.95	2.04	74.67
Lampsilis cardium	-	-	-	-	S(2);V(2)	-	-	-
Lasmigona compressa	-	-	-	-	V(2)	-	-	-
Lasmigona costata	-	-	-	-	1	0.53	0.01	1.33
Strophitus undulatus	-	-	-	-	S(1)	-	-	-
Total abundance	**212**				**189**			
Total live richness	**3**				**4**			
Total species richness	**3**				**8**			

SG11 Teeswater River								
	2011				2019			
Species	**Abundance**	**Relative Abundance (%)**	**Density (mussels/m^2)**	**Occurrence (% of quadrats)**	**Abundance**	**Relative Abundance (%)**	**Density (mussels/m^2)**	**Occurrence (% of quadrats)**
Alasmidonta marginata	2	1.35	0.03	2.67	V(3)	-	-	-
Alasmidonta viridis	1	0.68	0.01	1.33	1	2.94	0.01	1.33
Cambarunio iris	17	11.49	0.23	18.67	S(28);V(253)	-	-	-
Eurynia dilatata	128	86.49	1.71	76.00	32	94.12	0.43	32.00
Lampsilis cardium	-	-	-	-	V(4)	-	-	-
Lasmigona costata	-	-	-	-	1	2.94	0.01	1.33
Total abundance	**148**				**34**			
Total live richness	**4**				**3**			
Total species richness	**4**				**6**			