STANDARDIZED FIELD SAMPLING METHOD FOR MONITORING THE DISTRIBUTION AND RELATIVE ABUNDANCE OF THE CARMINE SHINER (NOTROPIS PERCOBROMUS) POPULATION IN CANADA

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The Species at Risk Program's objective for Carmine Shiner *(Notropis percobromus)* is to quantify and maintain current population levels throughout its Canadian range. In an effort to provide science advice to meet the Species at Risk Program objective, this report aims to provide a consistent sampling method and survey design that accurately informs on changes in the distribution and relative abundance of the Carmine Shiner in Manitoba, where it is listed as *Threatened*. This report details (1) the sampling gear, (2) recommended sampling effort and timing, and (3) sampling sites for Carmine Shiner distribution and relative abundance. This standardized sampling protocol is intended to improve the monitoring of the species throughout its Canadian range, the assessment of population trends, and consequently allow for a better-informed management of the species over time.

RÉSUMÉ

Macnaughton, C.J., Watkinson, D.A., and Enders, E.C. 2020. Standardized field sampling method for monitoring the distribution and relative abundance of the Carmine Shiner *(Notropis percobromus)* population in Canada. Can. Tech. Rep. Fish. Aquat. Sci. 3356: viii + 35 p.

Une des mesures de gestion provenant de la Loi sur les Espèces en Péril (LEP) pour la conservation de la tête carminée consiste à élaborer un plan de surveillance suffisamment solide afin de quantifier l'abondance, la distribution et l'habitat du poisson utilisé par l'espèce. Dans le cadre d'établir des cibles quantitatives pour la tête carminée en vue d'assurer sa protection et son rétablissement, ce rapport sert à définir un protocole et un design d'échantillonnage qui serviront à faire l'inventaire de la population de la tête carminée dans les rivières du bassin versant Birch et Whitemouth au Manitoba, où elle est menacée. Ce rapport vise à décrire (1) l'engin de pêche recommandé, (2) l'effort et le moment de l'année idéal pour l'échantillonnage, et (3) la localisation des sites d'échantillonnage qui se retrouvent dans l'ensemble de l'aire de répartition de l'espèce, ainsi qu'à l'extérieur de cette zone pour faire le suivi de l'abondance à long-terme. Ce rapport contribue directement à la conservation de l'espèce en mettant en œuvre un plan de surveillance dans les cours d'eau canadiennes pour assurer la viabilité à long-terme de l'espèce.

ACKNOWLEDGEMENTS

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1.0 INTRODUCTION

The purpose of the *Species at Risk Act* (SARA) is to protect wildlife species at risk from becoming extinct or extirpated in Canada, help with the recovery of extirpated, endangered, and threatened species, and ensure that species of special concern do not become extirpated or threatened as a result of human activity (*Species at Risk Act S.C. 2002, c.29*). Under provisions in the *Act*, wildlife species, designatable units (DUs) thereof, and the critical habitats of populations listed under the SARA as threatened or endangered receive protection. The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) is an independent body of experts tasked with identifying and assessing the status of wildlife species at risk. Once a species' outcome (i.e., designation) has been decided by COSEWIC and subsequent listing pursuant to SARA, assessments on the distribution and relative abundance of the species concerned are necessary for determining population trends and whether recovery strategies are effective or not. COSEWIC assessments determine the status of a species on a ten-year cycle, setting the timeline for when the information is required to update a species' status and to ensure the species' recovery is on the anticipated trajectory. The challenge in this process lies in the achieving consistent and current population trend updates by establishing a frequency of sampling events that potentially aligns with COSEWIC status review timelines and surveying methods for a given species.

Various field sampling methods for quantifying the distribution and relative abundance of small-bodied freshwater fishes in wadeable streams are currently in use. However, different field methods (e.g., beach seining vs. electrofishing) often yield different information, leading to conflicting, complementary and/or incomplete data records for any given species. Inconsistent or different sampling effort and survey designs may, therefore, preclude pooling data from different sources for obtaining reliable estimates (e.g., distribution and relative abundance) of target species. In fact, relatively few and scattered data records exist for the known Canadian distribution of Carmine Shiner (*Notropis percobromus*, Cope 1871), making the task of estimating their current relative abundance and population trends very difficult.

In an effort to provide science advice to meet the Species at Risk Program's objective of monitoring population trends within the ten-year cycle, this report aims to provide a consistent sampling method and survey design that may accurately inform on changes in the distribution and relative abundance of the Carmine Shiner population where the species was assessed as *Threatened* under SARA and *Endangered* (COSEWIC 2018). Properly designed sampling programs should include knowledge of the biology of the species and the deployment of the appropriate gear under the direction of experienced personnel. This report details which sampling gear to use and how much effort is required, where to sample the Carmine Shiner population(s), and where range extension sampling should be planned as part of a long-term monitoring for the species. This Carmine Shiner sampling protocol uses elements of an existing fish surveying protocol for first-time surveys of small streams (Fisheries Management Standards Committee 2008 by Fish and Wildlife Alberta) as a template, which applies to wadeable streams (<1 m in water depth). Using the most complete seining field sampling data records for the species (collected from May to October 2011), this technical report offers a sampling approach, which can provide advice to the Species at Risk program on baseline Carmine Shiner Catch per Unit Effort (CPUE) for the Whitemouth River and its tributary, the Birch River.

Since the latest data available are nearly a decade old, a standardized sampling protocol for monitoring Carmine Shiner populations and distribution targets that includes the frequency of sampling events over time should lead to improved and better-informed management of the species (i.e., assure the persistence of healthy, viable populations in all locations where the species currently exist, Fisheries and Oceans Canada 2013).

2.0 CARMINE SHINER

2.1 TAXONOMY

The Carmine Shiner is a small minnow of the genus Notropis. Carmine Shiner was originally undifferentiated from the Rosyface Shiner (*Notropis rubellus*), but the Manitoba populations are now considered to be Carmine Shiner based on the biogeographic information (Wood *et al.* 2002; Stewart and Watkinson 2004; Page *et al.* 2013).

2.2 MORPHOLOGY

Carmine Shiner is a slender, elongate minnow species (Figure 1). This species is olive green dorsally, with silvery blue and purple hues on the sides and silvery-white underbelly. Black pigment outlines the dorsal scale pockets and fins are transparent. Average fork length of individuals collected in the Birch River in 2011 was 23.7 mm (range: 16-52 mm). The largest documented Carmine Shiner in Manitoba was collected in the Whitemouth River and measured 67 mm in fork length (Watkinson, unpublished data). In a recent field study conducted in 2018 on the Carmine Shiner in the Birch River, average fork length ± SD was 53.04 ± 6.45 mm and average wet weight ± SD was 1.43 ± 0.53 g (Watkinson, unpublished data).

What distinguishes the Carmine Shiner from other minnows include: the origin of the dorsal fin is located behind the insertion of the pelvic fins (Stewart and Watkinson 2004). It can be distinguished from the Golden Shiner (*Notemigonus crysoleucas*) by the absence of both a fleshy keel on the abdomen and strongly decurved lateral line. It is distinguished from the Emerald Shiner by a conical snout in equal length to the eye diameter. Carmine Shiner also has five to seven short gill rakers on the lower limb of the first gill arch, the longest of these rakers is as long as the width of its base. Carmine Shiner have four slender, hooked, main row pharyngeal teeth (Stewart and Watkinson 2004).

Breeding males develop fine, nuptial tubercles on the head, some predorsal scales, and the upper surface of the pectoral fin rays. Spawning fish turn a bright carmine colour around their cheeks, operculum (sometimes the entire head), along the pectoral girdle and sides around the base of each fin (Stewart and Watkinson 2004). Breeding females are usually a paler colour on the sides.

Figure 1. Carmine Shiner (*Notropis percobromus*) (Photo courtesy of D.A. Watkinson).

2.3 BIOLOGY

Life Cycle and Reproduction

This species matures after the first year and may live to at least three and up to five years of age. The average age of mature individuals sampled in the Birch River is approximately 2.9 years. Spawning males and females attain fork lengths in the range of 55 to 67 mm in Manitoba (Watkinson and Sawatzky 2013) and the number of eggs per female increases with size (from 144 to 2806 eggs per female). The variation in egg count is likely explained by the protracted spawning period of the species, along with the number of eggs per female increasing with increasing fish size. Little is known of the species' spawning habitat, however, Carmine Shiner in spawning condition have been caught downstream of the Pinawa Channel (i.e., Lee River), the Whitemouth River, and the Birch River in riffle areas with sand, gravel, cobble, boulder, and bedrock substrates. In Manitoba, spawning occurs between mid-June and into July. Females collected in the Birch River in 2011 had mature eggs in July when water temperatures were between 20 to 30 °C, while males displayed breeding coloration around the same time periods when sampling in 2018. There is some evidence from collected specimens of repetitive spawning during the spawning season.

It is possible that Carmine Shiner hybridizes with other species given that the Rosyface Shiner, a close relative, hybridizes naturally with several species including Common Shiner (*Luxilus cornutus*), whose distribution overlaps with Carmine Shiner in Manitoba (Watkinson and Sawatzky 2013).

Carmine Shiner occupies a mid-trophic level, preying mainly on aquatic, semi aquatic, and terrestrial insects (Coleoptera, Hymenoptera, Diptera, and Hemiptera) (Enders *et al.* in review) and occasionally fish eggs (Hoover 1989). In the Birch River, insects that dwell on the water surface and terrestrial plants are the dominant items found in Carmine Shiner stomachs. Hoover (1989) found the diet variety decreased in the presence of Smallmouth Bass (*Micropterus dolomieu*) and increased at higher light levels, which suggests that Carmine Shiner are visual predators.

Physiology and Adaptability

Little is known of the Carmine Shiner physiology or ability to adapt to different environmental conditions, therefore, most of the information is inferred from the closely related Rosyface Shiner. Carmine Shiner are thought to have a narrow range of habitat requirements and respond quickly to changes in habitat and water quality (Cherry *et al.* 1977; Smith 1979; Trautman 1981; Humphries and Cashner 1994; Houston 1996). For example, the Rosyface Shiner exhibits long-term avoidance of pollutants (Cherry *et al.* 1977) and avoids water temperatures greater than 27.2 °C (Stauffer *et al.* 1975). Carmine Shiner prefer temperatures of 23.6 ºC (± 1.4 ºC) (Stol *et al.* 2013). Specific to the Birch River, Manitoba, water temperatures were found to vary between 20–30 °C from June to August (2011), with maximum diel temperature fluctuations of 6–7 °C (Enders *et al.* 2019). However, temperature data for the Birch River (2011–2012; the last year of data recorded) showed that it was ≥ 25 °C for less than three days in total (Macnaughton *et al.* 2018). Respirometry experiments conducted in the laboratory on Carmine Shiner indicated standard metabolic rate increased significantly with body mass and rising water temperature, ranging from 0.05 mg O_2 h^{-1} at 10 °C to 0.89 mg O_2 h^{-1} at 20 °C for individuals weighing 0.6–2.4 g.

Dispersal and migration

Carmine Shiner are not known to migrate, yet, they may move to deeper water when water temperatures drop in September. In the Whitemouth River, Carmine Shiner may disperse downstream or along flooded riparian habitat during heavy rainfall and subsequent higher flows. Carmine Shiner and Rosyface Shiner distributions suggest that both species can disperse via large lakes and rivers but will colonize and establish populations only in the tributaries to the larger systems.

