

Larval drift sampling of the Sydenham River fish community, Ontario, in 2017

Robin C. Gáspárdy, Jason Barnucz, and D. Andrew R. Drake

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

2026

**Canadian Data Report of
Fisheries and Aquatic Sciences 1477**

Canadian Data Report of Fisheries and Aquatic Sciences

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Cat. No. Fs97-13/1477E-PDF ISBN 978-0-660-98670-8 ISSN 1488-5395

Correct citation for this publication:

Gáspárdy, R.C., Barnucz, J., and Drake, D.A.R. 2026. Larval drift sampling of the Sydenham River fish community, Ontario, in 2017. Can. Data Rep. Fish. Aquat. Sci. 1477: vi + 40 p.

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ABSTRACT

Gáspárdy, R.C., Barnucz, J., and Drake, D.A.R. 2026. Larval drift sampling of the Sydenham River fish community, Ontario, in 2017. Can. Data Rep. Fish. Aquat. Sci. 1477: vi + 40 p.

The Sydenham River in southwestern Ontario supports among the highest richness of freshwater fishes in Canada, including several federally listed species at risk. Assessing the occurrence and spatial distribution of fish larvae can provide insight into the timing and environmental drivers of reproduction for multiple species concurrently. Between April and August of 2017, Fisheries and Oceans Canada (DFO) conducted a multi-week drift sampling program for ichthyoplankton (larval fishes and eggs) in the East Sydenham River. A total of 69 sampling events across 46 field days were conducted with a sampling design that used five 500 µm drift nets (30-minute sets) at three locations (Alvinston, Oil Springs, Florence). In total, 1,670 drift net sets sampled nearly 200,000 m³ of water. Using DNA barcoding to identify individuals to species and metabarcoding to identify species occurrence in bulk samples, this effort resulted in the detection of 48 species representing 16 families as larvae (n=18,418 individuals, 43 species), eggs (n=7,547 individuals, 26 species), and larger (i.e., non-larval) individuals (n=181 individuals, 16 species). Species present in larval drift included *Species at Risk Act* (SARA)-listed species: Eastern Sand Darter (*Ammocrypta pellucida*; Threatened), Northern Sunfish (*Lepomis peltastes*; Special Concern), and Spotted Sucker (*Minytrema melanops*; Special Concern); and invasive species Round Goby (*Neogobius melanostomus*) and Common Carp (*Cyprinus carpio*).

RÉSUMÉ

Gáspárdy, R.C., Barnucz, J., and Drake, D.A.R. 2026. Larval drift sampling of the Sydenham River fish community, Ontario, in 2017. Can. Data Rep. Fish. Aquat. Sci. 1477: vi + 40 p.

La rivière Sydenham, dans le sud-ouest de l'Ontario, abrite l'une des plus riches populations de poissons d'eau douce au Canada, dont plusieurs espèces inscrites sur la liste fédérale des espèces en péril. L'évaluation de la présence et de la répartition spatiale des larves de poissons peut fournir des informations sur le moment et les facteurs environnementaux qui déterminent la reproduction de plusieurs espèces simultanément. Entre avril et août 2017, Pêches et Océans Canada (MPO) a mené un programme d'échantillonnage par dérive de plusieurs semaines pour l'ichtyoplancton (larves et œufs de poissons) dans la partie est de la rivière Sydenham. Au total, 69 échantillonnages ont été effectués au cours de 46 jours sur le terrain, selon un plan d'échantillonnage utilisant cinq filets dérivants de 500 µm (30 minutes par filet) à trois endroits (Alvinston, Oil Springs et Florence). Au total, 1 670 filets dérivants ont permis d'échantillonner près de 200 000 m³ d'eau. Grâce au codage à barres de l'ADN pour identifier les individus par espèce et au métacodage pour identifier la présence d'espèces dans des échantillons en vrac, cette initiative a permis de détecter 48 espèces représentant 16 familles sous forme de larves (n = 18 418 individus, 43 espèces), œufs (n = 7 547 individus, 26 espèces) et individus plus grands (c'est-à-dire non larvaires) (n = 181 individus, 16 espèces). Parmi les espèces présentes dans la dérive larvaire, on comptait des espèces inscrites dans *la Loi sur les espèces en péril* (LEP) : le dard des sables (*Ammocrypta pellucida*; espèce menacée), le crapet du Nord (*Lepomis peltastes*; espèce préoccupante) et le meunier tacheté (*Minytrema melanops*; espèce préoccupante); ainsi que les espèces envahissantes gobie à taches noires (*Neogobius melanostomus*) et carpe commune (*Cyprinus carpio*).

