Investigating the presence of *Truncilla donaciformis* (Fawnsfoot) in Muskrat Creek, Lake Huron drainage

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Muskrat Creek is a small tributary of the Teeswater River (Lake Huron drainage) in southwestern Ontario. In 2005, a live *Truncilla donaciformis* (Fawnsfoot) was found in Muskrat Creek representing the first detection of this endangered species in the Lake Huron drainage outside of its known range in the Lake Erie and Lake St. Clair drainages. Muskrat Creek is considered a current location of *T. donaciformis* by the Committee on the Status of Endangered Wildlife in Canada. In 2019, Fisheries and Oceans Canada completed timed-search surveys and quadrat excavation in Muskrat Creek and the Teeswater River to investigate the presence of *T. donaciformis* in the survey area and determine if this is still a current location for this species. No evidence of *T. donaciformis* was detected in either waterbody and no live unionids were detected in Muskrat Creek. Based on the search effort completed, the probability of detecting at least one *T. donaciformis* individual was >99.9% for the timed-search surveys and 96% – >99.9% for the quadrat surveys. The lack of evidence despite a high probability of detection indicates that Muskrat Creek is unlikely to support a viable population of *T. donaciformis*.

RESUMÉ

Goguen, M.N., McNichols-O'Rourke, K.A., and Morris, T.J. 2021. Investigating the presence of *Truncilla donaciformis* (Fawnsfoot) in Muskrat Creek, Lake Huron drainage. Can. Manuscr. Rep. Fish. Aquat. Sci. 3212: v + 21 p.

Le ruisseau Muskrat est un petit affluent de la rivière Teeswater (bassin hydrographique du lac Huron) dans le sud-ouest de l'Ontario. En 2005, on a observé une troncille pied-de-faon (*Truncilla donaciformis*) vivante dans le ruisseau Muskrat. Il s'agissait de la première détection de cette espèce en voie de disparition dans le bassin hydrographique du lac Huron, soit à l'extérieur de son aire de distribution connue dans les bassins hydrographiques du lac Érié et du lac Sainte-Claire. Le Comité sur la situation des espèces en péril au Canada (COSEPAC) considère le ruisseau Muskrat comme un emplacement où l'on trouve actuellement la troncille pied-de-faon. En 2019, Pêches et Océans Canada a effectué des relevés de recherche programmée et des échantillonnages par quadrats dans le ruisseau Muskrat et la rivière Teeswater pour étudier la présence de troncilles pied-de-faon dans la zone de relevé et pour déterminer si cette espèce se trouve toujours dans ces cours d'eau. La présence de la troncille pied-de-faon n'a pas été détectée dans les deux cours d'eau et aucun unionidé vivant n'a été détecté dans le ruisseau Muskrat. Selon les activités de recherche effectuées, la probabilité de détection d'au moins une troncille pied-de-faon était de plus de 99,9 % pour les relevés de recherche programmée et de 96 % à 99,9 % pour les échantillonnages par quadrats. L'absence de preuve en dépit d'une forte probabilité de détection indique qu'il est peu probable que le ruisseau Muskrat puisse maintenir une population viable de troncilles de pied-de-faon.

INTRODUCTION

Freshwater mussels are critically important components of the aquatic ecosystems in which they occur as they are natural environmental filters, provide habitat for algae and invertebrates, provide physical stability to the substrate, and transfer energy from aquatic to terrestrial environments [Committee on the Status of Endangered Wildlife in Canada (COSEWIC) 2008; Haag 2012]. In recent decades, the Bivalvia taxon has experienced global declines and is one of the most imperilled taxa in the world (Lopes-Lima et al. 2018). This trend of drastic declines has also been seen nationally and has resulted in 35% of Canada's 55 native species being considered at-risk (Ricciardi et al. 1998; Government of Canada 2020). Declines have been primarily driven by the invasion of dreissenid mussels (*Dreissena polymorpha*, Zebra Mussel; *Dreissena rostriformis bugensis*, Quagga Mussel), habitat loss and degradation, and decreasing water quality (Ricciardi et al. 1998; COSEWIC 2008).

Of Ontario's 42 native freshwater mussel species, 15 are currently listed as Endangered, Threatened, or of Special Concern under the federal *Species at Risk Act* (SARA; Table 1). *Truncilla donaciformis* (Fawnsfoot) was assessed as Endangered by COSEWIC (2008) and listed as such under SARA in 2019 (Government of Canada 2020). *Truncilla donaciformis* is a small mussel that typically occurs in the lower reaches of larger rivers in sand or gravel substrate [COSEWIC 2008; Fisheries and Oceans Canada (DFO) 2011]. Freshwater Drum (*Aplodinotus grunniens*) and Sauger (*Sander canadensis*) are the known hosts of *T. donaciformis* in the United States and are the assumed hosts for Canadian populations of *T. donaciformis*, with Freshwater Drum considered the likely primary Canadian host due to overlapping distributions (COSEWIC 2008; DFO 2011).

Truncilla donaciformis is currently thought to occur in five waterbodies in Ontario: St. Clair delta, Sydenham River, Thames River, Grand River, and Muskrat Creek (COSEWIC 2008). Muskrat Creek is a cold water tributary of the Teeswater River in the Lake Huron drainage in southwestern Ontario where land use is primarily agricultural; the creek flows between farm fields until joining the Teeswater River just east of the town of Teeswater, Ontario [Drinking Water Source Protection (DWSP) 2015; Saugeen Valley Conservation Authority (SVCA) 2019]. While the occurrence of *T. donaciformis* in the St. Clair delta and the Sydenham, Thames, and Grand rivers is consistent with the known habitat preferences of the species and its host, the Muskrat Creek occurrence appears unusual as the reported collection site is small (stream order, river width, etc.) and isolated from the known range of the suspected host species.

In 2005, Environment Canada (now Environment and Climate Change Canada) completed benthic invertebrate surveys in Muskrat Creek and discovered a single live *T. donaciformis* (COSEWIC 2008; DFO 2011). This detection represents a range extension into the Lake Huron drainage and is well outside the known range of the species in the Lake Erie and Lake St. Clair drainages. No previous mussel surveys had been conducted in Muskrat Creek, but numerous qualitative and quantitative surveys have been conducted in the Saugeen River watershed, including the Teeswater River, both prior to and since 2005 and no other evidence of *T. donaciformis* has ever been detected (Morris and Di Maio 1998–1999; Morris et al. 2007; McNichols-O'Rourke et al. 2012; Sheldon et al. 2020). In 2019, DFO conducted an extensive survey of Muskrat Creek and the Teeswater River in the area around the confluence with Muskrat Creek in an attempt to confirm the presence of *T. donaciformis* and to delineate its range within this waterbody.