2.4 KNOWN DISTRIBUTION IN CANADA

Manitoban populations - Endangered (COSEWIC 2018)

Carmine Shiner are widely distributed throughout highland and glaciated regions of eastern North America. In Canada, Carmine Shiner are at the northwestern limit of the species' range. The Canadian populations of Carmine Shiner have been collected only in the Whitemouth River and Winnipeg River watersheds, including Birch River, in eastern Manitoba, Canada (Figure 2). It is a single designatable unit found only in the Saskatchewan-Nelson River National Freshwater Biogeographic Zone.

Carmine Shiner were designated *Special Concern* in April 1994 and the status was re-examined and designated *Threatened* in November 2001 and in April 2006. The status was subsequently re-examined and Carmine Shiner was designated *Endangered* in April 2018. Carmine Shiner is currently listed as *Threatened* under the Canadian *Species at Risk Act*.

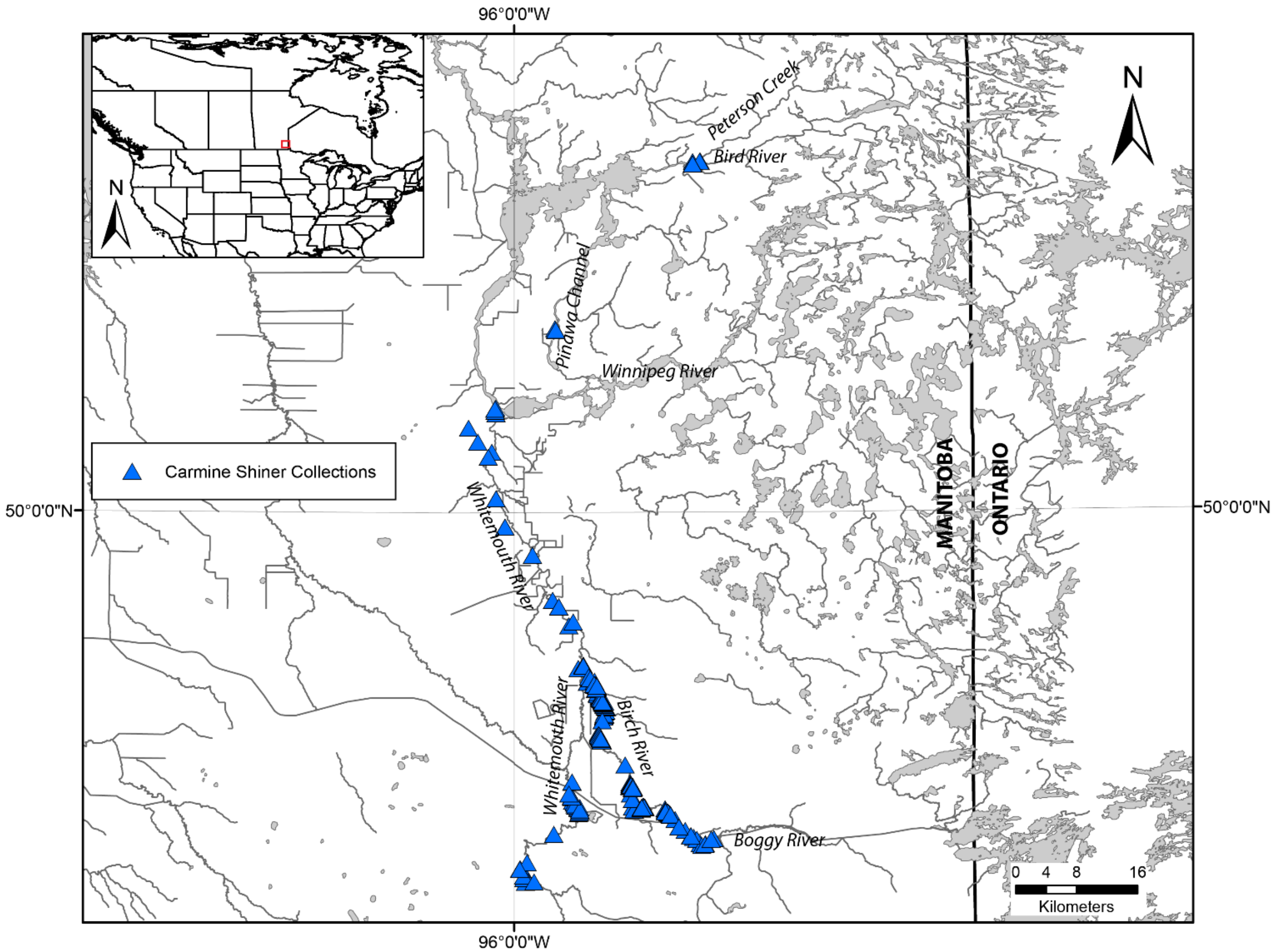

Figure 2. Carmine Shiner distributions in the Whitemouth River and Winnipeg River watersheds, Manitoba.

2.5 HABITAT

Habitat Features

Carmine Shiner were collected in the Whitemouth River watershed, in flowing water <3 m deep, in habitats where substrates varied from silt, sand, gravel, cobble, boulder, and bedrock (COSEWIC 2018). Smaller individuals (< 42.5 mm) generally preferred sites with sand more than larger individuals. Carmine Shiner may be intolerant to sustained turbidity (Trautman 1981; Becker 1983), but may be tolerant to pulses of turbidity in the Whitemouth River watershed associated with natural flooding events (Stewart and Watkinson 2004). Recent field surveys in the Birch River indicated that Carmine Shiner inhabited tea-colored waters of slow to moderate flows (Appendix 1). Historical records indicated that Carmine Shiner did not occur in the headwaters, lower course or other tributaries of the Whitemouth River, where habitats were composed of silty bottom and fewer riffles (Smart 1979). Overwintering Carmine Shiner are thought to move into deeper pools and eddies in rivers or occasionally near river mouths in lakes. Similar to the closely-related Rosyface Shiner, Carmine Shiner appear to occupy a relatively narrow ecological niche, which suggests limited adaptive ability to changes in habitat and water quality (Watkinson and Sawatzky 2013). For example, Rosyface Shiner exhibits long-term avoidance of pollutants and avoid water temperatures >27.2 °C (Watkinson and Sawatzky 2013).

Field surveys conducted in Birch River in 2011 provided general site descriptors of the habitat where Carmine Shiner occurred. Overall, habitat characteristics for Birch River surveys align with previous accounts of the species habitat preferences for rivers of small to intermediate size (average wetted width of 18.94 ± 8.23 m) with shallow water depths (average depth = 0.66 ± 0.41 m), moderate water velocities (0.078 ± 0.16 $cm \cdot s^{-1}$), warm water temperatures (average temperature = 15.0 ± 5.56 °C), and varied, small substrate types (mainly silt, sand, and gravel) (Appendix 1). Carmine Shiner occasionally inhabited wooded and vegetated habitats (~9% and 17% cover within a site, respectively). In fact, the greatest relative abundance (count n = 97 individuals) was recorded in a site with a high presence of algae. Habitat use in the systems expanded as flows increased and lessened during the period of low water flows. Water temperatures caused fish to converge to fewer habitats when flow conditions were adverse. In fact, geomorphic structure analysis conducted in the Birch River provided linkages between hydrologic attributes and species habitat preferences; where 58% of immature Carmine Shiner preferred geomorphologically variable reaches, while 50% of mature individuals preferred reaches of low-sinuosity, punctuated with increased slopes (Carr *et al.* 2015).

Figure 3. Birch River (49.782882, -95.842913) in Manitoba sampled in July 2018 (photo courtesy of S. Wolowich).

Habitat Trends and Threats

Ongoing degradation of the watersheds where Carmine Shiner occur caused by deforestation, additional drainage for agriculture, and peat mining is projected to continue over the next ten years due to increased sediment entering watercourses and modified river flows. In addition, transportation and service corridors will likely contribute minor changes to drainages and sediment load (COSEWIC 2018). In fact, Carmine Shiner habitat in the United States has been transformed on account of changes in agricultural practices (i.e., silt deposition) and dam construction leading to the decline of the species in the Smoky Hill River, Arkansas and the lower Kansas River basin (Gido *et al.* 2010), and to the extirpation of Carmine Shiner in rivers upstream of reservoirs in Kansas (Falke and Gido 2006).

Conversely, the projected effect of climate change leading to warming on small-scale temperature variations (0.8–4 °C) in >86 000 km of rivers in Wisconsin may increase suitable habitat for Carmine Shiner from 22.6–36.8% (Lyons *et al.* 2010). At the global scale, Pandit *et al.* (2017) predicted a northward shift (i.e., into Manitoba) in suitable Carmine Shiner habitat based on climate models that combined temperature and precipitation. As the southern extend of the distribution would become unsuitable, Carmine Shiner should be able to spread into these suitable habitats, however, barriers to movement may pose a substantial obstacle for migration (Pandit *et al.* 2017).

2.6 POPULATION SIZE AND CPUE TRENDS IN CANADA

There has been some effort since 2002 (i.e., electroshocking, seine netting, and eDNA) focused on improving knowledge of the distribution of Carmine Shiner throughout the Whitemouth River and Winnipeg River watersheds in Manitoba. Birch River surveys conducted in 2011 ranged from 0–97 individuals per standardized seine haul (total of 1,813 individuals caught; average ± SD = 5.9 ± 12.0 individuals per site; Appendix 1). Average abundance estimates were calculated for the 130 sites sampled in 2011, where up to three seine passes were conducted per site (Figure 4). Carmine Shiner were caught across over half of the sites surveyed between June and November 2011 (presence = 58% vs. absence = 42%), however, only 27% of sites saw more than 5.9 individuals per site. Catch-per-unit-effort (CPUE) from the seine netting was calculated as the total number of fish per unique site of approximately 100 m^2 over three standardized net passes, which is equivalent to site density. The sampling for CPUE since 2002 were rarely conducted at the same site as most of the survey effort was directed at collecting fish in new sites and delineating the extent of the species' distribution. Greater Carmine Shiner densities were observed from mid-August to mid-October for surveys conducted in the Birch River in 2011 (Figure 5). The average densities ± SD for surveys conducted in 2011 was 0.059 ± 0.12 fish m^{-2} (max. density = 0.97 fish m^{-2}). Qualitative information indicated that the higher catch numbers occurred with a single seine pass per site, however further analysis is required to determine the optimal number of passes to sample the species. With the limited data available, there is no evidence that Carmine Shiner distribution or abundance has declined over time. Since the species has a limited distribution and suspected low abundance, the species may be vulnerable to future anthropogenic disturbances including substantial mortalities due to handling stress or lethal sampling. While the sampling protocol detailed in subsequent sections is catch-and-release, it is important to observe the condition the Carmine Shiner are in (i.e., reproductive state, lethargic etc.) prior to sampling in order to reduce unnecessary stress and mortalities.