INTRODUCTION

Fisheries and Oceans Canada (DFO) has the responsibility to provide for the protection and recovery of fishes listed under the *Species at Risk Act* (SARA). To inform scientific aspects of the recovery process, DFO regularly conducts field sampling to satisfy research objectives for SARA-listed fishes, such as evaluating the distribution and abundance of species, determining species-habitat relationships, and better understanding the influence of threats and recovery actions. DFO data reports are published to support the Species at Risk Program by providing an overview of field activities and to provide a medium for archiving data associated with sampling SARA-listed fishes and their habitat.

There is a paucity of knowledge about the early life history of many non-game fishes, including SARA-listed species. In particular, the timing of reproduction is poorly understood for many species, which limits an understanding of the environmental drivers of recruitment. For SARA-listed fishes, a better understanding of the occurrence and spatial distribution of larvae is needed to inform the identification of critical habitat, understand the response of species to environmental changes, and identify and assess threats.

Part of the Lake Erie basin, the Sydenham River has two main branches, the North Sydenham and the East Sydenham, that meet in Wallaceburg, which outlets to the Chenail Ecarté (The Snye) in the St. Clair River. The Sydenham River is a freshwater biodiversity hotspot (Staton et al. 2003) in Canada's Carolinian zone with at least 80 known species of fish. Of these, at least seven fish species are SARA-listed (DFO 2018), including Pugnose Minnow (*Opsopoeodus emiliae* – Threatened), Eastern Sand Darter (*Ammocrypta pellucida* – Threatened), Northern Madtom (*Noturus stigmosus* – Endangered), Spotted Sucker (*Minytrema melanops* – Special Concern), Blackstripe Topminnow (*Fundulus diaphanous* – Special Concern), Grass Pickerel (*Esox vermiculatus americanus* – Special Concern), and Northern Sunfish (*Lepomis peltastes* – Special Concern) (Dextrase et al. 2003; Edwards and Staton 2009; Beauchamp et al. 2012; COSEWIC 2012a, 2012b, 2014; DFO 2012; Government of Canada 2024).

A multi-week larval drift sampling program was implemented in the East Sydenham River in 2017. The objectives of the program were to:

- 1) detect the occurrence of ichthyoplankton (fish eggs and larvae), including SARA-listed species, and relate species detections to environmental variables such as water temperature and river discharge;
- 2) collect ichthyoplankton samples for the development of high-throughput metabarcoding methods for bulk genetic identification of Ontario fishes (Gallage 2020; Van Nynatten et al. 2023; Gallage et al. 2023); and,
- 3) generally, address knowledge gaps about the early life history of non-game species, including SARA-listed species.

This data report summarizes the field methods and results of the 2017 sampling program.

METHODS

STUDY SYSTEM AND SITE SELECTION

Three index stations in the East Sydenham River were selected for the collection of ichthyoplankton in 2017 (Figure 1, Appendix 1). From upstream to downstream, the three stations are referred to as Alvinston, located immediately downstream of Courtright Line; Oil Springs, downstream of Oil Springs Line/Pratt Siding Rd; and Florence, downstream of Lambton Line. These locations were selected due to ease of access and existing land-owner permission, proximity to Environment and Climate Change Canada (ECCC) hydrometric gauging stations at Alvinston (station 02GG002) and Florence (02GG003), and existing DFO fish assemblage data from these locations. Several at-risk fishes are known from these locations based on past sampling (Staton et al. 2003; Mandrak et al. 2006; Poos et al. 2008, 2010; Marson and Mandrak 2009).

Sampling occurred over an 11-week span between May 18 and August 2, 2017, with two pilot days on April 19 and 20 to refine collection techniques. Each index station was sampled twice per sampling week on a rotating basis, such that each station was sampled once in the morning and once in the afternoon each week.