METHODS

SAMPLING METHODS

Between June and August 2019, a total of eleven sites were surveyed with six sites in Muskrat Creek and five in the Teeswater River (Table 2; Figure 1). In Muskrat Creek, three sites were located upstream of the 2005 *T. donaciformis* record and three sites were downstream of the record (Figure 2). In the Teeswater River, one site was immediately upstream of the confluence with Muskrat Creek and a second site was immediately downstream of the confluence (Figure 3). Each of the eight sites was 500 m in length for a total of 3,000 m of river length in Muskrat Creek and 1,000 m in the Teeswater River. In Muskrat Creek, the sites downstream of the record were continuous, with the end of one site marking the beginning of the next site. Two of the upstream sites were continuous but the third was ~500 m upstream of the end of the second site. This gap was due to site accessibility. The same total amount of river length was searched downstream and upstream of the *T. donaciformis* record. The two Teeswater River sites were continuous with the end of the first site marking the beginning of the second site at the confluence point with Muskrat Creek.

All eight of these sites were surveyed using both a semi-quantitative timed-search method and a quantitative quadrat method. A crew of 5–6 people completed a 4.5 person-hour timed-search survey using mussel viewers throughout the entire 500 m length of each site (Metcalfe-Smith et al. 2000a). Additionally, ten 1 m^2 quadrats were excavated within the 500 m length of each site following the modified procedure of Metcalfe-Smith et al. (2007; Figure 4). Each quadrat was searched using three different techniques: 1) visual search with the naked eye, 2) visual search with a viewing box, and 3) excavation to a depth of 10–15 cm. While the location of each quadrat was determined randomly, the process for selecting the location was handled differently in Muskrat Creek and the Teeswater River as the waterbodies differed greatly in river width. In Muskrat Creek, a random number generator was used to select a distance from the beginning of the site (i.e., 0 m to 500 m) to determine the location along the length of the site at which the quadrat would be placed. No randomization was given to the position of the quadrat along the width of the river at a given distance as the creek was narrow [average width = 2.58 m ± 0.33 (standard error, SE)]; the quadrat was placed in the center of the creek at the random distance along the length of the site. In the wider Teeswater River, the location of each quadrat was determined using a three-point random number generator sequence: 1) distance along the site (length); 2) distance off the center line (width); and, 3) left (0) or right (1) off the center line. The location of each quadrat was marked before the timed-search survey began to prevent interference between the two search methods. In Muskrat Creek, timed-search surveys were completed throughout the entire 3,000 m length of creek and 60 m^2 of area was excavated. In the Teeswater River, timed-search surveys were completed throughout the entire 1,000 m length of river and 20 m^2 of area was excavated. In both survey methods, each mussel found alive was identified to species, counted, measured (length in millimeters), and sexed visually (if sexually dimorphic) before being returned to the river. Shells of species not observed live at a site were also counted and recorded.

The three remaining sites in the Teeswater River were surveyed using only a qualitative timed-search method (Figure 3). One site (SG01) was ~1.5 km upstream of the two 500 m long sites around the Muskrat Creek confluence. This site had been previously surveyed by DFO in 2006 and 2011 (Morris et al. 2007; McNichols-O'Rourke et al. 2012). In 2006, one live species (*Cambarunio iris*, Rainbow) was detected with an additional four common species found as shells/valves (Morris et al. 2007). Shells of *C. iris* were the only evidence of unionids observed during the 2011 survey of the same site (McNichols-O'Rourke et al. 2012). No evidence of *T. donaciformis* was found during either previous survey. The other two sites were downstream of the dam in Teeswater, Ontario approximately 2.5 km and 5 km downstream from the 500 m long sites at the confluence with Muskrat Creek. At each of these three sites, a crew of 5–6 people completed a 4.5 person-hour timed-search with mussel viewers and/or mussel scoops (Metcalfe-Smith et al. 2000a). Each mussel found alive was identified to species, counted, measured (length in millimeters), and sexed visually (if sexually dimorphic) before being returned to the river. Shells of species not observed live at the site were also counted and recorded.

Environmental data were also collected at each site. Before the timed-search survey began, water velocity (OTT MF Pro flow meter), water clarity (0.60 m turbidity tube), and water chemistry (EXO2 Multiparameter YSI) were measured. After the timed-search was completed, substrate composition (%) was estimated across the entire site. Definitions of substrate sizes were taken 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). Before quadrat excavation began, water velocity (OTT MF Pro flow meter), depth (meter stick), and water clarity (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), shading (open, partly open, dense), and presence or absence of aquatic macrophytes. Substrate composition was estimated using the definitions from Stanfield (2010) as detailed above. 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 (i.e., a site may have low overall siltation compared to another site but still has variation among quadrats so low, medium, and high siltation is recorded within the 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.

DATA ANALYSIS

The probability of detection of *T. donaciformis* in Muskrat Creek and the Teeswater River was calculated to determine the confidence of the survey results. The probability of detecting at least one *T. donaciformis* if present in the survey area was calculated using the following equation from Smith (2006):

$$P = 1 - e^{-\beta\alpha\mu}$$

where:

P is the probability of detecting at least one individual,
e is the base of natural logarithms,
β is the search efficiency of the method used,
α is the search area, and
μ is the density of the species in the search area.

A separate probability of detection was calculated for the quadrat surveys and the timed-search surveys to investigate the likelihood that a *T. donaciformis* would have been detected during each sampling method independently from the other. The search area for both the timed-search and quadrats surveys was combined across the eight 500 m long sites in Muskrat Creek and the Teeswater River; the three additional timed-search survey sites in the Teeswater River were not included as the total length of river searched was not measured as in the 500 m sites.

For the timed-search surveys, the search efficiency (β) was estimated to be 5%. Timed-search surveys only involved a visual search of the riverbed with no excavation such that only individuals at the surface would be detected. Reid and Morris (2017) found that ~5–7% of the total unionids detected during two full site excavations were found at the surface of the riverbed and could be detected visually. Although Reid and Morris (2017) found that *T. donaciformis* were not detected at the surface during their study, this species has been detected during timed-search surveys and at the surface during other quadrat excavations in southern Ontario (Metcalfe-Smith et al. 2000b; Morris and Edwards 2007; DFO unpublished data). This indicates the timed-search survey technique is a suitable, though inefficient, method for detecting *T. donaciformis*. The low end of the Reid and Morris (2017) range (i.e., 5%) was chosen for the search efficiency to account for the potentially low proportion of individuals at the surface. The search area (α) was calculated differently for Muskrat Creek and the Teeswater River before being added together. The area surveyed in Muskrat Creek was calculated to be 7,740 m^2 using the total river length sampled (3,000 m), the average width of the river across the six sites (2.58 m), and 100% of the riverbed searched. As Muskrat Creek was very narrow, crew members were able to walk shoulder to shoulder while completing the timed-search survey viewing 100% of the surface of the riverbed throughout each site. The total area surveyed in the Teeswater River was calculated to be 1,960 m^2 using the total river length sampled (1,000 m), the average width of the river across the two sites (9.80 m), and 20% of the riverbed searched. As the Teeswater River was wider than Muskrat Creek, crew members were not able to view the entire surface of the riverbed throughout the sites. The estimate of 20% of the riverbed searched was based on the number of crew members and the approximate field of view while walking upstream with a mussel viewer. The total search area (α) used for the timed-search surveys was 9,700 m^2.