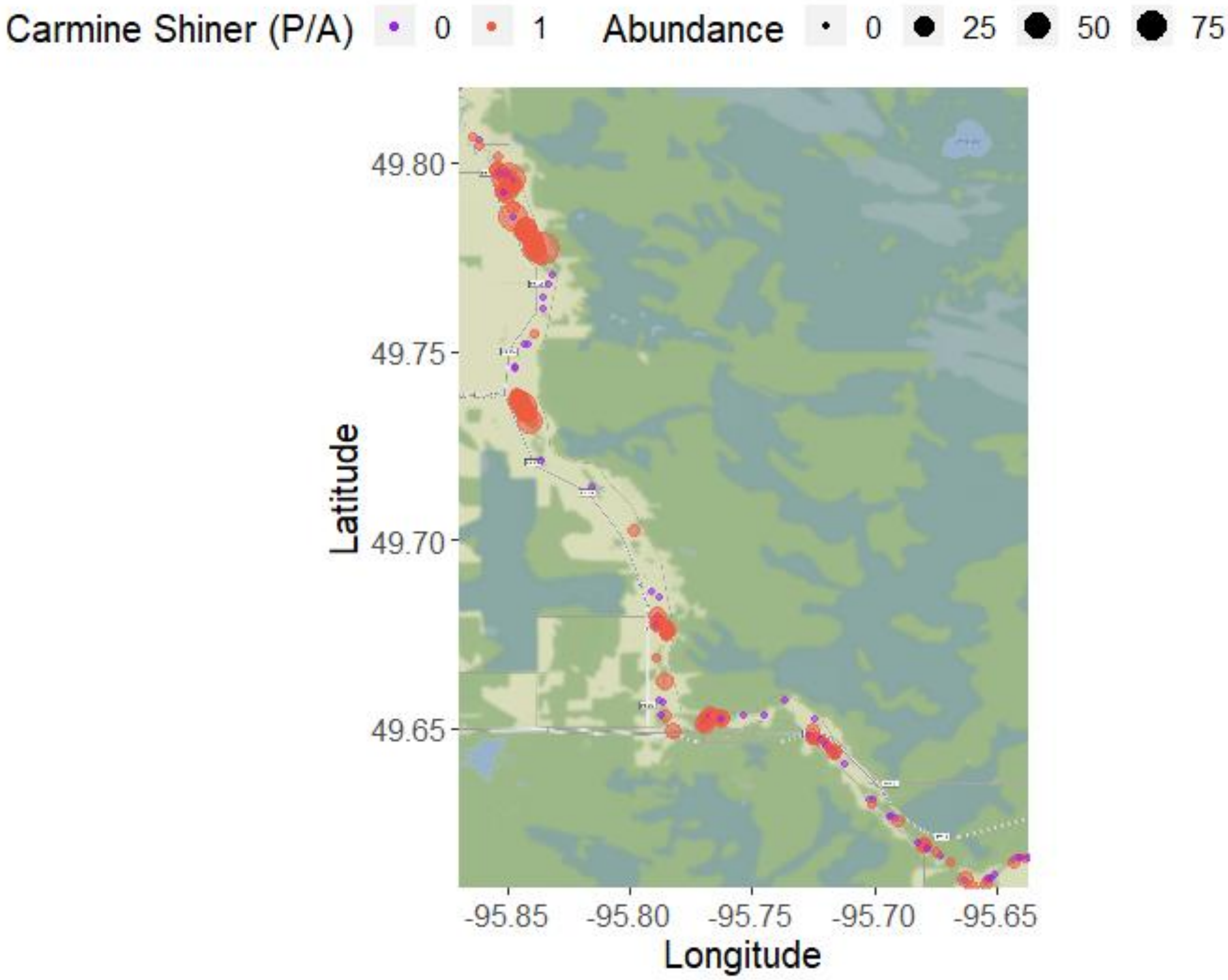

Figure 4. Carmine Shiner presence and absence (P/A) and abundance sampled in the Birch River in 2011.

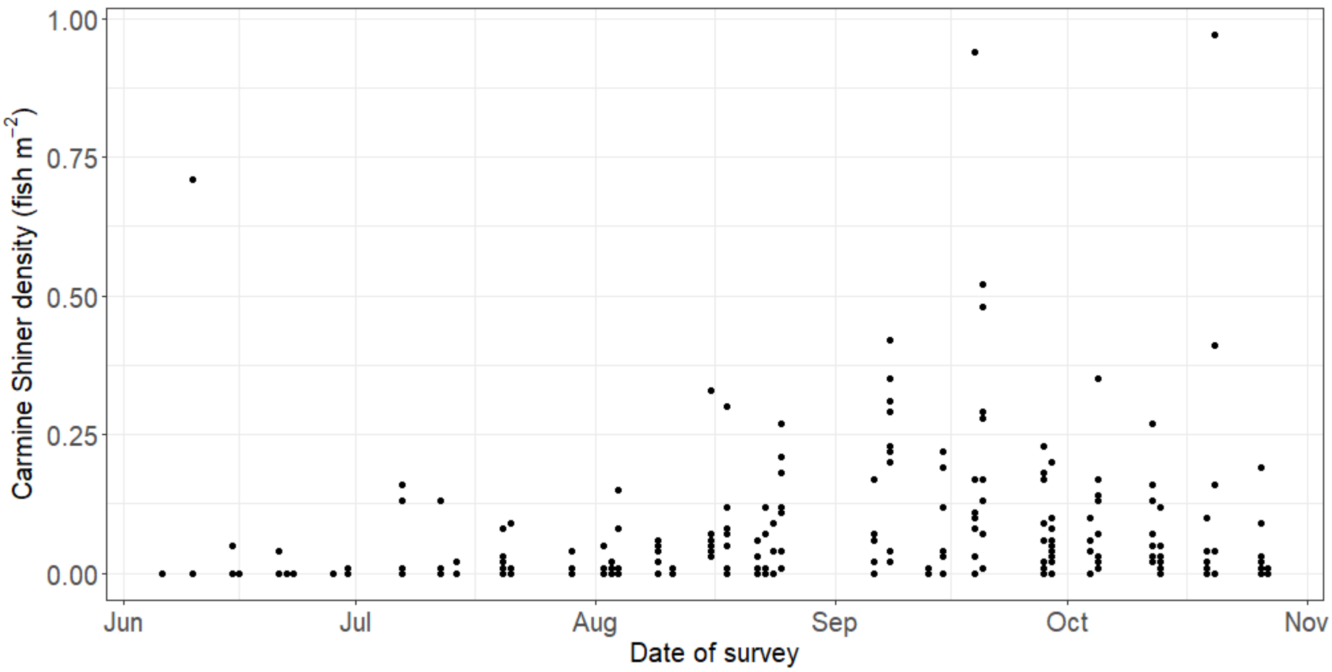

Figure 5. Seasonal densities (#fish m^{-2}) for Carmine Shiner surveys conducted in the Birch River. Each point corresponds to a seining site of approximately 100 m^2, for a total of 308 sites surveyed in 2011.

3.0 SAMPLING PROTOCOL

To obtain consistent fish survey data and ensure that monitoring is effective, a standard sampling protocol using a seine net has been developed to monitor the occurrence and abundance of Carmine Shiner. The sampling protocol describes a standardized approach for fish and habitat data collection throughout the species' distribution in the Whitemouth River and Winnipeg River watersheds, Manitoba. A Carmine Shiner Catch Database has now officially been published on an open data site: https://open.canada.ca/data/en/dataset/a6a606a4-8cdc-48e9-812c-7bdcd84840e7. Additional sampling should be catalogued on the open data site to ensure the most complete fish survey database.

Access points

Sixteen access points have been identified for sampling Whitemouth and Birch rivers within the known distribution range of the Carmine Shiner (Figure 6). The access points presented below (see Appendix 2 for the full list of access points and associated coordinates) are recommended for accessing locations in the watershed for sampling and monitoring population trends over time. Sampling proposed range extension locations may provide information on whether the species' distribution could be expanding.

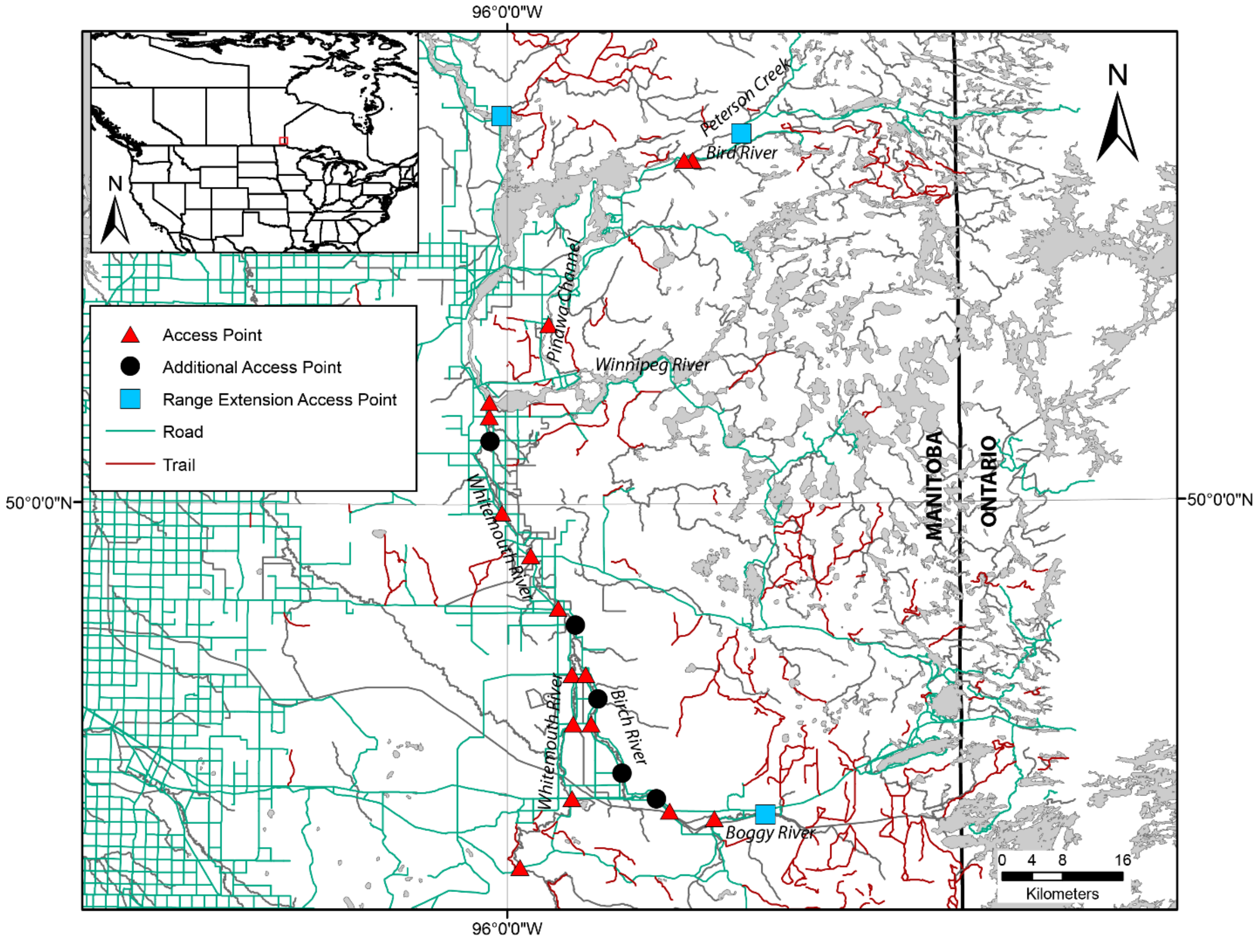

Figure 6. Map of the 16 access points (triangles) and three potential range extension sampling sites for Whitemouth River and Winnipeg River watersheds in Manitoba.

Sites

Birch River merges with the Whitemouth River and extends northward to its confluence with the Winnipeg River at Whitemouth Falls Provincial Park. Sites represent the sampling unit for which CPUE is calculated and should be evenly distributed among recommended access points along each river. If the availability of stream habitat is not limited, sites should be consistent and measure ~100 m^2 (i.e., semi-circle of ~8 m radius seine pull with a 9.1 m seine) and distributed among recommended access points along each river (i.e., three sites per access point), maximizing the spatial extent of the surveying effort (~48 sites throughout the known distribution of the species). In order to balance the spatial distribution of sampling sites with the effort of moving between these sites, we recommend that sites are spaced out from 50–100 m apart from one another. To avoid disturbing fish habitats during surveys, sampling should commence at the most downstream site at any given access point, moving upstream with each new site. To minimize pseudoreplication among sites (i.e., double counts between adjacent sites), fish sampled per site should be retained in separate containers until the sampling is completed for the access point. Furthermore, habitat and environmental descriptors that specifically measure water quality (i.e., water turbidity and conductivity) should be obtained prior to entering the stream for sampling.