Upon arrival at the index location during each visit, the initial placement of a wadeable transect across the riffle closest to the access point was identified. For effective use of drift nets, the transect was selected where water depth was wadeable across the river (<1 m) and where flow did not exceed 1 m/s. GPS coordinates were recorded at each visit where the sampled transect met the right bank (while facing upstream) using a handheld Garmin Montana GPS unit. The location of the transect in relation to the nearest road crossing was measured using a Nikon laser rangefinder and recorded as part of the narrative locality description. The wetted width of the transect was measured with a Nikon laser rangefinder or with a measuring tape and recorded. The wetted width was used to determine the equidistant spacing of five drift nets across the transect (assuming that the required depth and velocity conditions were met) (Figure 2). If depth or water velocity did not meet drift net requirements, then nets were spaced evenly within the suitable segment of the transect. Once spacing was finalized, two pieces of rebar per drift net were pounded into the substrate to secure each net. Drift nets could be positioned on and removed from rebar without removing the rebar from the substrate. The rebar was left in place for the entirety of the visit to ensure each drift net was always placed in the same position on that day. Drift nets were labelled 1 through 5, with drift net 1 always closest to the right bank, facing upstream (Figure 2). Drift net positions were measured and recorded as distances from the right bank.

ICHTHYOPLANKTON COLLECTION

Standard stationary drift nets constructed of a 0.30 m x 0.46 m x 0.99 m (W x H x D) stainless steel frame, covered with 500 µm Nitex mesh, were used to collect drifting ichthyoplankton. A custom-made 200 mL collection bucket made of 50.8 mm (2") ABS pipe fitted with a threaded adapter was affixed to the cod end of the drift net. The collection bucket had a 500 µm mesh across the posterior end for collecting drifting material, while maintaining consistent water flow through the net.

During sample collection, each drift net was positioned just above the river bottom and set to fish for a standard sample time of 30 minutes. To calculate an approximate volume-based sampling effort per net set, water depth and velocity were measured immediately upstream of the mouth of each drift net using a metre stick and a Swiffer 2100 velocity metre. Where water depth exceeded the height of the drift net (0.30 m) and the net was fully submerged (Figure 2),

volume was calculated as the opening area of the drift net (0.30 m x 0.46 m) multiplied by the water velocity (m/s) and by the 30-minute (1800 seconds) sampling time (or time recorded at each net set). If the water depth was less than the height of the drift net, then the water depth was used in place of the net height in the volume calculation to account for the lower cross-section of water at the opening of the drift net (Figure 2). Catch per unit effort was calculated for larval fishes and eggs separately, per net set, and cumulatively, as larval fishes or eggs per 100 m³ of water sampled to standardize catch across net sets.

In each visit, the five drift net locations across the river were fished for five consecutive replicate net sets for a total of 25 net sets per visit. Pilot sampling conducted in April used five or six nets across the river and only two replicates per visit (due to time available to test equipment). The first net of each transect was staggered by five minutes to allow each net to be retrieved, fished, and reset prior to fishing the next net (Figure 2, Appendix 2). After each net was fished for 30 minutes, the drift net was retrieved by carefully lifting it vertically out of the water column. The net was held with the opening facing upwards, and river water was poured on the outside of the net to wash any sample material into the collection bucket affixed to the cod end of the net. Any larger material and organisms (adult fishes, turtles, sticks, etc.) that were observed were removed and released with care, while ensuring all smaller material was retained. The collection bucket was carefully removed from the drift net, and all contents, including vegetation and debris, were flushed into a 500 mL Nalgene sample bottle using a spray bottle with 95% ethanol, and then topped with 95% ethanol, to preserve the entire sample. The drift net was then thoroughly flushed before being redeployed for subsequent replicates. Preserved net contents were stored at ambient temperature in the field vehicle through the sampling week and transferred at the end of each week to non-flammable storage cabinets at room temperature in the laboratory until processed.

ENVIRONMENTAL DATA COLLECTION

The substrate, aquatic vegetation, and riparian vegetation, immediately within the transect, were visually assessed and recorded as the percent composition of each type of substrate or vegetation present. Substrate classifications were modified from Bain (1999) and were based on particle size: organic, clay, silt, sand, gravel, cobble, boulder, hardpan, bedrock, or rubble. Classifications of aquatic vegetation were open water (no vegetation), submerged, emergent, and floating vegetation. Riparian vegetation within 2 m of the bank in line with the sampled transect was classified as: deciduous, coniferous, herbaceous, shrubs, or none (no vegetation).

Five times per visit (approximately every 35 minutes), the air temperature was measured using a Kestrel anemometer, water clarity was measured using a 0.60 m long Fieldmaster secchi tube, and water quality parameters (water temperature, conductivity, dissolved oxygen, pH, and turbidity) were measured with a YSI EXO2 multiparameter sonde. Kestrel and YSI measurements were taken from the centre of the transect (mid-channel) and immediately behind the drift nets so as not to interfere with ichthyoplankton collection, with the YSI probes submerged to approximately 0.10 m from the water surface and allowed to stabilize for approximately 1 minute before recording water quality parameters.