For the quadrat surveys, the search efficiency (β) was considered to be 1 representing 100% detectability within the 1 m^2 quadrat as each quadrat was searched until no further animals were detected. The total search area (α) was 80 m^2 as 80 individual 1 m^2 quadrats were excavated throughout Muskrat Creek and the Teeswater River.

The same densities (μ) were used to calculate the probability of detection for the timed-search and quadrat surveys. As the density of *T. donaciformis* is not known in Muskrat Creek or the Teeswater River as only a single animal has ever been detected, the density of known populations at long-term monitoring index stations in the Thames River and Sydenham River in southwestern Ontario were used (DFO unpublished data). The Thames and Sydenham river populations were selected as they are believed to represent stable populations currently demonstrating signs of reproduction. The probability of detection was calculated using two densities from each watershed: 1) the lowest site density within the watershed and 2) the average watershed density. In the Thames River, the lowest site density of *T. donaciformis* is 0.04 mussels/m^2 and the average watershed density is 0.59 mussels/m^2. In the Sydenham River, the lowest site density and average watershed density are both 0.05 mussels/m^2.

RESULTS

No evidence of *T. donaciformis* was detected in Muskrat Creek or the Teeswater River during the 2019 surveys. In Muskrat Creek, no live unionids of any species were detected in either the timed-search surveys or the quadrat surveys. Very weathered shells/valves of four common species were found during the timed-search surveys (Table 3): *Alasmidonta viridis* (Slippershell), *Anodontoides ferussacianus* (Cylindrical Papershell), *Lasmigona compressa* (Creek Heelsplitter), and *Strophitus undulatus* (Creeper). The majority of the shells/valves were found in the sites upstream of the *T. donaciformis* record; shells/valves of all four species were found in the site directly upstream of the record (LHU-MSK-05) while no shells/valves of any species were found in the site directly downstream of the record (LHU-MSK-04). A single weathered valve of *L. compressa* was found during the quadrat surveys in LHU-MSK-05 (Table 4).

In the Teeswater River, 144 live individuals representing five species were observed during the five timed-search surveys (Table 5): *C. iris*, *Lampsilis siliquoidea* (Fatmucket), *L. compressa*, *Lasmigona costata* (Flutedshell), and *S. undulatus*. Of those animals, 85% (123 individuals) were found at the site directly upstream of the confluence with Muskrat Creek (LHU-TWR-07). *Lampsilis siliquoidea* was the most abundant species found representing 83% (120 individuals) of the unionids detected. *Cambarunio iris* was the only SAR found in the Teeswater River and accounted for 2% (3 individuals) of the total unionids detected. Six additional common species were found as shells/valves during the timed-search surveys. During the quadrat surveys, no evidence of unionids was detected in the site directly downstream of the Muskrat Creek confluence (LHU-TWR-06) but two live individuals representing two common species were found at LHU-TWR-07: *Eurynia dilatata* (Spike) and *L. siliquoidea* (Table 6). Six additional species were also found as shells/valves in LHU-TWR-07 including ten shells/valves of *C. iris*. All of the species detected in 2019 had been previously observed in the Teeswater River.

The probability that a *T. donaciformis* individual would have been detected in the timed-search surveys or quadrat surveys is high (Table 7). The timed-search surveys had a probability of detection in excess of 99.9% likelihood across all of the tested densities. There is a 96% – >99.9% likelihood that *T. donaciformis* would have been detected in the survey area during the quadrat surveys even if it occurred at the lowest known site density (Thames River 0.04 mussels/m^2).

Only environmental data from the timed-search surveys is presented as these averages are representative of the entire site rather than single locations within a site as measured by the quadrats (Table 8, 9). Muskrat Creek was shallow and narrow across all six sites with an average depth of 0.33 (± 0.03) m and average width of 2.58 (± 0.33) m. Water temperature warmed throughout the summer but remained cool with an average of 16.72 (± 1.20) °C. The substrate in Muskrat Creek was composed of ~60% sand and gravel across all sites. Water clarity was high throughout Muskrat Creek exceeding the 0.60 m turbidity tube. In the Teeswater River, average depth was similar at 0.29 (± 0.04) m but the average width was much greater at 9.80 (± 1.02) m. The site water temperature varied greatly upstream and downstream of the Muskrat Creek confluence with a water temperature of 25.1°C in the site directly upstream of the confluence and 16.6°C directly downstream. The average water temperature across the five sites was 21.92 (± 1.77) °C. The substrate in the Teeswater River was much coarser than Muskrat Creek with boulder and cobble accounting for 60 % of the composition. Water clarity in the Teeswater River was also high with four of five sites exceeding the 0.60 m turbidity tube and one site with clarity of 0.48 m.

DISCUSSION

The lack of evidence of *T. donaciformis* detected during the 2019 surveys indicates it is unlikely that there is a population in the surveyed area of Muskrat Creek and the Teeswater River. The survey techniques and search effort employed would have been sufficient for detecting a small, rare species such as *T. donaciformis* with a very high degree of certainty. The high probability of detection for both the timed-search and quadrat surveys indicates that an adequate search effort was completed for both survey methods. While the probability of detection would still have been very high if just one method had been employed, the completion of both timed-search and quadrat surveys provides even stronger support for the conclusion that this species no longer occurs in the survey area. The 4.5 person-hour search effort used during the timed-search surveys is known to be effective at detecting most rare species at a site; however, this method is biased towards larger individuals that are more easily detected visually (Metcalfe-Smith et al. 2000a). Quadrat excavation is effective at detecting small individuals; therefore, even if missed during the timed-search surveys, *T. donaciformis* should have been detected during the quadrat excavation, for which sufficient search area was completed to reach a high probability of detection (Metcalfe-Smith et al. 2007; Reid and Morris 2017). While it is possible in any survey that a very rare individual remained undetected, the 2019 surveys provide strong confidence that if *T. donaciformis* occur in the survey area they do not represent a viable, self-sustaining population.