3.1 TIMING OF SAMPLING

Seasonality

It is important that sampling be timed to match the most appropriate conditions every year and/or timed to have relatively consistent sampling across year to reduce environmental variation. The sampling sites should therefore be georeferenced and photographed in the field to ensure that the same approximate locations are used repeatedly across years.

Sampling feasibility in the Birch and Whitemouth rivers is contingent on seasonal water levels and water temperatures that allow sampling to consistently occur. Rather than aiming to sample particular calendar dates each year, annual studies should be conducted for targeted flows within a particular calendar period or under a similar flow stage (~Days 201-3001; Figure 7). Real-time hydrometric data for the systems are available from the Water Survey of Canada to inform on seasonal flow and water level variability in Whitemouth and Birch rivers (Source: Water Survey of Canada 2019; Table 1) and should be checked prior to field surveying.

Table 1. List of real-time hydrometric stations (ID) and recommended sampling time in the Birch River and Whitemouth River where Carmine Shiner occur.

Waterbody	Hydrometric station	Location of hydrometric station	Suggested sampling time	Station source
Birch River	05PH007 (seasonally operated)	near the town of Prawda	June 30 to mid-October	Water Survey of Canada
Whitemouth River	05PH003	near the town of Whitemouth	mid-July to mid-October	Water Survey of Canada

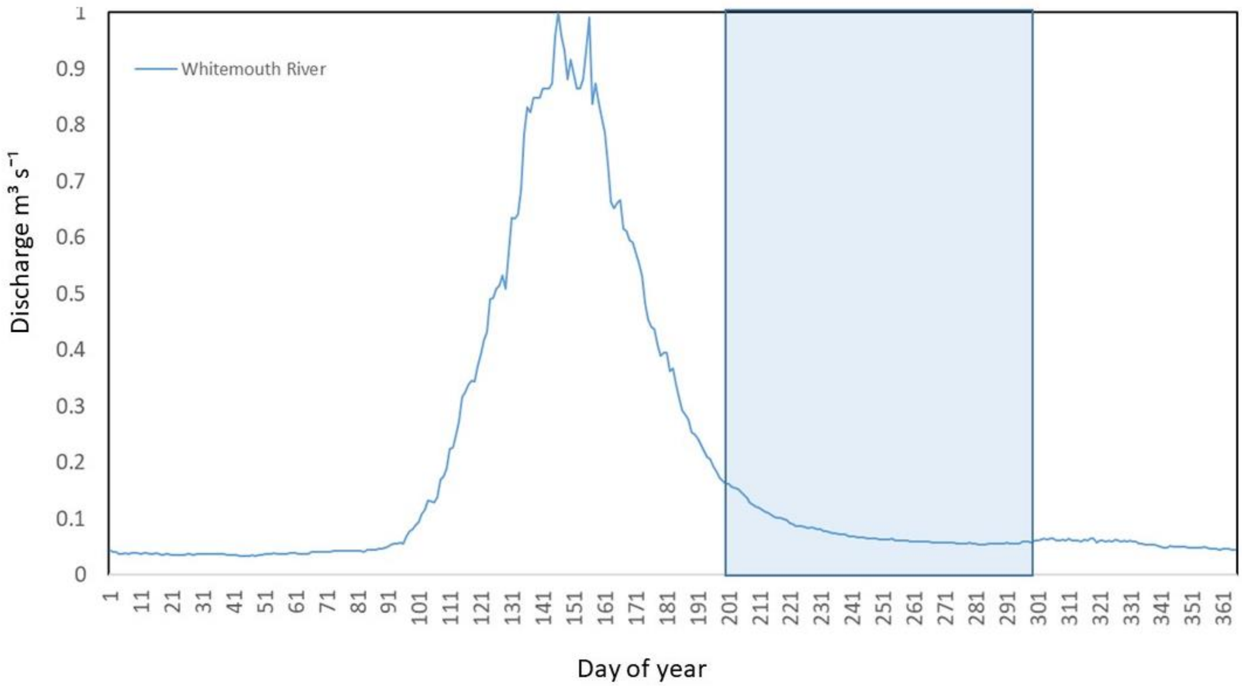

Figure 7. Hydrographs illustrating the standardized median discharge ($m^3\ s^{-1}$) for the Whitemouth River, over the days of a year (Day 1 = January 1).

Surveying Frequency

Baseline CPUE data is available for the Birch River, however, the last population assessment was conducted in 2011. To determine whether the range of the Carmine Shiner distribution is stable, population trend assessments require more frequent surveying of the same sites and should include range extension sampling (e.g., eDNA sampling). COSEWIC assessments determine the status of a species on a ten-year cycle, setting the timeline for when the information is required to update a species' status. To maximize the temporal extent of surveys and to provide a minimum of two estimates of the distribution and relative abundance of the species, sampling should be conducted twice in the ten-year cycle. Ideally, sites should be sampled at least every five years, preferably not in subsequent years, once baseline data has informed the survey effort necessary to achieve reliable population trends.

3.2 SAMPLING GEAR AND METHOD

A minimum crew size of two people is required to pull a seine net (length = 9.14 m by width = 1.82 m, mesh size = 4.76 mm, and 1.82 m^3 center pocket) out from a pivot (individual or anchor) centered along the shoreline within a site. One end of the seine is held stationary on the shore (pivot) and the other end stretched out upstream from the pivot, before being fully deployed in the water and swept downstream in an arc to complete the pass. It is recommended to sample when discharge is low enough for wadeable conditions (Figure 8). Once the individual has completed the semi-circle, crew members quickly grab the seine's lead line and pull up the net along the shoreline (Figure 8).

Sampling effort corresponds to approximately the semi-circle of ~8 m radius, multiplied by the number of seine net passes. Three passes per site are recommended. Since sites and sampling area are variable throughout the watershed on account of seasonal water level changes, it is essential that sampling effort is measured consistently for each site, ensuring that fish density and biomass estimates may be compared across sites and between years. Relative fish depletion estimates can be measured within the site by immediately placing fish in buckets after each pass of the seine and releasing fish back into the site once the sampling is complete. To reduce pseudoreplication among sites (i.e., double counts between adjacent sites), we recommend that fish sampled in adjacent sites be retained in separate buckets until the last site for a given access point is completed. Once all three sites are sampled for the access point, the fish can be released back into their respective sites.

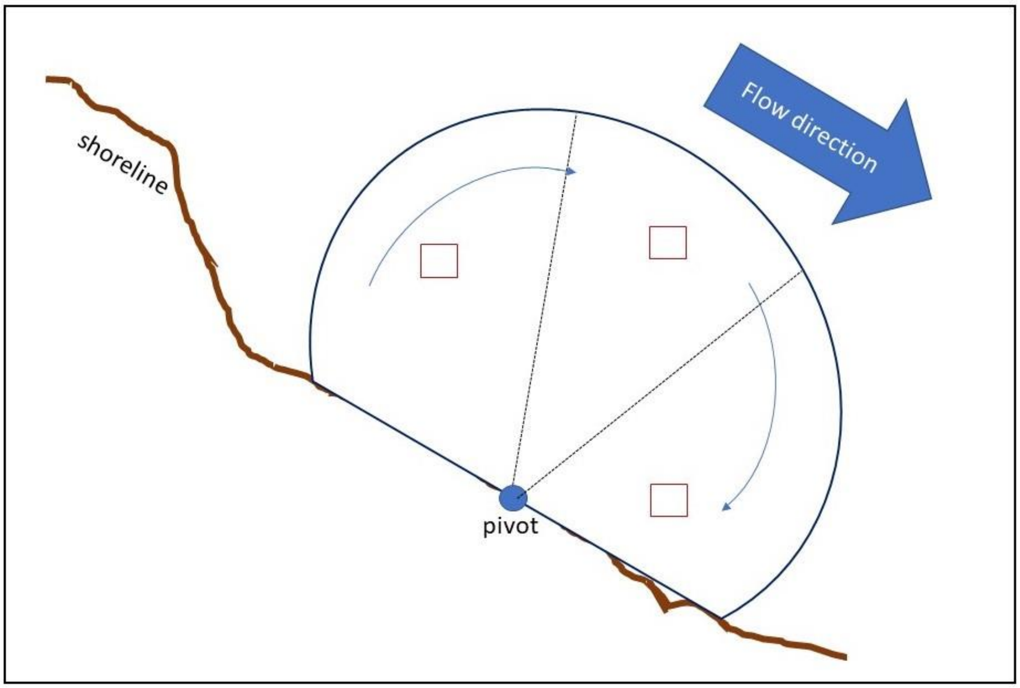

Figure 8. Schematic of the direction of sampling along the shoreline. Squares correspond to the quadrats where physical variables are surveyed.

At each sampling site, habitat data (described in following section) must be collected to complement fish data and to quantify habitat. Water temperature trends (i.e., among and within rivers) are thought to drive species' distribution via their cumulative impacts with water flow, dissolved oxygen concentration, and other habitat variables. Along with the habitat descriptors collected for each site, temperature loggers programmed for long-term monitoring of thermal trends at each access point should be considered to better understand habitat trends over time. Not included in the report is an approach for quantifying thermal trends in rivers, however, details on launching temperature loggers and their placement in rivers can be found in Chu *et al.* (2009) and Mandrak and Bouvier (2014).

Many factors affect the success of seining in small stream habitats, namely water turbidity, substrate complexity, and the presence of woody debris that can snag the lead line and allow fish to escape under the net. Other variables, including water depth and velocity, may also affect seining efficiency, which is why habitat variables must be recorded to complement fish data and to quantify usability at each sampling site. The Carmine Shiner protocol applies to wadeable streams (<1 m in water depth) in Manitoba, where the distribution of Carmine Shiner is currently being monitored. Refer to Appendix 2 for the database template.

Collecting habitat data

Collecting habitat data from sampling locations is an important activity as changes to habitat through time may help explain the future presence/absence or changes in the abundance of Carmine Shiner at any given location. Habitat data collected while sampling for Carmine Shiner includes water turbidity, depth, velocity, substrate complexity, and plant cover (see items 9, 12, 13, 15, and 16 in the environmental/habitat descriptors). There are inherent biases to sampling habitat conditions and what one perceives as similar or different may not be so if sites are not selected randomly for collecting habitat data. In an attempt to capture the variability within a site and to aid in the random sampling of habitat variables/conditions/features at each site, it is recommended to divide the site (semi-circle) into thirds. To select the placement of the three quadrats per site, randomly position the quadrat within each third (e.g., random stone toss marking the center of the quadrat; Figure 8). The habitat data collected from the three quadrats are then entered in the database template shown below (Appendix 3). Habitat data must be collected from each sampled stretch of river regardless of whether Carmine Shiner are captured.

Environmental/habitat descriptors

1. Waterbody name – List the name of the river surveyed (e.g., Birch River).
2. Date of surveying – Use the format (dd/mm/yyyy). Do not abbreviate.
3. Crew – List the names of crew members so that appropriate persons may be contacted to verify data.
4. Latitude and longitude coordinates – Units should be in decimal degrees (projection WGS84). Provide geographic reference locations of each sample site.
5. Site location notes – Give concise description of the geographic location of the reach or site surveyed using map and site observations (e.g., 10 m upstream from confluence with tributary X).
6. Site number – Give a unique number to the site surveyed.
7. Water temperature – Measure the water temperature (ºC) at mid-water depth using an appropriately calibrated thermometer. Temperature influences the distribution of biota and the catchability of certain species. Avoid taking measurements in stream margins, outflows

from tributaries or stagnant pools (unless the site is located in these habitats). Record the time of day (24 h).