Two water temperature data loggers (HOBO Water Temp Pro, H20-001) per index station were placed in the river on March 23, 2017, and were programmed to measure water temperature (°C) every 30 minutes, 24 hours per day, starting at 12:00 AM on March 24, 2017. Each data logger was placed within a stilling well made of 2"-diameter ABS pipe, with many 3/4" holes drilled to minimize biofouling and allow water flow-through. The data logger was attached to a threaded lid on the stilling well to allow regular maintenance and data downloading from the data logger. The stilling well was attached to a cinder block using 1/8" stainless steel aircraft cable. The cinderblock was placed on the bottom of the river, in a wadeable area near the

bridge crossing, such that the temperature logger would remain within the flow even in low water periods, and the water would flow through the openings of the cinderblock and over the temperature logger. Each cinderblock was tethered using 1/8" stainless steel aircraft cable to a piece of T-bar pounded into the nearest bank. Temperature data loggers remained in the river from March 23, 2017, until retrieval on November 14, 2018, and were downloaded and inspected approximately every 3-4 months (or seasonally) over that time. For this data report, an estimate of the mean daily water temperature was calculated for each station by averaging all readings from both temperature loggers for each full day between March 24 and August 31, 2017.

ICHTHYOPLANKTON PROCESSING

Due to the volume of samples collected, there were several stages to sample processing and species identification (Figure 3). All raw drift samples were processed identically, separating ichthyoplankton from detritus and other drift material. At this stage, ichthyoplankton from each drift sample was sorted into up to three sub-samples: larval fishes, fish eggs, and non-larval fishes (fishes in juvenile or older life stages). There was no secondary processing of fish eggs or non-larval fish prior to identification. Secondary processing of larval fish subsamples followed two methods based on the station from which they were collected: an individual processing method for subsamples from the Florence site, and a bulk processing method for subsamples from the Alvinston and Oil Springs sites. The individual processing method was used to obtain information about the relative abundance of individual eggs and larvae in each sample, and to allow determination of individual length and weight for subsequent analyses. However, due to time constraints, individual processing was only used at the Florence station. All samples were kept in 95% ethanol at room temperature through all primary and secondary processing stages.

Primary processing

Primary processing of drift material from each drift net sample consisted of separating detritus or any non-fish drift material from ichthyoplankton, which was then separated and enumerated by type: larval fish, fish eggs, or non-larval fish. The primary processing procedure was modified from the USGS Great Lakes Science Centre Ichthyoplankton Sample Picking Standard Operating Procedure (Dr. E. Roseman, pers. comm). In the laboratory, technicians carefully strained all collected material from one drift net sample (preserved in 95% ethanol) through a 500 µm mesh strainer basket, then transferred the collected material to a sorting tray with water. One technician methodically picked through the sample material, carefully rinsing larger pieces of debris such as sticks and leaves before removing them to ensure all ichthyoplankton remained in the sample. All eggs and larval fishes observed during picking were transferred to separate petri dishes with water. A second technician then scanned the sorting tray for any remaining ichthyoplankton that may have been missed by the first technician prior to discarding the remainder of the sample debris. Larval fishes from the sample were counted, placed in bulk into a uniquely labelled sub-sample vial, and topped with fresh 95% ethanol. Eggs found in the sample material were counted separately from the larval fishes and placed in bulk into a separate, uniquely labelled sub-sample vial, and topped up with fresh 95% ethanol. This process was repeated separately for all drift samples prior to secondary processing of ichthyoplankton. All primary processing was completed by 12 DFO staff over 39 lab days between June 20 and November 20, 2017.

Secondary processing – bulk method

Secondary processing of larval fish samples from Alvinston and Oil Springs stations was conducted following a bulk processing method to obtain the minimum and maximum total length

of larvae collected within each sample and bulk images. A Nikon SMZ800 stereomicroscope was used with a DS-Fi3 camera for microscopy. A Nikon DS-L4 tablet interface with built-in measurement, annotation, and image capture functions was used for image processing.