Based on the environmental data collected during the 2019 surveys, the habitat requirements for *T. donaciformis* are not met in Muskrat Creek making it unlikely that this species would persist here. This species typically occurs in the lower reaches of large rivers such as the lower Thames River and lower Grand River (COSEWIC 2008; DFO 2011). While the dominant sand and gravel substrate in Muskrat Creek is consistent with the substrate preferences of *T. donaciformis*, the narrow width, shallow depth, and cool water temperature of the creek do not provide suitable habitat for *T. donaciformis*. Cold water systems are not ideal habitat for unionid species as cold water temperatures can disrupt reproduction and slow growth rate (Haag 2012). The paucity of any live unionids in Muskrat Creek suggests this waterbody does not provide suitable habitat for supporting unionid populations of any species.

The habitat of Muskrat Creek is not only unsuitable for *T. donaciformis*, but also for Freshwater Drum and Sauger, its two presumed hosts. Neither host species has been recorded in the Saugeen River watershed although both are known to occur in Lake Huron (COSEWIC 2008; Holm et al. 2009; DFO 2011; D. Andrews, DFO, personal communication, 2019; R. Gaspardy, DFO, personal communication, 2019). Freshwater Drum inhabit lakes and large, slow moving rivers while Sauger occur in cool water habitats in lakes and streams but must remain in deep waters or very turbid shallow waters due to their light sensitive eyes (Holm et al. 2009). The shallow depth, narrow width, and high water clarity in Muskrat Creek make this waterbody unsuitable for Freshwater Drum and Sauger; additionally, the cold water temperature contributes to unsuitable habitat for Freshwater Drum. The likelihood of either host species occurring in Muskrat Creek is further reduced by the number of physical barriers between Lake Huron and Muskrat Creek. There are 52 dams over three metres in height across the Saugeen River watershed with 14 in the Teeswater River subwatershed (Smith 2002; SVCA 2019; SVCA 2020); six dams lie between the location of the *T. donaciformis* record and Lake Huron (COSEWIC 2008; Land Information Ontario 2021). A number of these dams are very large such as Denny's Dam (Southampton, Ontario) which is less than 5 km upstream of Lake Huron. Several of the large dams in the lower portion of the Saugeen River watershed (e.g., Denny's Dam, Maple Hill Dam in Walkerton) have fish ladders to facilitate salmon migration which could potentially allow Freshwater Drum and Sauger to move further upstream in the watershed from Lake Huron, but the lack of records in the watershed suggest these species do not move upstream from the lake (Ontario Steelheaders 2020). As recognized in COSEWIC (2008), it is very unlikely that the *T. donaciformis* collected from Muskrat Creek was brought into the waterbody while encysted on a Freshwater Drum or Sauger.

In spite of sufficient effort, there was no indication of *T. donaciformis* and only extremely limited evidence of any unionids within Muskrat Creek. Habitat conditions within the creek do not correspond to the conditions required by *T. donaciformis* or its suspected hosts. Although it cannot be explained how a single individual of *T. donaciformis* was collected from Muskrat Creek in 2005, it is clear that the creek does not support an established population at this time.

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REFERENCES

Committee on the Status of Endangered Wildlife in Canada (COSEWIC). 2008. COSEWIC assessment and status report on the Fawnsfoot *Truncilla donaciformis* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vii + 39 pp.

Fisheries and Oceans Canada (DFO). 2011. Recovery Potential Assessment of Eastern Pondmussel (*Ligumia nasuta*), Fawnsfoot (*Truncilla donaciformis*), Mapleleaf (*Quadrula quadrula*), and Rainbow (*Villosa iris*) in Canada. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2010/073.

Drinking Water Source Protection (DWSP). 2015. Watershed Characterization. Approved Assessment Report for the Saugeen Valley Source Protection Area. Drinking Water Source Protection Act for Clean Water. Available from http://home.waterprotection.ca/wp-content/uploads/AR/SVCA/SVSPA_Ch2_2016_Final.pdf [accessed 17 November 2020].

Government of Canada. 2020. Species at Risk Public Registry. Available from https://wildlife-species.canada.ca/species-risk-registry/sar/index/default_e.cfm [accessed 17 November 2020].

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

Holm, E., Mandrak, N.E., and Burridge, M.E. 2009. The ROM field guide to freshwater fishes of Ontario. Royal Ontario Museum, Toronto, Ontario, Canada.

Land Information Ontario. 2021. Ontario dam inventory. Ontario GeoHub. Available from https://geohub.lio.gov.on.ca/datasets/mnrf::ontario-dam-inventory [accessed 4 January 2021].

Lopes-Lima, M., Burlakova, L.E., Karatayev, A.Y., Mehler, K., Seddon, M., and Sousa, R. 2018. Conservation of freshwater bivalves at the global scale: diversity, threats and research needs. Hydrobiologia 810:1–14.

McNichols-O'Rourke, K.A., Robinson, A., and Morris, T.J. 2012. Summary of freshwater mussel timed search surveys in southwestern Ontario in 2010 and 2011. Can. Manuscr. Rpt. Fish. Aquat. Sci. 3009: vi + 42 pp.

Metcalfe-Smith, J.L., Di Maio, J., Staton, S.K., and Mackie, G.L. 2000a. Effect of sampling effort on the efficiency of the timed search method for sampling freshwater mussel communities. J. N. Am. Benthol. Soc. 19(4):725–732.

Metcalfe-Smith, J.L., Mackie, G.L., Di Maio, J., and Staton, S.K. 2000b. Changes over time in the diversity and distribution of freshwater mussels (Unionidae) in the Grand River, southwestern Ontario. J. Great Lakes Res. 26(4):445–459.

Metcalfe-Smith, J.L., McGoldrick, D.J., Zanatta, D.T., and Grapentine, L.C. 2007. Development of a monitoring program for tracking the recovery of endangered freshwater mussels in the Sydenham River, Ontario. Water Science and Technology Directorate, Environment Canada, Burlington, Ontario. 07-510:61 p.

MolluscaBase eds. 2021. MolluscaBase. Available from https://www.molluscabase.org/ [accessed 9 September 2021].

Morris, T.J., and Di Maio, J. 1998–1999. Current distribution of freshwater mussels (Bivalvia: Unionidae) in rivers of southwestern Ontario. Malacological Review. 31:9–17.

Morris, T.J., and Edwards, A. 2007. Freshwater mussel communities of the Thames River, Ontario: 2004-2005. Can. Manuscr. Rpt. Fish. Aquat. Sci. 2810: v + 30 pp.

Morris, T.J., Granados, M., and Edwards, A. 2007. A preliminary survey of the freshwater mussels of the Saugeen River Watershed, Ontario. Burlington, ON. Fisheries and Oceans Canada. Can. Manuscr. Rep. Fish. Aquat. Sci. 2809: v + 30 p.