8. Conductivity – Measure the conductivity, the capacity of transmitting electricity, at mid-water depth within the site using a portable conductivity meter (µS cm^{-1}, standardized to 25 ºC). Conductivity influences catchability for electrofishing surveys and may provide the means to stratify data.
9. Turbidity – Measure the turbidity within the site using a portable turbidity meter (NTU) and Secchi disk (cm). Turbidity influences catchability and may provide the means to stratify data. Carmine Shiner have also been known to be sensitive to increased turbidity due to sedimentation.
10. Wetted and rooted widths of the upstream cross section – Measure the channel wetted and rooted widths (m) using a tape measure at the upstream location of the river site surveyed. Wetted width corresponds to the width of the channel at the surface of the water at the time of survey. Wetted width may influence seining effort and efficiency, affecting catchability and CPUE. Rooted or bank-full width corresponds to the channel width at the base of permanently rooted vegetation. For braided channels, the measurement should include any islands not covered by permanent vegetation.
11. Maximum depth – Measure the depth of the water at the deepest point between the wetted banks using a metre stick.
12. Water depth – Measure the depth of the water (m) at three points within a site using a metre stick (one per third of the semi-circle), making sure to obtain measurements from the center of the randomly selected quadrats.
13. Water velocity – Measure the water velocity of the water (m s^{-1}) within each of the three quadrats in a site using a flow metre and wading rod (Marsh-McBirney Flo-Mate), making sure to obtain measurements from the center of the quadrats.
14. Site discharge – Measure the water velocity (m s^{-1}) and depth (m) at three points along the upstream-most cross-section of the site, using a flowmeter and wading rod (Marsh-McBirney Flo-Mate). Divide the river width into thirds and measure water depth and velocity at each point. Note that the accuracy of flow measurements may vary as habitat complexity changes laterally.
15. Substrate complexity – Calculate the proportion of the substrate within a ~1 m^2 quadrat (visual and tactile assessment) that are: bedrock, boulder, cobble, large gravel, small gravel, sand, silt, and clay (modified Wentworth scale). Repeat substrate complexity estimates at each of the three quadrats within a site.
16. Plant cover – Calculate the proportion of plant cover within a ~1 m^2 quadrat (visual assessment), at each of the three quadrats within a site.
17. Site characterization – Characterize the site surveyed based on the pool/riffle/run categories observed to provide a broad idea of productivity and a mechanism for stratifying data.
18. Photo number – Take at least one picture and record the number of the photographs taken during the stream survey.

19. Photo description – Briefly describe the picture taken for later reference. Indicate whether you are facing upstream or downstream.
20. Comments – Briefly describe any details relating to surveying, location, and sources of error (e.g., outflow from tributary) or change (e.g., seepage or barrier).

Beach seining descriptors

21. Distance/effort – Record the approximate area (m^2) over which the seine net is pulled within a site. Seining effort corresponds to the semi-circle covered by the a fully deployed seine with radius of ~8 m (~100 m^2).
22. Pass – Record the number of the pass or seine haul in the case that multiple passes are done.

Fishing descriptors

23. Capture method – Since the recommended capture method for Carmine Shiner is a seine, note the specifications of the beach seine (i.e., 9.14 m length x 1.82 m width, mesh size 4.76 mm).
24. Sample Number – Sequentially number fish, an entry per fish sampled. Assign to seine hauls (1, 2 or 3).
25. Species – Enter the name code for all the species sampled. Recording all the species that are present allows for a better understanding of fish community dynamics occurring at the time of sampling (e.g., co-occurrence of species).
26. Fork length/total length – Record the fork (tip of the snout to the natural fork of the tail) and total (tip of the snout to the end of the tail) lengths (mm) for each fish sampled. Ensure that fish are placed on a flat measuring board.
27. Injuries/comments – Note body condition and injury observations (e.g., lesions or parasite burden (presence or absence).
28. Sample picture – Place the fish on a flat, non-reflective surface and take a photograph of the fish on its left side, next to a ruler. Identify the picture number- (CRSH-number-date-river).
29. Sample specimen – Retain a voucher specimen if necessary (e.g., atypical morphologies, uncertain identification or good examples of species' traits), indicating the access point, location, time and date where the specimen was taken.
30. Should additional tissues or life-history structures be required, refer to specimen collections for archives and life history (Macnaughton *et al.* 2019; Appendix 3).
31. Given the increasing use of environmental DNA (eDNA) for monitoring this species of conservation concern, water samples should be collected for eDNA analysis (see Macnaughton *et al.* 2019; Appendix 4). Carmine Shiner-specific eDNA assays have been developed (M. Docker, University of Manitoba) that have detected the species in the Birch and Whitemouth rivers (COSEWIC 2018). Water should be refrigerated and transferred to the eDNA lab for subsequent filtering and DNA stabilization within ~1 day (Macnaughton *et al.* 2019; Appendix 4).

32. A Carmine Shiner Catch Database has now officially been published on an open data site: https://open.canada.ca/data/en/dataset/a6a606a4-8cdc-48e9-812c-7bdcd84840e7. All new data should be catalogued on this open data registry.

4.0 SUMMARY AND RECOMMENDATIONS FOR FUTURE SAMPLING INVESTIGATIONS

The basis of any effective monitoring program is reliable baseline data against which to monitor and compare future conditions. Generally, a couple of years of data should be collected to establish baseline trends for targeted species and monitoring should continue for several years with the same methods, sites and timing of sampling. Adopting monitoring programs that include integrated and consistent surveying protocols should provide more efficient, comparable, and powerful assessments of population trends over time.

The appropriate method for a particular project, or combination of methods for fish sampling, will require consideration of the capture probability of the species/life stages of interest, as well as the physical conditions of the site (Lewis *et al.* 2013). Although this report describes a protocol for sampling a minimum area (~100 m^2) based on a seining technique, the timing of surveys will dictate whether seining can take place and the full seining area can be achieved. Specifically, higher river flow and depths will likely drive the distribution of fishes throughout the system. As such, the timing of surveys should consider annual flow conditions as well as inter-annual flow trends, to ensure that surveys are conducted for similar flow stages.

Fish sampling and environmental DNA (eDNA) sampling efforts over the last 15 years in areas where Carmine Shiner occur suggest that all populations currently persist (CSAS 2013). However, the absence of consistent survey estimates since 2011 precluded the assessment of population trends since then and contributed to the uncertainty in population estimates for Carmine Shiner in Canada. While Carmine Shiner remain a poorly monitored species in Canada, new biological information from Canadian populations regarding diet, life history, and physiology has reduced the dependence on information from related species, e.g., Rosyface Shiner, and Carmine Shiner from other locations. The greatest threats to the survival and persistence of the species are related to the cumulative effects of landscape changes causing habitat loss and degradation, especially as a result of flow alteration (dams and water regulation), habitat alteration from agriculture runoff and sediment, and the introduction of invasive species (COSEWIC 2018). In the face of uncertain changes to suitable fish habitat and scarcity of data to derive population trends for Carmine Shiner in Manitoba, the need has never been more critical for more consistent sampling protocols, frequent assessments, and reporting of fish and fish habitat data collections. A Carmine Shiner

Catch Database has now officially been published on an open data site: https://open.canada.ca/data/en/dataset/a6a606a4-8cdc-48e9-812c-7bdcd84840e7, which should assist with monitoring future population trends in Manitoba and provide a central data resource for ongoing research on the species.

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APPENDICES

APPENDIX 1. Data table listing habitat variables (wetted width, water depth, water velocity, water temperature, conductivity and substrate and vegetation cover) by site location (310 unique sites) for Carmine Shiner surveys (fish count) conducted in the Birch River in 2011 by Fisheries and Oceans Canada. NA refers to non-available data.

Location		Habitat variables					Substrate and vegetation cover (%)							
Latitude	Longitude	Width (m)	Depth (m)	Velocity (cm s^{-1})	Temp. (°C)	Cond. (μS cm^{-1})	Silt	Sand	Gravel	Cobble	Boulder	Wood	Vegetation	Fish count
49.61575	-95.63684	12.90	1.60	0.01	19.70	380	30	70	0	0	0	0	10	0
49.61543	-95.63822	9.00	1.37	0.01	20.90	290	95	0	0	0	0	5	0	1
49.61568	-95.63973	14.20	1.00	0.01	4.00	430	40	30	30	0	0	0	0	0
49.61567	-95.64109	NA	2.07	0.33	16.00	120	80	0	10	10	0	0	0	0
49.61492	-95.64289	NA	0.90	0.04	16.20	136	0	5	5	10	0	0	0	0
49.61469	-95.64303	26.10	0.50	0.02	12.80	NA	20	0	50	20	10	0	0	6
49.61095	-95.65035	13.90	0.75	0.00	19.70	380	30	60	10	0	0	0	15	0
49.60991	-95.65218	10.20	1.36	0.01	20.90	290	90	10	0	0	0	0	0	0
49.60994	-95.65295	13.40	0.61	0.01	4.00	430	80	20	0	0	0	0	0	0
49.60954	-95.65352	NA	1.88	0.18	16.00	120	100	0	0	0	0	0	0	0
49.60962	-95.65366	NA	1.95	0.07	19.70	NA	95	0	0	0	0	5	0	2
49.60871	-95.65452	20.60	1.21	0.02	12.80	NA	100	0	0	0	0	0	0	4
49.60757	-95.6599	13.60	2.10	0.01	19.70	380	30	70	0	0	0	0	10	7
49.60964	-95.66242	NA	1.50	0.01	19.70	NA	95	0	0	0	0	5	50	0
49.61	-95.66277	11.50	1.69	0.01	20.90	290	90	10	0	0	0	0	15	15
49.61465	-95.66901	15.00	2.10	0.01	19.70	380	50	50	0	0	0	0	10	1
49.61626	-95.6729	NA	1.74	0.18	16.00	120	100	0	0	0	0	0	0	0
49.61692	-95.67459	NA	1.60	0.01	19.70	NA	100	0	0	0	0	0	30	2
49.61778	-95.67808	20.10	0.75	0.02	4.00	430	20	70	10	0	0	0	0	0
49.618	-95.67821	21.60	0.59	0.02	12.80	NA	40	0	30	15	15	0	0	0
49.61912	-95.67944	46.20	0.75	0.02	12.80	NA	50	0	20	20	10	0	0	10
49.61915	-95.67944	NA	0.70	0.10	19.70	NA	40	10	30	15	5	0	10	0
49.61889	-95.67989	55.00	2.48	0.02	20.90	290	70	0	0	0	0	0	15	8