Larval fishes from a single drift net sample were arranged within the field of view on the microscope, fitting as many larvae in the field of view as were visible in a single layer. Images were then captured using the microscope camera and tablet interface. If all larval fishes from a sample did not fit in a single image, then this process was repeated until all larval fishes were captured in a series of images. The processing technician visually selected the largest and smallest individuals from the sample and measured the total lengths (mm) using the Nikon DS-L4 tablet's interface measurement and annotation functions. If preservation curled a specimen, its total length was measured by tracing its length in segments. All larval fishes were returned to the bulk sample bottle and stored at room temperature for subsequent bulk species identification. All secondary bulk processing was completed by four DFO staff over 15 days between January 1 and February 16, 2018.

Secondary processing – individual method

Secondary processing of larval fish samples from Florence station was conducted following an individual processing method to obtain total length (mm), weight (g), and a photograph of each larval fish collected. The same stereomicroscope, microscopy camera, and tablet interface, as described in the bulk processing method, were used in the same way to individually measure total length and capture a photograph of every larval fish. Once measured and photographed, each fish was dabbed dry and placed on a VWR International analytical balance (160 g x 0.1 mg) to obtain the preserved weight. All larvae were then placed individually into uniquely labelled 5 mL vials, topped with 95% ethanol, allowing each specimen to be catalogued to the sampling event and the drift net from which it was collected. All samples were stored at room temperature prior to species identification. All secondary individual processing was completed by 13 DFO staff across 84 days between February 15 and July 5, 2018.

SPECIES IDENTIFICATION

Conventional taxonomy – non-larval fishes

During primary processing, non-larval fishes were separated from larvae based on size and visibility of morphological features to enable conventional taxonomic assessment. These specimens were identified to species level using taxonomic keys and other reference materials, with key identification features recorded. Fishes were enumerated by species per drift net, and the minimum and maximum total lengths per species per drift net sample were measured and recorded. Identifications were reviewed and verified by one of two experienced taxonomists. If a specimen was too small or too damaged to be identified to species-level, it was returned to the bulk vial for secondary processing and genetic identification by metabarcoding or DNA barcoding, where possible. Conventional taxonomic identification of non-larval fishes was completed by seven DFO staff across 21 days between January 22 and May 5, 2018, and March 20-29, 2019.

Metabarcoding – bulk larval fishes

Larval fishes in bulk samples by drift net from Alvinston and Oil Springs (n=775 samples), along with bulk egg samples by drift net from all three stations (n=302 samples), were sent to the University of Toronto Scarborough on May 4, 2018, for metabarcoding using high-throughput methods. The genetic methods are described in Gallage (2020) and Gallage et al. (2023).

DNA Barcoding – individual larval fishes

All larval fishes from the Florence station were sent to the Canadian Centre for DNA Barcoding (CCDB) at the University of Guelph in January 2019 to determine individual species identification via genetic analysis. A project entry was created in the Barcode of Life Database (BOLD) containing collection data, including unique sample identification numbers to track samples individually through the genetic analysis process. Larval samples were drained of ethanol, transferred to 50 mL Falcon sample tubes, and lysed with lysis buffer containing proteinase K. The homogenized samples were subsampled and transferred into 96-well plates and extracted using a validated silica membrane-based DNA extraction process. Polymerase Chain Reaction (PCR) amplification was completed using primers specific to freshwater fishes targeting the cytochrome c oxidase 1 (CO1) gene (Ivanova et al. 2006, 2007). The amplicons from individual larvae were then unidirectionally Sanger sequenced. Solid Phase Reversible Immobilization (SPRI)-based robotic cleanup was used to remove non-incorporated BigDye from the sequencing reactions. DNA sequence information was gathered with ABI3730XL instruments. The Sanger sequences were manually edited, identified, and validated against the CCDB's reference library, and then submitted to BOLD. Failure tracing was completed for sample DNA extracts that did not deliver a full-length amplicon, and an alternative primer was used for the secondary analysis. The species identifications were available for download through BOLD and were audited collaboratively by DFO and CCDB. In cases where the sequences were matched to species not known from the Great Lakes, the identity was matched to the next most geographically likely species (i.e., *Moxostoma pisolabrum* initially identified but not geographically likely; records updated to *Moxostoma macrolepidotum*).

SAMPLING PERMITS AND DATA ARCHIVING

Sampling for this project was conducted under *Species at Risk Act* Permit Number 17-PCAA-00008. Data associated with the collections in this report are housed under the project code "2017-LFS-SR" in the Great Lakes Biodiversity Database (DFO 2025). Every effort has been made to ensure the accuracy of data contained in this report; however, results may be updated as part of ongoing data verification procedures.