Ontario Ministry of Natural Resources and Forestry (OMNRF). 2020. Species at Risk in Ontario (SARO) List. Available from https://www.ontario.ca/environment-and-energy/species-risk-type?name=Fish+and+Mussels [accessed 17 November 2020].

Ontario Steelheaders. 2020. Saugeen River. Available from http://www.steelheaders.ca/saugeen-river/#.XtadC_lKi70 [accessed 20 November 2020].

Reid, S.M., and Morris, T.J. 2017. Tracking the recovery of freshwater mussel diversity in Ontario rivers: Evaluation of a quadrat-based monitoring protocol. Diversity. 9:1–17.

Ricciardi, A., Neves, R.J., and Rasmussen, J.B. 1998. Impending extinctions of North American freshwater mussels (Unionoida) following the zebra mussel (*Dreissena polymorpha*) invasion. J. Anim. Ecol. 67:613–619.

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

Smith, D.C. 2002. Improving the sustainability of river systems through the removal of dams on the Saugeen River, southwestern Ontario. M.Sc. thesis, School of Environment & Sustainability, Royal Roads University, Victoria, B.C.

Smith, D.R. 2006. Survey design for detecting rare freshwater mussels. J. N. Am. Benthol. Soc. 25(3):701–711.

Stanfield, L. 2010. Ontario stream assessment protocol. Version 8. Fisheries Policy Section. Ontario Ministry of Natural Resources. Peterborough, Ontario, Canada. Available from https://docs.ontario.ca/documents/2575/226871.pdf [accessed 4 January 2021].

Saugeen Valley Conservation Authority (SVCA). 2019. Teeswater River watershed report card. Available from https://www.svca.on.ca/downloads/Teeswater_1.pdf [accessed 17 November 2020].

Saugeen Valley Conservation Authority (SVCA). 2020. Dams. Available from https://www.svca.on.ca/page.php?page=waterdams [accessed 20 November 2020].

Table 1. Species at Risk in Ontario and their current federal (Government of Canada 2020) and provincial (OMNRF 2020) designation status as of November 2020. Nomenclature here and throughout follows MolluscaBase eds. (2021).

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

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 location and sampling date details for the eleven sites surveyed in Muskrat Creek and the Teeswater River by Fisheries and Oceans Canada in 2019. Sites are presented in downstream to upstream order.

Site Code	Drainage	Waterbody	Latitude	Longitude	Date
LHU-MSK-02	Lake Huron	Muskrat Creek	43.99298	-81.27518	2019-08-13
LHU-MSK-03	Lake Huron	Muskrat Creek	43.98995	-81.27161	2019-08-13
LHU-MSK-04	Lake Huron	Muskrat Creek	43.98480	-81.26690	2019-07-04
LHU-MSK-05	Lake Huron	Muskrat Creek	43.98447	-81.26682	2019-06-25
LHU-MSK-06	Lake Huron	Muskrat Creek	43.98123	-81.26379	2019-06-26
LHU-MSK-07	Lake Huron	Muskrat Creek	43.96752	-81.25562	2019-07-04
LHU-TWR-04	Lake Huron	Teeswater River	43.99510	-81.33715	2019-08-12
LHU-TWR-05	Lake Huron	Teeswater River	43.99704	-81.31120	2019-08-12
LHU-TWR-06	Lake Huron	Teeswater River	44.00026	-81.28305	2019-08-15
LHU-TWR-07	Lake Huron	Teeswater River	44.00009	-81.27733	2019-08-14
SG01	Lake Huron	Teeswater River	44.00344	-81.26431	2019-08-15

Table 3. Results of the timed-search surveys at six sites in Muskrat Creek. S(#) represents individuals that were detected as a shell (both valves) and the number of shells detected. V(#) represents individuals that were detected as a single valve and the number of valves detected. Sites are presented in downstream to upstream order.

Scientific Name	Common Name	LHU-MSK-02	LHU-MSK-03	LHU-MSK-04	LHU-MSK-05	LHU-MSK-06	LHU-MSK-07	Totals
Alasmidonta viridis	Slippershell	-	V(1)	-	V(1)	V(2)	V(5)	-
Anodontoides ferussacianus	Cylindrical Papershell	-	-	-	V(1)	-	-	-
Lasmigona compressa	Creek Heelsplitter	V(1)	-	-	V(1)	S(2);V(3)	V(2)	-
Strophitus undulatus	Creeper	-	-	-	V(1)	-	-	-
Total Abundance		0	0	0	0	0	0	0
Live Species Richness		0	0	0	0	0	0	0
Total Species Richness		1	1	0	4	2	2	4

Table 4. Results of the quadrats excavated throughout six sites in Muskrat Creek. V(#) represents individuals that were detected as a single valve and the number of valves detected. Sites are presented in downstream to upstream order.

Scientific Name	Common Name	LHU-MSK-02	LHU-MSK-03	LHU-MSK-04	LHU-MSK-05	LHU-MSK-06	LHU-MSK-07	Totals
Lasmigona compressa	Creek Heelsplitter	-	-	-	V(1)	-	-	-
Total Abundance		0	0	0	0	0	0	0
Live Species Richness		0	0	0	0	0	0	0
Total Species Richness		0	0	0	1	0	0	1

Table 5. Results of the timed-search surveys at five sites in the Teeswater River. Species at Risk are highlighted. S(#) represents individuals that were detected as a shell (both valves) and the number of shells detected. V(#) represents individuals that were detected as a single valve and the number of valves detected. Sites are presented in downstream to upstream order.

Scientific Name	**Common Name**	**LHU-TWR-04**	**LHU-TWR-05**	**LHU-TWR-06**	**LHU-TWR-07**	**SG01**	**Totals**	**Relative Abundance (%)**	**Frequency of Occurrence (%)**
Actinonaias ligamentina	Mucket	-	V(16)	-	-	-	-	-	-
Alasmidonta marginata	Elktoe	V(14)	V(6)	-	V(8)	V(6)	-	-	-
Alasmidonta viridis	Slippershell	V(4)	-	V(1)	S(1); V(5)	V(7)	-	-	-
Cambarunio iris	Rainbow	-	S(1); V(9)	V(4)	3	S(19); V(144)	3	2.08	20.00
Eurynia dilatata	Spike	V(1)	-	-	-	-	-	-	-
Lampsilis cardium	Plain Pocketbook	-	V(5)	-	-	-	-	-	-
Lampsilis siliquoidea	Fatmucket	S(1); V(1)	V(32)	S(1); V(32)	120	S(1); V(1)	120	83.33	20.00
Lasmigona compressa	Creek Heelsplitter	1	9	-	-	V(1)	10	6.94	40.00
Lasmigona costata	Flutedshell	S(1); V(2)	7	-	-	-	7	4.86	20.00
Pyganodon grandis	Giant Floater	V(3)	V(43)	-	V(5)	V(2)	-	-	-
Strophitus undulatus	Creeper	V(3)	4	-	-	-	4	2.78	20.00
Total Abundance		1	20	0	123	0	144		
Live Species Richness		1	3	0	2	0	5		
Total Species Richness		8	9	3	5	6	11		

Table 6. Results of the quadrats excavated throughout two sites in the Teeswater River. S(#) represents individuals that were detected as a shell (both valves) and the number of shells detected. V(#) represents individuals that were detected as a single valve and the number of valves detected. Sites are presented in downstream to upstream order.