49.61893	-95.68002	30.70	2.50	0.00	19.70	380	80	20	0	0	0	0	5	12
49.61955	-95.68217	17.10	0.65	0.02	4.00	430	50	30	20	0	0	0	0	0
49.6256	-95.6903	21.20	0.78	0.02	12.80	NA	25	10	40	15	10	0	0	4
49.62596	-95.6909	NA	1.35	0.38	16.00	120	40	0	60	0	0	0	0	0
49.62627	-95.69289	NA	1.20	0.01	19.70	NA	50	30	0	0	5	15	0	0
49.62663	-95.69373	18.80	1.01	0.02	4.00	430	70	0	20	10	0	0	0	0
49.63112	-95.70058	NA	0.90	0.20	16.00	120	40	40	20	0	0	0	0	0
49.62971	-95.70102	20.40	0.99	0.03	4.00	430	50	0	30	10	10	0	0	1
49.63078	-95.70216	16.10	0.60	0.02	12.80	NA	20	20	30	20	10	0	0	0
49.64034	-95.71196	NA	0.93	0.63	16.90	138	0	10	50	20	20	0	0	0
49.64257	-95.71568	20.10	0.32	0.01	NA	NA	10	10	35	35	10	0	0	0
49.64298	-95.71576	18.00	0.92	0.02	NA	NA	90	0	0	0	10	0	5	0
49.64338	-95.71587	18.20	1.03	0.02	12.50	376	0	80	20	0	0	0	0	3
49.64362	-95.71618	20.60	0.38	0.01	15.10	296	50	30	20	0	0	0	30	0
49.64373	-95.7165	15.40	1.02	0.02	12.50	376	30	30	35	5	0	0	0	12
49.64378	-95.71671	18.00	1.06	0.00	NA	NA	5	40	40	15	0	0	5	4
49.64401	-95.71689	19.20	0.85	0.01	15.10	296	30	40	30	0	0	0	0	1
49.64436	-95.71762	19.20	0.53	0.02	12.50	376	50	0	45	5	0	0	0	0
49.64442	-95.71819	18.30	0.64	0.04	15.10	296	0	40	50	10	0	0	0	1
49.64471	-95.71854	17.20	0.47	0.00	NA	NA	10	20	55	10	5	0	5	0
49.64496	-95.71886	17.20	0.77	0.03	12.50	376	40	0	50	10	0	0	0	1
49.64489	-95.7189	16.30	0.60	0.01	21.50	234	10	20	60	5	5	0	15	2
49.64507	-95.7191	18.40	0.78	0.02	15.10	296	20	40	30	10	0	0	10	0
49.64541	-95.71935	16.90	0.71	0.01	NA	NA	10	10	70	10	0	0	5	0
49.64551	-95.71953	18.20	1.04	0.02	12.50	376	20	40	35	5	0	0	0	0
49.64529	-95.71956	16.90	1.00	0.01	21.50	234	60	0	25	5	0	10	0	0
49.64604	-95.71975	20.70	0.39	0.02	15.10	296	30	0	40	30	0	0	0	1
49.64608	-95.71983	17.10	0.46	0.00	NA	NA	10	15	50	20	5	0	5	0
49.64591	-95.71984	15.60	0.43	0.02	21.50	234	0	15	70	10	5	0	5	1
49.64632	-95.72066	16.80	0.66	0.02	12.50	376	40	20	20	20	0	0	0	3
49.6465	-95.72068	17.80	0.48	0.03	15.10	296	10	10	50	30	0	0	10	1
49.64679	-95.72149	18.00	0.67	0.01	21.50	234	25	0	60	10	5	0	40	1

49.64677	-95.72185	20.40	0.46	0.00	NA	NA	10	20	50	20	0	0	5	0
49.64681	-95.72218	18.30	0.78	0.01	12.50	376	50	15	25	10	0	0	0	0
49.64685	-95.72247	20.20	0.65	0.02	15.10	296	30	50	20	0	0	0	20	0
49.64703	-95.72281	18.20	0.27	0.01	21.50	234	20	30	20	30	0	0	5	0
49.64709	-95.72297	17.20	0.77	0.02	NA	NA	35	35	20	5	5	0	5	9
49.64709	-95.72324	17.20	1.02	0.01	12.50	376	60	20	20	0	0	0	20	2
49.64693	-95.72342	18.50	0.53	0.02	15.10	296	40	30	30	0	0	0	30	0
49.64706	-95.72359	17.30	0.50	0.01	21.50	234	20	10	50	10	10	0	40	0
49.65246	-95.7246	NA	0.97	0.47	16.90	138	10	0	80	10	0	0	0	0
49.64705	-95.72477	15.90	0.14	0.01	12.50	376	10	0	0	0	0	0	0	1
49.64697	-95.725	18.30	0.52	0.01	NA	NA	20	20	0	0	10	0	5	0
49.64701	-95.72509	18.00	0.90	0.02	21.50	234	5	5	30	0	0	0	0	2
49.64914	-95.72517	NA	0.72	0.01	NA	NA	10	45	20	20	5	0	0	9
49.64722	-95.72534	17.20	0.52	0.01	15.10	296	0	30	40	20	10	0	0	0
49.64907	-95.72565	NA	0.67	0.15	NA	NA	20	10	50	10	10	0	0	1
49.64755	-95.72577	17.30	0.60	0.03	12.50	376	0	40	50	10	0	0	0	5
49.64757	-95.72598	17.10	0.38	0.01	15.10	296	0	50	40	0	0	0	0	0
49.64799	-95.72623	17.50	0.39	0.01	21.50	234	0	10	70	10	10	0	30	0
49.64799	-95.72633	17.90	0.28	0.01	NA	NA	0	10	75	10	5	0	10	0
49.6572	-95.73662	NA	1.05	0.16	16.90	138	70	30	0	0	0	0	0	0
49.65348	-95.74492	NA	0.67	0.27	16.90	138	0	0	0	0	0	0	0	0
49.65339	-95.75379	NA	0.96	0.15	16.90	138	0	10	10	50	30	0	0	0
49.65254	-95.76243	20.50	0.49	0.03	4.50	360	0	0	0	0	10	0	0	0
49.65264	-95.76263	70.20	0.59	0.02	14.40	273	10	0	0	0	0	0	0	12
49.65247	-95.76279	58.60	0.90	0.02	14.20	267	20	0	0	0	0	0	0	17
49.65301	-95.76308	NA	0.78	0.45	16.90	138	0	10	70	0	20	0	0	0
49.65296	-95.76326	52.50	0.30	0.02	NA	NA	10	40	30	10	10	0	30	4
49.65266	-95.76342	68.30	0.70	0.02	14.40	273	10	20	40	30	0	0	0	4
49.65308	-95.76379	19.60	0.82	0.02	14.20	267	10	50	40	0	0	0	0	0
49.65305	-95.76408	24.00	1.29	0.03	4.50	360	30	0	70	0	0	0	0	9
49.65321	-95.76415	22.40	0.70	0.02	NA	NA	5	30	45	15	5	0	20	1
49.65348	-95.76425	15.20	0.86	0.01	14.20	267	0	30	50	20	0	0	0	6

49.65334	-95.76444	15.30	1.08	0.03	4.50	360	30	0	70	0	0	0	0	3
49.65354	-95.76511	23.10	0.82	0.02	14.40	273	50	20	30	0	0	0	0	0
49.65353	-95.76531	18.80	0.84	0.04	4.50	360	30	20	50	0	0	0	0	1
49.6537	-95.76572	14.80	1.06	0.03	14.20	267	30	0	40	30	0	0	0	2
49.65371	-95.76576	25.20	1.01	0.02	14.40	273	40	10	50	0	0	0	0	3
49.65366	-95.76585	20.50	0.84	0.02	NA	NA	20	20	40	15	5	0	0	0
49.65377	-95.76627	25.40	0.72	0.01	NA	NA	15	15	50	20	0	0	30	1
49.65385	-95.76659	24.00	1.30	0.04	4.50	360	40	60	0	0	0	0	0	1
49.65389	-95.76677	30.00	1.02	0.04	14.40	273	70	20	10	0	0	0	0	4
49.65388	-95.76682	24.00	1.08	0.02	14.20	267	40	0	20	20	0	0	0	2
49.65329	-95.76714	17.20	0.52	0.02	4.50	360	0	30	60	10	0	0	0	0
49.65385	-95.76717	32.00	0.61	0.01	NA	NA	10	0	0	0	10	0	0	0
49.65305	-95.76729	14.10	0.32	0.03	14.20	267	20	0	60	10	10	0	0	0
49.65317	-95.76735	16.20	0.64	0.04	14.40	273	0	0	50	40	10	0	0	19
49.65273	-95.76778	12.20	0.47	0.05	NA	NA	10	35	35	15	5	0	0	0
49.6526	-95.7681	17.60	0.29	0.01	14.20	267	0	40	40	10	10	0	0	7
49.65258	-95.76826	14.30	0.24	0.02	14.40	273	0	20	70	5	5	0	0	0
49.65257	-95.76834	13.80	0.33	0.03	4.50	360	0	45	45	0	10	0	0	19
49.65249	-95.76873	14.00	0.32	0.01	NA	NA	10	35	35	15	5	0	0	1
49.65255	-95.76912	12.20	0.45	0.01	4.50	360	0	20	50	20	10	0	0	2
49.65118	-95.76914	14.10	0.56	0.02	14.20	267	10	10	40	30	10	0	0	6
49.65131	-95.76933	19.80	0.45	0.02	14.40	273	0	30	40	20	10	0	0	22
49.65258	-95.76936	13.60	0.26	0.02	14.20	267	0	20	60	10	10	0	0	7
49.65248	-95.76947	16.80	0.34	0.03	NA	NA	30	25	25	15	5	0	0	0
49.65248	-95.76956	18.20	0.64	0.02	14.40	273	0	20	70	10	0	0	0	0
49.65104	-95.76958	9.20	0.40	0.04	4.50	360	0	0	20	10	0	0	0	1
49.65189	-95.76969	14.40	0.14	0.01	4.50	360	0	30	50	20	0	0	0	0
49.65193	-95.76975	13.60	0.18	0.01	14.20	267	10	0	60	30	0	0	0	0
49.65188	-95.76977	14.70	0.14	0.03	NA	NA	0	20	40	20	0	0	0	0
49.65109	-95.76985	NA	0.21	0.32	NA	NA	0	15	50	10	5	0	0	0
49.65107	-95.77007	19.30	0.25	0.02	NA	NA	0	35	20	20	5	0	0	0
49.65103	-95.77014	NA	0.50	0.18	21.60	NA	0	0	10	70	20	0	0	1