RESULTS

ICHTHYOPLANKTON COLLECTION

A total of 69 half-day sampling events were conducted over 47 field days across the three index stations, during which 1,670 drift net sets were fished for a total of 50,101 minutes and sampled 199,626 m³ of water. Oil Springs station was sampled 20 times (29% of total sampling events), including one pilot sampling event, and sampled 44,139.9 m³ of water (22% of the total water volume). Sampling at Oil Springs concluded on July 19 due to low flow, at which point additional sampling effort in the subsequent two weeks was allocated at Florence and Alvinston only (four sampling events each). Florence station was sampled 25 times (36%), including two pilot sampling events, and accounted for 40% of the total water volume (80,667.5 m³) due to greater depth and water velocity. Alvinston station was sampled 24 times, including one pilot sampling event, and sampled 74,611 m³ of water (37%) (Table 1, Figure 4, Appendix 3).

The overall sampling effort resulted in the collection of 18,418 larval fishes, 7,547 eggs, and 181 non-larval fishes. Larval fishes were detected in 80% of drift net samples across 99% of half-day sampling events, while eggs were detected less frequently from 20 of the total drift net sets across 72% of sampling events (Table 1).

Overall larval catch per unit effort (CPUE) was 9.2 larvae/100 m³; when calculated per drift net, mean drift net CPUE was 8.4 larvae/100 m³ (range 0-266.2 larvae/100 m³). Overall CPUE of eggs was 3.8 eggs/100 m³. Mean egg CPUE per drift net was 2.8 eggs/100 m³ (range 0-368.8 eggs/100 m³) (Table 1). Overall, larval drift was highest in June (18.0 larvae/100 m³), and egg drift was highest in May (16.1 eggs/100 m³) (Figure 5). This pattern was consistent across sites except for Oil Springs, where egg density was highest in April (4.2 eggs/100 m³) (**Error! Reference source not found.**).

ENVIRONMENTAL DATA COLLECTION

Across all net sets, mean water depth was 0.48 m (0.18-0.89 m) and mean water velocity was 0.52 m/s (0.25-0.86 m/s) (Table 1). The Oil Springs site was deepest with the slowest velocity (mean 0.64 m depth and 0.37 m/s velocity), while Alvinston and Florence had similar mean velocity (0.59 and 0.57 m/s, respectively), but depth was greater at Florence than at Alvinston (mean 0.48 m and 0.31 m, respectively).

Across all net sets, air temperatures during sampling ranged from 12.1-33.5°C, instantaneous water temperatures from 11.96-28.60°C, conductivity from 205.2-685.3 µS/cm, dissolved oxygen from 5.73-12.27 mg/L, pH from 7.98-8.59, turbidity from 6.52-147.55 NTU, and water clarity from 0.06-0.71 m (Table 2, Appendix 4). Across all in-stream water temperature data loggers, the daily mean water temperature on sampling days was 21.23°C (12.85-25.50°C). Between March 24 and August 30, the daily mean water temperature was lowest on March 31 (4.64 °C) and was highest on July 21 (25.90°C; Table 2). The average instantaneous water temperature of each sampling event is plotted against the aggregate mean daily water temperature from continuous logged data across all locations in Figure 6.

There was little to no aquatic vegetation present within the sampling transects, except for 1-2% emergent vegetation along the shoreline between June and August. Riparian vegetation was predominantly herbaceous, shrubs, and deciduous trees (Appendix 5). Substrate across stations was predominantly cobble, gravel, and boulder (Appendix 6). Monthly variations in substrate composition relate to shifting the placement of the transect either upstream or downstream in the riffle, based on the depth and flow conditions during each sampling event.

SPECIES IDENTIFICATION

Identification processes

Genetic metabarcoding analysis by the University of Toronto Scarborough successfully identified 964 (90%) bulk samples, with 113 samples (10% total; 40 egg samples [13.2%] and 73 bulk larval samples [9.4%]) failing to produce genetic identification results. When the metabarcoding primer library failed to distinguish closely related species, genus-level identification was provided. This includes Buffalo species (genus: *Ictiobus*), and the two closely related members of the genus *Paranotropis* [Ghost Shiner (*P. buchanaui*) and Mimic Shiner (*P. volucellus*)]. Since no other specimens were identified as Buffalo to species in other identification methods, all Buffalo records are presented as *Ictiobus* sp. owing to the unknown number of species represented. Conversely, since both Ghost Shiner and Mimic Shiner were identified to species using other methods and are both known from all samples, genus level records are presented as *Paranotropis* spp., as it is known to represent two species.