Scientific Name	Common Name	LHU-TWR-06	LHU-TWR-07	Totals	Relative Abundance (%)	Frequency of Occurrence (%)
Actinonaias ligamentina	Mucket	-	V(2)	-	-	-
Alasmidonta marginata	Elktoe	-	V(1)	-	-	-
Alasmidonta viridis	Slippershell	-	V(4)	-	-	-
Cambarunio iris	Rainbow	-	S(4);V(6)	-	-	-
Eurynia dilatata	Spike	-	1	1	50.00	50.00
Lampsilis siliquoidea	Fatmucket	-	1	1	50.00	50.00
Pyganodon grandis	Giant Floater	-	V(1)	-	-	-
Strophitus undulatus	Creeper	-	V(1)	-	-	-
Total Abundance		0	2	2		
Live Species Richness		0	2	2		
Total Species Richness		0	8	8		

Table 7. Probability of detection of T. donaciformis *during the timed-search surveys and quadrat surveys completed in Muskrat Creek and the Teeswater River calculated by using the lowest site density and average watershed density from the Thames River and Sydenham River.*

	Thames River		**Sydenham River**	
	Site low (0.04 mussels/m^2)	Watershed average (0.59 mussels/m^2)	Site low (0.05 mussels/m^2)	Watershed average (0.05 mussels/m^2)
$P_{\text{Timed-search}}$	>0.999	>0.999	>0.999	>0.999
P_{Quadrat}	0.959	>0.999	0.982	0.982

Table 8. Environmental data collected during the timed-search surveys at six sites in Muskrat Creek. SE represents the standard error of the average. Sites are presented in downstream to upstream order.

	LHU-MSK-02	LHU-MSK-03	LHU-MSK-04	LHU-MSK-05	LHU-MSK-06	LHU-MSK-07	Average	SE
Average site depth (m)	0.25	0.4	0.35	0.35	0.4	0.25	0.33	0.03
Average site width (m)	4	3	2	2	2.5	2	2.58	0.33
Water temperature (°C)	19.1	21.1	14.6	14	14.2	17.3	16.72	1.20
Bedrock (%)	5	0	0	0	0	0	0.83	0.83
Boulder (%)	10	10	5	2	0	0	4.50	1.89
Cobble (%)	60	30	10	15	10	25	25.00	7.75
Gravel (%)	5	0	30	40	38	45	26.33	7.82
Sand (%)	5	30	50	35	50	30	33.33	6.79
Silt (%)	5	0	0	0	0	0	0.83	0.83
Clay (%)	5	0	0	0	0	0	0.83	0.83
Muck (%)	0	20	0	0	0	0	3.33	3.33
Detritus (%)	5	10	5	8	2	0	5.00	1.51

Table 9. Environmental data collected during the timed-search surveys at five sites in the Teeswater River. SE represents the standard error of the average. Sites are presented in downstream to upstream order.

	LHU-TWR-04	**LHU-TWR-05**	**LHU-TWR-06**	**LHU-TWR-07**	**SG01**	**Average**	**SE**
Average site depth (m)	0.4	0.25	0.15	0.35	0.3	0.29	0.04
Average site width (m)	12	12	7	10	8	9.80	1.02
Water temperature (°C)	24.1	25	16.6	25.1	18.8	21.92	1.77
Bedrock (%)	0	0	0	0	0	0.00	0.00
Boulder (%)	10	5	30	5	0	10.00	5.24
Cobble (%)	55	40	45	50	60	50.00	3.54
Gravel (%)	20	30	10	25	15	20.00	3.54
Sand (%)	0	25	13	15	5	11.60	4.31
Silt (%)	5	0	2	0	5	2.40	1.12
Clay (%)	0	0	0	0	0	0.00	0.00
Muck (%)	0	0	0	0	10	2.00	2.00
Detritus (%)	10	0	0	5	5	4.00	1.87