49.65093	-95.77015	12.20	0.23	0.01	14.40	273	10	0	0	0	0	0	0	4
49.65094	-95.77018	NA	0.32	0.17	NA	NA	0	20	15	10	5	0	0	0
49.64959	-95.78259	NA	0.98	0.07	21.60	NA	80	10	0	0	10	0	0	13
49.67625	-95.78406	18.40	0.56	0.02	4.50	315	20	20	40	20	0	0	0	2
49.67636	-95.78416	19.10	0.54	0.02	13.60	281	20	15	50	15	0	0	0	8
49.67638	-95.78417	15.60	0.52	0.02	20.20	NA	0	40	40	20	0	0	0	1
49.67587	-95.78426	17.20	0.40	0.02	13.60	281	0	20	50	20	10	0	0	4
49.67671	-95.7843	10.40	0.34	0.03	21.80	274	0	20	70	10	0	0	0	1
49.67584	-95.78448	16.30	0.39	0.04	21.80	274	0	45	35	10	10	0	0	0
49.67575	-95.78451	16.60	0.38	0.02	20.20	NA	0	40	30	30	0	0	0	0
49.67551	-95.78474	19.10	0.45	0.02	4.50	315	0	20	40	30	10	0	0	10
49.67708	-95.78478	13.00	0.30	0.01	20.20	NA	0	20	35	35	10	0	0	0
49.67699	-95.78481	13.20	0.49	0.02	13.60	281	0	50	40	5	5	0	0	6
49.67706	-95.78481	13.80	0.46	0.03	4.50	315	0	10	40	40	10	0	0	4
49.6771	-95.78485	10.90	0.38	0.01	21.80	274	0	10	50	30	10	0	0	1
49.67496	-95.78526	16.30	0.70	0.02	13.60	281	0	30	25	35	10	0	0	10
49.65323	-95.78535	NA	0.91	0.03	17.70	168	80	20	0	0	0	0	0	5
49.67479	-95.7855	18.80	0.60	0.01	20.20	NA	10	30	30	20	10	0	0	0
49.67491	-95.78551	16.80	0.48	0.01	21.80	274	0	75	15	5	5	0	0	1
49.67493	-95.78555	18.20	0.54	0.03	4.50	315	0	50	35	15	0	0	0	1
49.66231	-95.78572	NA	0.90	0.26	21.60	NA	0	10	20	70	0	0	0	16
49.67464	-95.78614	NA	1.50	0.71	15.00	128	0	80	0	0	20	0	0	0
49.67693	-95.78625	16.90	0.32	0.03	21.80	274	0	20	70	10	0	0	0	0
49.67699	-95.78635	15.20	0.24	0.03	20.20	NA	0	35	50	15	0	0	0	0
49.65692	-95.78642	NA	1.00	0.45	17.70	168	50	30	20	0	0	0	0	0
49.67698	-95.78647	17.80	0.39	0.02	4.50	315	0	60	25	15	0	0	0	1
49.67706	-95.78667	15.10	0.42	0.02	13.60	281	0	65	25	10	0	0	0	2
49.65321	-95.78741	NA	0.70	0.31	21.60	NA	10	30	60	0	0	0	0	0
49.6772	-95.78772	21.40	0.18	0.00	21.80	274	15	0	40	40	5	0	0	0
49.65742	-95.78774	NA	0.60	0.42	21.60	NA	0	40	50	0	10	0	0	0
49.68467	-95.78774	NA	1.20	0.60	15.00	128	0	90	0	0	0	10	0	0
49.67702	-95.78818	17.20	0.36	0.02	4.50	315	0	60	15	20	5	0	0	1

49.67707	-95.78821	17.00	0.30	0.02	20.20	NA	40	10	20	30	0	0	10	0
49.67706	-95.78825	17.10	0.26	0.02	13.60	281	0	60	25	15	0	0	0	0
49.67877	-95.78844	16.40	0.78	0.02	4.50	315	30	20	30	20	0	0	0	2
49.67853	-95.78847	17.90	0.60	0.02	13.60	281	0	55	40	0	5	0	0	4
49.67864	-95.78868	17.50	0.56	0.02	20.20	NA	15	50	20	15	0	0	0	0
49.67836	-95.7888	NA	1.50	0.73	15.00	128	10	90	0	0	0	0	0	0
49.67928	-95.78885	17.80	0.64	0.02	4.50	315	0	60	30	10	0	0	0	0
49.67937	-95.78887	15.60	0.44	0.01	21.80	274	0	30	60	10	0	0	0	6
49.67948	-95.78895	17.20	0.48	0.02	20.20	NA	10	30	40	20	0	0	0	0
49.67841	-95.78897	17.40	0.42	0.02	21.80	274	0	20	40	30	10	0	0	1
49.67707	-95.78899	20.10	0.28	0.03	4.50	315	25	60	0	10	5	0	0	0
49.67836	-95.7891	NA	1.44	1.16	15.00	128	0	0	30	70	0	0	0	0
49.67969	-95.78913	20.80	0.89	0.02	13.60	281	40	20	30	0	10	0	0	20
49.6687	-95.78923	NA	0.90	0.19	21.60	NA	20	50	0	30	0	0	0	1
49.67803	-95.78942	12.20	0.30	0.01	21.80	274	0	5	70	20	5	0	0	0
49.67729	-95.78944	17.20	0.44	0.02	21.80	274	15	40	40	0	5	0	0	3
49.67817	-95.78949	13.20	0.41	0.02	4.50	315	0	30	50	20	0	0	0	1
49.67791	-95.78957	10.40	0.42	0.01	20.20	NA	0	20	40	30	10	0	0	0
49.67745	-95.78959	17.90	0.75	0.02	13.60	281	0	40	40	20	0	0	0	5
49.67724	-95.78962	17.20	0.50	0.01	20.20	NA	35	10	20	35	0	0	10	0
49.678	-95.78967	12.20	0.50	0.02	13.60	281	0	30	40	30	0	0	0	3
49.68639	-95.79091	NA	1.20	0.58	17.20	145	0	20	60	20	0	0	0	0
49.7027	-95.79855	NA	0.99	0.51	17.20	145	0	10	50	20	20	0	0	4
49.71405	-95.81577	NA	0.67	0.83	17.20	145	0	60	40	0	0	0	0	0
49.77016	-95.83208	NA	0.99	0.18	19.70	143	60	20	10	10	0	0	0	0
49.76786	-95.83362	NA	0.71	0.10	18.90	NA	10	5	5	40	40	0	5	0
49.7772	-95.83539	16.80	0.45	0.01	10.50	450	0	30	50	20	0	0	0	94
49.76121	-95.8358	NA	0.71	0.08	18.90	NA	60	10	10	0	10	10	0	0
49.7639	-95.83598	NA	0.94	0.31	19.70	143	0	80	10	0	0	10	0	0
49.72083	-95.83664	NA	0.84	0.55	17.20	145	20	80	0	0	0	0	0	0
49.77527	-95.83746	NA	0.74	0.30	18.90	NA	15	15	15	15	15	0	0	13
49.77612	-95.83784	NA	0.90	0.38	19.70	143	20	50	20	10	0	0	0	1

49.77657	-95.83887	22.00	0.38	0.02	12.80	381	20	80	0	0	0	0	0	3
49.77703	-95.8391	18.40	0.82	0.02	12.80	381	0	85	0	10	5	0	0	27
49.77825	-95.83922	20.00	0.38	0.01	NA	NA	0	50	30	20	0	0	0	5
49.77683	-95.83923	19.40	0.38	0.02	16.90	380	0	20	60	20	0	0	0	42
49.77808	-95.83923	20.60	0.38	0.02	12.80	381	0	75	10	15	0	0	0	2
49.77821	-95.83923	19.00	0.25	0.02	16.90	380	0	50	30	20	0	0	0	23
49.77772	-95.83924	17.40	0.16	0.02	16.90	380	0	40	25	30	5	0	0	4
49.77888	-95.83927	23.20	0.39	0.02	12.80	381	0	60	20	15	5	0	0	5
49.77796	-95.83938	19.10	0.42	0.02	10.50	450	0	20	50	20	10	0	0	10
49.77852	-95.83948	22.90	0.44	0.03	10.50	450	25	25	40	10	0	0	0	8
49.77898	-95.83964	21.40	0.50	0.01	NA	NA	0	40	20	10	10	0	0	4
49.7547	-95.83968	NA	0.74	0.13	18.90	NA	0	5	70	20	5	0	0	1
49.77928	-95.8397	24.20	0.50	0.03	16.90	380	10	50	25	15	0	0	0	29
49.77973	-95.83988	18.60	0.48	0.03	NA	NA	0	30	40	20	10	0	0	6
49.77962	-95.84004	19.40	0.56	0.03	12.80	381	0	30	40	20	10	0	0	13
49.77969	-95.84005	19.80	0.43	0.01	10.50	450	0	20	40	30	10	0	0	17
49.77962	-95.84007	20.40	0.50	0.03	16.90	380	0	20	20	40	20	0	0	31
49.73126	-95.84027	11.00	0.24	0.02	12.70	362	0	20	80	0	0	0	0	7
49.77999	-95.84097	20.60	0.32	0.00	NA	NA	0	60	15	15	10	0	0	3
49.73278	-95.84132	9.50	0.56	0.03	23.60	289	0	60	30	5	5	0	0	0
49.73117	-95.84137	13.20	0.40	0.02	12.70	362	20	60	10	10	0	0	0	52
49.73237	-95.84141	13.60	0.36	0.02	13.20	375	20	60	20	0	0	0	0	3
49.73223	-95.84144	13.20	0.14	0.01	12.70	362	0	100	0	0	0	0	0	1
49.75158	-95.84146	NA	0.52	0.10	19.70	143	100	0	0	0	0	0	0	0
49.78038	-95.8415	24.90	1.00	NA	12.80	381	40	0	25	25	10	0	0	16
49.73312	-95.84161	9.10	0.37	0.02	12.70	362	0	30	40	20	10	0	0	17
49.73325	-95.84161	7.80	0.47	0.02	13.20	375	0	40	40	15	5	0	0	14
49.7804	-95.84177	21.40	0.68	0.02	16.90	380	50	0	30	15	5	0	0	2
49.78041	-95.84201	22.40	0.54	0.02	10.50	450	30	40	20	10	0	0	0	0
49.78244	-95.84209	NA	1.80	0.52	13.40	138	20	80	0	0	0	0	0	0
49.78246	-95.84211	NA	1.08	0.11	19.70	143	80	0	0	0	20	0	0	0
49.78052	-95.84219	23.80	0.66	0.01	NA	NA	0	30	60	0	10	0	0	5

49.73339	-95.84238	17.10	0.64	0.03	23.60	289	0	75	20	0	5	0	0	0
49.7825	-95.84243	21.20	0.88	0.03	16.90	380	0	0	0	10	20	0	0	35
49.78251	-95.84264	20.40	1.06	0.01	NA	NA	0	0	0	20	10	0	0	33
49.73347	-95.84266	6.20	0.31	0.02	NA	NA	0	80	20	0	0	0	0	1
49.73362	-95.84267	12.90	0.31	0.02	13.20	375	0	95	0	0	5	0	0	35
49.734	-95.84292	9.00	0.36	0.03	23.60	289	0	50	30	15	5	0	0	5
49.78075	-95.84292	26.80	0.66	NA	12.80	381	30	30	20	20	0	0	0	2
49.7339	-95.84293	5.60	0.29	0.01	NA	NA	0	30	60	10	0	0	0	0
49.73398	-95.84295	7.90	0.22	0.03	12.70	362	0	50	30	20	0	0	0	28
49.78279	-95.84302	14.60	0.46	0.03	10.50	450	30	20	15	20	0	15	0	3
49.78206	-95.84304	19.00	0.48	0.04	16.90	380	0	40	40	15	5	0	0	42
49.7343	-95.84307	16.50	0.32	0.04	23.60	289	10	70	15	0	5	0	0	1
49.73358	-95.84309	NA	1.04	0.40	17.20	145	40	20	40	0	0	0	0	0
49.78294	-95.84325	NA	1.55	0.87	13.40	138	0	30	70	0	0	0	0	0
49.78117	-95.84338	26.20	0.76	0.02	16.90	380	10	40	30	20	0	0	0	20
49.78111	-95.84341	19.80	0.53	0.03	10.50	450	10	20	40	20	10	0	0	11
49.73482	-95.84349	12.50	0.38	0.01	NA	NA	0	100	0	0	0	0	0	8
49.78208	-95.84349	19.40	0.64	0.01	NA	NA	0	20	40	20	20	0	0	7
49.78163	-95.84358	22.10	0.68	0.03	10.50	450	30	20	20	20	10	0	0	0
49.78113	-95.8436	22.10	0.62	0.02	12.80	381	40	0	30	20	10	0	0	7
49.78162	-95.84364	22.40	0.60	0.02	16.90	380	20	40	25	15	0	0	0	22
49.75179	-95.84367	NA	0.78	0.07	18.90	NA	60	10	5	0	5	20	35	0
49.73511	-95.84368	17.10	0.10	0.01	12.70	362	40	60	0	0	0	0	0	48
49.78164	-95.8437	20.40	0.62	0.01	NA	NA	0	40	40	10	10	0	0	4
49.73517	-95.84379	13.80	0.22	0.03	13.20	375	40	60	0	0	0	0	0	17
49.73573	-95.84405	13.90	0.21	0.01	12.70	362	40	60	0	0	0	0	0	1
49.73556	-95.84406	12.00	0.30	0.02	23.60	289	0	60	25	10	5	0	0	0
49.73612	-95.84422	13.50	0.22	0.01	23.60	289	35	30	30	0	5	0	0	0
49.73593	-95.84424	12.50	0.39	0.00	NA	NA	20	80	0	0	0	0	0	5
49.73608	-95.84425	14.10	0.23	0.02	13.20	375	20	80	0	0	0	0	0	2
49.78385	-95.8444	NA	0.53	0.00	13.40	138	100	0	0	0	0	0	50	0
49.73655	-95.8446	12.50	0.26	0.03	NA	NA	0	80	20	0	0	0	0	30