Genetic barcoding analysis of individual larvae from Florence station by the University of Guelph successfully identified 12,468 individuals, with 596 individual samples failing to return a genetic identification result (4.6% failure rate). Failed identification could have been the result of sample degradation from preservation error, the size of the sample, the presence of non-fish genetic material, laboratory error, or other sources of error.

One hundred and eighty-one non-larval fishes were separated from 128 drift samples during primary processing for traditional taxonomic identification, based on their size: 30 from Alvinston, 11 from Oil Springs, and 140 from Florence. Total length ranged from 12-60 mm. The species list by identification method is provided in Appendix 7.

Species occurrence

Overall, 48 species representing 16 families were detected from bulk and individual-level genetic processing. Of these, larvae of 43 species, eggs of 26 species, and non-larval individuals of 16 species, were present (Table 3). Two species were detected in bulk samples that are not known from this system: Lake Chubsucker (*Erimyzon sucetta*, SARA-listed Endangered) and Lake Trout (*Salvelinus namaycush*). These species are included in the raw results in this data report; however, it is noted in Gallage (2020) that these species were detected at a very low resolution, and these results are therefore uncertain.

Two species [Sea Lamprey (*Petromyzon marinus*) and *Ictiobus* sp.] were detected only at the egg stage from metabarcoding methods. Three species [Brook Stickleback (*Culea inconstans*), Northern Pike (*Esox lucius*), and Fathead Minnow (*Pimephales promelas*)] were detected only at the juvenile/non-larval or adult stage and identified by traditional taxonomic methods. Fifteen species were detected only at the larval stage (Table 3).

The Florence station was the most speciose station with 40 species detected: 38 as larvae, 15 as eggs, and 12 as non-larval individuals (Table 4). The Alvinston station produced 24 species as larvae, 14 species as eggs, and nine as non-larval individuals. The Oil Springs station produced 28 species as larvae, 13 species as eggs, and seven species as non-larval individuals. Three species were unique to the Alvinston station (*Ictiobus* sp., Fathead Minnow, and Sea Lamprey), two species were unique to the Oil Springs station (Lake Chubsucker and Lake Trout), and ten species were unique to the Florence station (Appendix 7).

Overall, Shorthead Redhorse, Greenside Darter (*Etheostoma blennioides*), Bluntnose Minnow (*Pimephales notatus*), and Spotfin Shiner (*Cyprinella spiloptera*) were the most frequently occurring species as larvae, detected at 78%, 75%, 65%, and 59% of sampling events, respectively (Appendix 8). However, Greenside Darter, Shorthead Redhorse, and Spotfin Shiner were the three most frequently occurring species as larvae across all drift nets (Figure 7). Spotfin Shiner, Shorthead Redhorse, and Greenside Darter were the three most frequently occurring species as eggs (Figure 7). This was consistent across stations except for Florence, where Freshwater Drum (*Aplodinotus grunniens*) was the second-most frequently occurring species at the egg stage, detected at 32% of sampling events and 2.5% of total drift nets at the station. The three most frequently occurring species in the non-larval stage were Ghost Shiner, Spotfin Shiner, and Bluntnose Minnow (Figure 7).

Detection timing

Figure 8 depicts the duration of detection (date range) per species by life stage (bulk and individual results combined), with tabular results provided in Appendix 8. For some species, such as Shorthead Redhorse, whose eggs and/or larvae were first detected on May 17 or 18 (the first days of sustained sampling), spawning was likely initiated before the sustained sampling period began. Similarly, several species, such as Spotfin Shiner and Bluntnose Minnow, were detected as eggs and/or larvae on the final days of sampling and likely persisted in the larval drift beyond the sampling period. Seven species were detected during the two pilot sampling days in April, and only one species, Walleye (*Sander vitreus*), was not detected again during the sustained sampling period from May 17 to August 2. Eggs of White Sucker (*Catostomus commersonii*), eggs and larvae of Logperch (*Percina caprodes*), larvae of Walleye,

non-larval Bluntnose Minnow, eggs of Greenside Darter and Mooneye (*Hiodon tergisus*), and larvae of Blackside Darter (*Percina maculata*) were detected in this earliest sampling window.