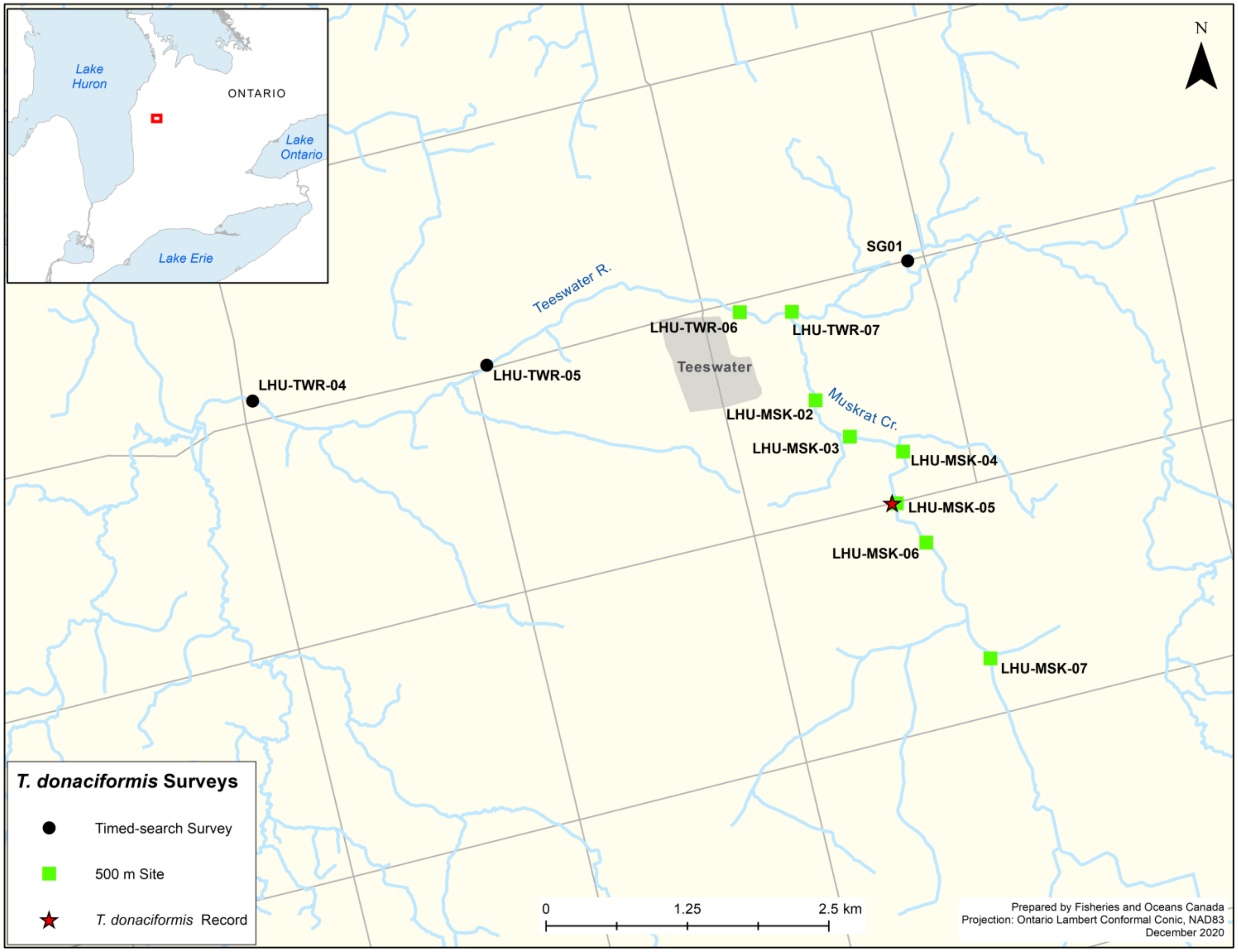

Figure 1. Eleven sites in Muskrat Creek and the Teeswater River surveyed by Fisheries and Oceans Canada in 2019. The eight 500 m long sites are marked at the starting (downstream) end of the site.

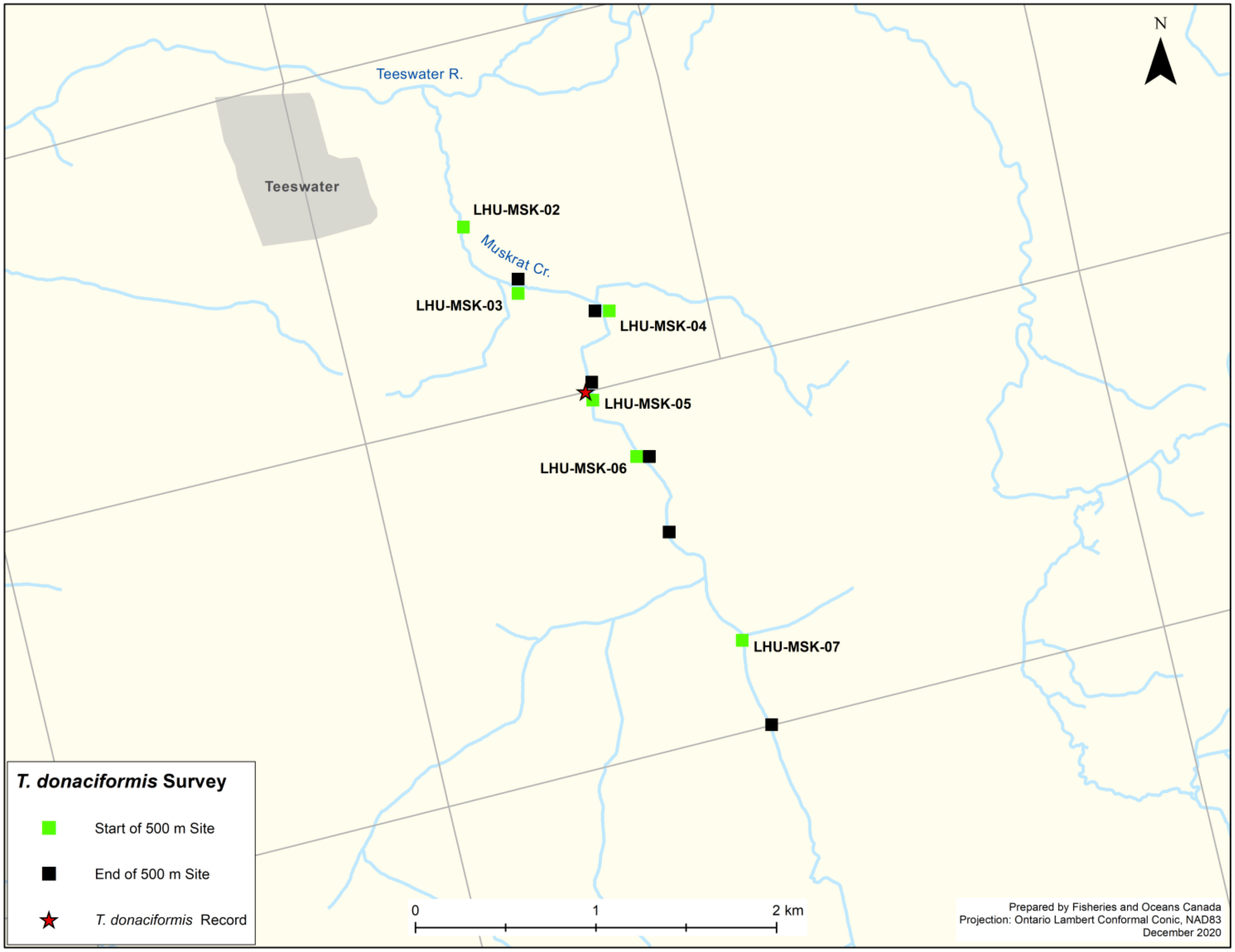

Figure 2. Six 500 m long sites in Muskrat Creek searched using a semi-quantitative timed-search survey and quadrat excavation.