49.73668	-95.84475	15.40	0.28	0.02	23.60	289	35	40	20	0	5	0	0	0
49.73679	-95.84489	13.10	0.22	0.03	13.20	375	20	80	0	0	0	0	0	13
49.73691	-95.84508	14.60	0.18	0.01	12.70	362	40	60	0	0	0	0	0	17
49.73708	-95.84531	12.90	0.15	0.01	12.70	362	40	60	0	0	0	0	0	29
49.73713	-95.84541	17.50	0.14	0.04	23.60	289	30	60	10	0	0	0	0	0
49.73764	-95.84595	16.70	0.19	0.01	12.70	362	40	60	0	0	0	0	0	13
49.73769	-95.84602	14.70	0.22	0.04	NA	NA	10	90	0	0	0	0	0	12
49.7376	-95.84607	16.20	0.22	0.02	13.20	375	30	70	0	0	0	0	0	1
49.7379	-95.84646	17.60	0.18	0.02	13.20	375	30	70	0	0	0	0	0	7
49.73836	-95.84673	16.20	0.22	0.02	NA	NA	10	80	10	0	0	0	0	7
49.74601	-95.84698	NA	0.88	0.18	19.70	143	70	30	0	0	0	0	0	0
49.74535	-95.847	NA	0.75	0.25	18.90	NA	40	40	10	10	0	0	0	0
49.78536	-95.84789	NA	1.50	0.12	13.40	138	0	100	0	0	0	0	0	0
49.78809	-95.84789	NA	0.66	0.08	25.30	220	0	25	60	10	5	0	0	8
49.78563	-95.84827	NA	2.05	0.67	13.40	138	0	40	60	0	0	0	0	71
49.79447	-95.84846	31.60	0.46	0.02	19.50	NA	20	15	40	15	10	0	0	0
49.795	-95.84866	NA	1.00	0.03	25.30	220	50	0	0	30	20	0	0	0
49.79476	-95.84871	29.40	0.46	0.02	3.60	301	0	50	40	10	0	0	0	0
49.79472	-95.84874	29.10	0.33	0.01	NA	NA	5	50	30	10	5	0	0	18
49.79491	-95.84877	36.20	0.32	0.01	19.50	NA	20	20	40	10	10	0	0	0
49.79424	-95.84883	30.30	0.46	0.01	13.00	351	5	35	40	0	20	0	0	23
49.79512	-95.84887	12.00	0.49	0.01	13.00	351	0	0	15	10	75	0	0	6
49.79591	-95.84934	38.60	0.34	0.01	NA	NA	5	10	10	60	15	0	0	12
49.79405	-95.84953	32.30	0.45	0.01	NA	NA	40	40	0	10	10	0	0	27
49.79336	-95.84969	26.20	0.36	0.00	19.50	NA	0	20	35	35	10	0	0	0
49.79344	-95.84971	22.80	0.25	0.02	3.60	301	0	30	30	30	10	0	0	4
49.79103	-95.8498	NA	1.00	NA	16.80	133	70	30	0	0	0	0	0	0
49.79565	-95.84982	40.10	0.71	0.02	3.60	301	0	20	30	40	10	0	0	97
49.79331	-95.84985	22.20	0.48	0.00	13.00	351	0	30	50	15	5	0	0	18
49.79642	-95.84995	17.80	0.52	0.01	19.50	NA	0	30	40	25	5	0	0	0
49.79638	-95.85	15.10	0.52	0.00	NA	NA	0	25	35	35	5	0	0	1
49.79287	-95.85004	19.90	0.74	0.02	3.60	301	0	20	30	40	10	0	0	16

49.79624	-95.85007	4.20	0.11	0.11	13.00	351	0	45	45	10	0	0	0	0
49.7932	-95.85016	23.60	0.42	0.01	NA	NA	5	55	15	10	15	0	0	4
49.79311	-95.85023	NA	1.40	0.80	13.40	138	0	80	20	0	0	0	0	0
49.79267	-95.85027	23.20	0.76	0.02	19.50	NA	30	20	30	10	10	0	0	2
49.79264	-95.85045	21.50	0.53	0.01	NA	NA	0	50	20	10	20	0	0	4
49.79662	-95.85045	11.80	0.08	0.12	13.00	351	0	10	60	30	0	0	0	1
49.79668	-95.8506	20.40	0.30	0.04	3.60	301	0	40	40	20	0	0	0	0
49.79222	-95.85074	19.40	0.38	0.02	3.60	301	0	60	10	30	0	0	0	41
49.7969	-95.85101	16.20	0.76	0.02	19.50	NA	0	20	50	20	10	0	0	2
49.79702	-95.85143	12.90	0.41	0.03	3.60	301	0	30	30	30	10	0	0	0
49.79694	-95.85159	12.30	0.46	0.02	13.00	351	5	40	10	20	20	5	0	2
49.79699	-95.85167	10.70	0.47	0.00	NA	NA	0	5	60	30	5	0	0	12
49.79211	-95.85179	13.40	0.62	0.01	NA	NA	0	0	5	5	70	0	0	12
49.79687	-95.85197	16.40	0.62	0.01	19.50	NA	0	25	60	15	0	0	0	4
49.79186	-95.85202	21.40	0.48	0.01	19.50	NA	10	0	0	0	10	0	0	5
49.79192	-95.85208	20.10	0.39	0.02	3.60	301	0	0	30	30	0	0	0	0
49.79699	-95.85265	19.40	0.49	0.02	NA	NA	0	15	65	15	5	0	0	21
49.79689	-95.8528	18.60	0.20	0.02	13.00	351	5	35	40	15	0	5	0	9
49.79699	-95.85302	19.80	0.52	0.02	19.50	NA	0	20	50	30	0	0	0	6
49.79703	-95.85319	20.20	0.50	0.03	3.60	301	0	30	60	10	0	0	0	0
49.79739	-95.85371	19.60	0.87	0.03	3.60	301	0	65	0	25	10	0	0	0
49.80153	-95.85417	NA	0.68	0.07	25.30	220	10	30	30	20	10	0	0	2
49.79774	-95.85424	24.00	0.58	0.02	13.00	351	45	5	20	5	20	5	0	17
49.79783	-95.85435	24.50	0.78	0.03	19.50	NA	40	0	20	30	10	0	0	0
49.79767	-95.85454	23.40	0.57	0.02	NA	NA	40	15	5	20	20	0	0	11
49.8041	-95.86145	NA	0.50	0.18	25.30	220	0	70	10	10	10	0	0	3
49.80583	-95.86203	NA	0.98	0.49	16.80	133	20	70	10	0	0	0	0	0
49.80661	-95.86473	NA	0.74	0.09	25.30	220	20	55	10	5	10	0	0	1
49.81967	-95.8706	NA	1.16	0.14	16.80	133	80	20	0	0	0	0	0	0
*49.65078	*-95.76938	NA	0.58	0.42	17.70	168	0	0	0	0	0	0	0	2
*49.64947	*-95.78163	NA	0.49	0.36	17.70	168	0	60	40	0	0	0	0	1

APPENDIX 2**.** Access point information for all current and potential range extension Carmine Shiner sampling locations in the Whitemouth River and Birch River watersheds, Manitoba.

Waterbody	Location	Site Type	Latitude	Longitude	Notes
Whitemouth River	HWY 505 crossing	Access Point	49.56756	-95.97697	Road Access
Whitemouth River	HWY 1	Access Point	49.64996	-95.88116	Road Access
Whitemouth River	HWY 507 crossing	Access Point	49.73844	-95.87878	Road Access
Whitemouth River	HWY 506 crossing	Access Point	49.79742	-95.8804	Road Access
Whitemouth River	HWY 11	Access Point	49.87617	-95.9064	Road Access
Whitemouth River	HWY 11 & 44 crossing	Access Point	49.93915	-95.95673	Road Access
Whitemouth River	HWY 408 crossing	Access Point	49.99033	-96.00952	Road Access
Whitemouth River	HWY 307 crossing	Access Point	50.10536	-96.0338	Road Access
Birch River	East of HWY 506	Access Point	49.79762	-95.85497	Road Access
Birch River	East of HWY 507	Access Point	49.73862	-95.84646	Road Access
Boggy River	HWY 308 crossing	Access Point	49.6253	-95.61983	Road Access
Birch River	McMunn	Access Point	49.63389	-95.70254	Road Access
Winnipeg River	Below Whitemouth Falls	Access Point	50.12252	-96.03356	Road Access
Pinawa Channel	Below Old Pinawa Dam	Access Point	50.21524	-95.92386	Boat Access
Bird River	Below first rapids	Access Point	50.41154	-95.65354	Boat Access
Bird River	Below confluence with Peterson Creek	Access Point	50.41128	-95.67015	Boat Access
Birch River	HWY 1	Additional Access Point	49.64874	-95.72591	Road Access
Birch River	1st Bridge N of HWY 1	Additional Access Point	49.6799	-95.78903	Road Access
Birch River	Near confluence with Lenchuk Creek	Additional Access Point	49.76817	-95.83323	Road Access
Whitemouth River	Silver Bridge Road, upstream of Elma	Additional Access Point	49.85639	-95.87413	Road Access
Whitemouth River	Road Crossing Near River Hills	Additional Access Point	50.07613	-96.03193	Road Access
Peterson Creek	HWY 314 access	Range Extension	50.44349	-95.56235	Road Access
Winnipeg River	Below Great Falls	Range Extension	50.46465	-96.01089	Boat Access
Boggy River	East end of Boggy River Road	Range Extension	49.62928	-95.52647	Road Access

APPENDIX 3. Database template developed for the standardized sampling protocol of Carmine Shiner in rivers in Manitoba.

Waterbody Body		Activity Date (day/month/year)	
Access Point		Time of Day	
Description of access		Crew	

Upstream Latitude (decimal degrees)	Upstream Longitude (decimal degrees)	Site #	Upstream Wetted Width (m)	Upstream Rooted Width (m)

Discharge (velocity/ depth) at upstream site	1	2	3

Water Temperature (°C)	Turbidity (NTU)	Max. Depth (m)	Secchi (cm)	Conductivity (µS cm^{-1})

SEINING

Seine dimensions (m)	Area fished (m^2)	Pass number
		1
		2
		3

QUADRAT	1	2	3
Water Depth (m)			
Water Velocity (m s^{-1})			
Bedrock (>1024 mm)			
Boulder (256─1024 mm)			
Cobble (64─256 mm)			
Large Gravel (34─64 mm)			
Small Gravel (2─34 mm)			
Sand (0.062─2 mm)			
Silt (0.004─0.062 mm)			
Clay (<0.004 mm)			
Vegetation/Woody debris Cover in%			

Photo Number:	Description:

Fish #	Species ID	Fork Length (mm)	Picture	Specimen Collected- lethally sampled (Y/N)	Comments