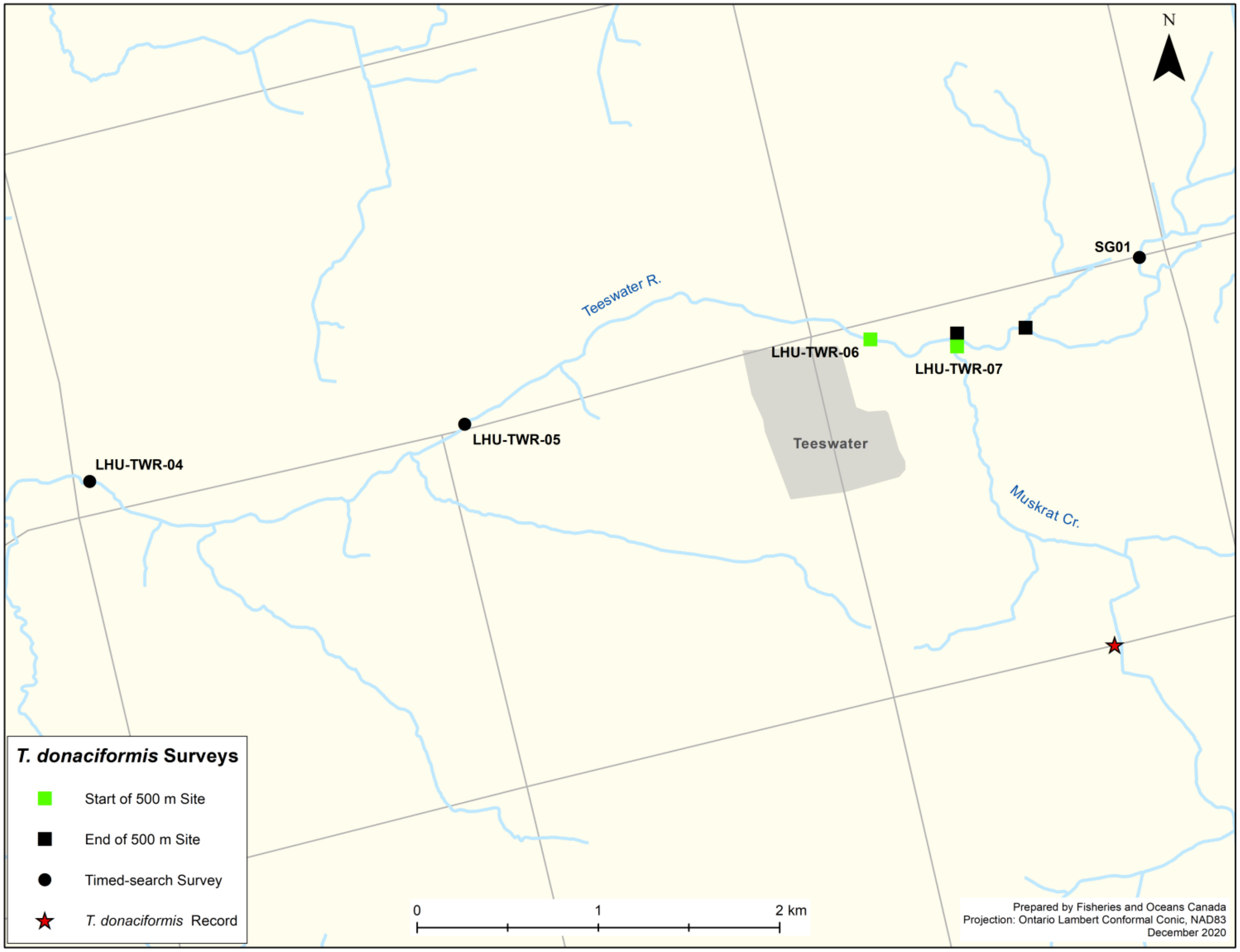

Figure 3. Five sites surveyed in the Teeswater River using a semi-quantitative timed-search survey and quadrat excavation or a qualitative timed-search survey.

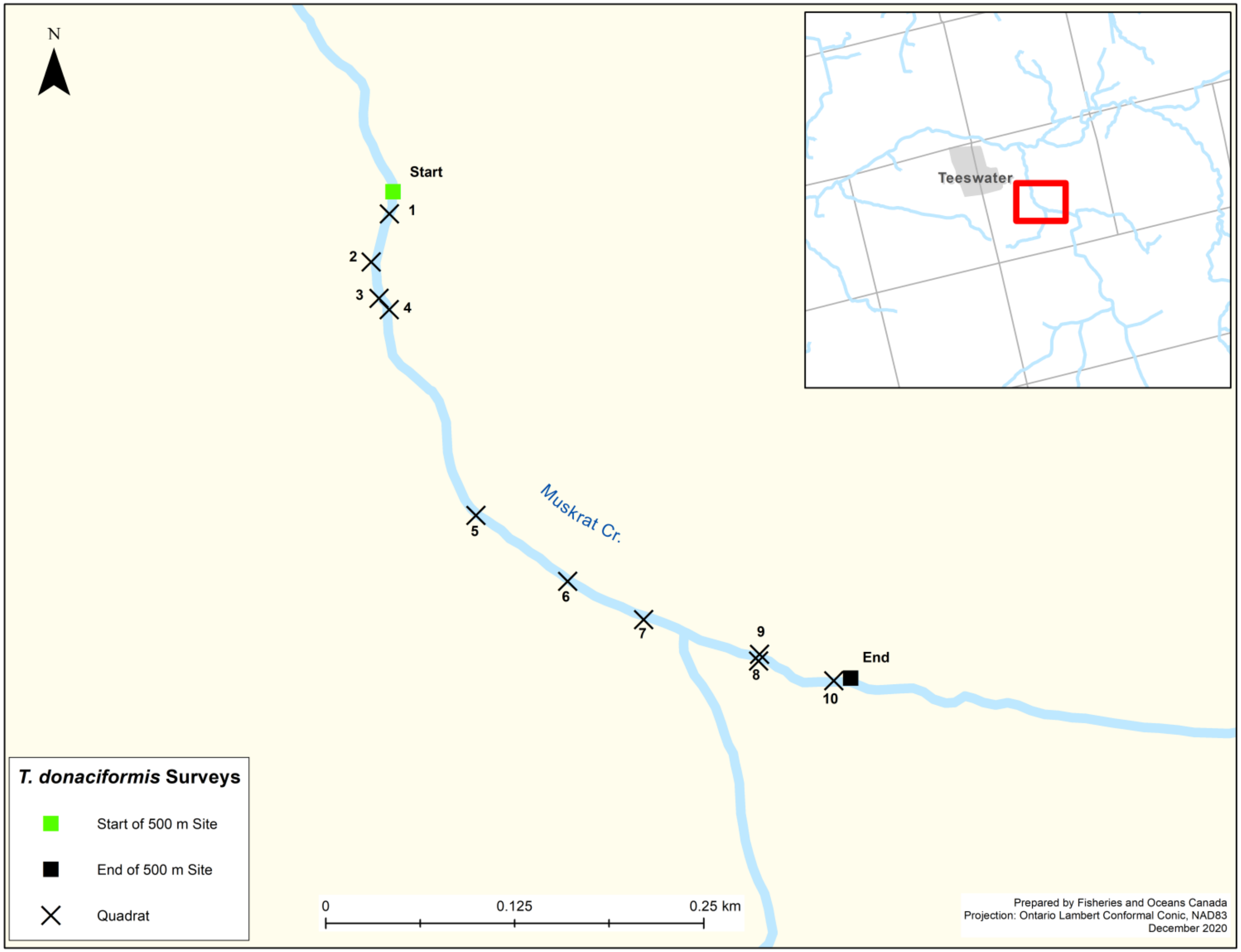

Figure 4. LHU-MSK-02 as an example of a 500 m long site with ten quadrats randomly placed and excavated.