Figure 9 depicts the range of water temperatures (by point measures per sampling event) when each species was detected at each life stage (text in Appendix 8). The water temperature on the dates of first and last detection is highlighted, as well as the minimum and maximum detection temperatures if they differ from the first/last detection temperatures. For example, the minimum detection temperature for Johnny Darter (*Etheostoma nigrum*) was the same as the temperature of the date of first detection (16.3°C on May 22), and the maximum detection temperature was the same as the temperature on the date of last detection (23.5°C on July 6). Alternatively, the minimum detection temperature for Shorthead Redhorse was 15.9°C on May 25th, but was 19.4°C on the date of first detection (May 18). Additionally, water temperature was 19.1°C on the date of last detection (July 25), which is cooler than the date of first detection and cooler than the maximum detection temperature of 26.7°C on June 12.

Individual species identification results

Thirty-eight species were identified within the 13,064 individually processed larval fishes from Florence (Table 4). Of these, 596 individuals (4.6%) were not successfully identified to species, and 8,278 individuals (63.4%) were identified as Shorthead Redhorse. The remaining 32% of individuals represented 37 species. Sixteen species were represented by fewer than ten individuals each. The next three most abundant species were Logperch (7.8%), Spottfin Shiner (6.4%), Ghost Shiner (6.1%), and Greenside Darter (4.1%) (Figure 10). The total count, total length summary (mean, range), and detection date range by species are provided in Table 4. Figure 11 provides a total length-weight relationship for all species detected at Florence.

SARA-listed fishes

Larvae of Eastern Sand Darter (ESD) were detected at all three sampling stations across 16 sampling days between June 6 and August 1 (the last day of sampling) (Figure 8, Figure 12). At Alvinston station, ESD larvae were detected in four drift net samples across three sampling days (July 12, July 18, and August 2). At Oil Springs station, ESD was detected in five drift net samples across five sampling days (June 6, July 5, July 11, July 12, and July 19). At Florence station, 16 ESD (15 larvae and one juvenile, based on total length) were collected from 16 drift nets across 11 days (June 8-19 and July 6-26). Larval Eastern Sand Darter were detected in the drift at instantaneous water temperatures ranging from 17.5-28.6°C (Figure 8, Appendix 8). Based on existing estimates of maximum ESD larval total length (DFO 2011), 15 of these were larvae ranging from 4.1-17.2 mm, and one was a juvenile at 19.1 mm, collected on July 13 (Table 4, Figure 12). Eastern Sand Darter eggs were detected on June 15 from two net sets in the location closest to the right (east) bank. These were identified using bulk metabarcoding methods (Appendix 7); however, each sample contained only one egg, and metabarcoding indicated that ESD was the only species present in each. Therefore, ESD eggs represented 0.03% of the total eggs collected in this project.

Spotted Sucker was detected at both larval and egg stages on 16 sampling days (Figure 13, Appendix 7). Larvae of Spotted Sucker were detected at all three sampling stations on 16 sampling days between May 30 and July 25 in instantaneous water temperatures ranging from 17.3-26.6°C. Eggs were detected on two sampling days (July 20 and 26) at Florence in instantaneous water temperatures of 21.4-25.9°C (Appendix 8). A total of 228 individual Spotted Sucker larvae were collected at Florence between May 30 and July 25, ranging in total length from 6.0-23.8 mm (Table 4). Spotted Sucker was the sixth-most abundant species in the samples at Florence, making up 1.75% of the total larval catch (Figure 10).

Larvae of Northern Sunfish were present in drift samples on 12 sampling days across all stations between June 15 and August 1 (Figure 8, Figure 13, Appendix 7). Larval Northern Sunfish were detected at instantaneous water temperatures between 20.2 and 25.9°C (Figure 9, Appendix 8). At Florence, 68 larval individuals from 4.2-7.9 mm TL were collected (Table 4), representing 0.52% of the total catch (Figure 10).

Northern Madtom was not detected. However, two madtom species were detected: one juvenile Brindled Madtom (*Noturus miurus*; TL = 15.7 mm) was collected on July 26 at Florence at 22.34°C, and Stonecat (*Noturus flavus*) larvae were present at Alvinston and Oil Springs on five sampling days (May 31-July 10) between 17.3°C and 21.8°C (Figure 9, Figure 13, Appendix 7).

Lake Chubsucker was detected in one bulk sample of larvae from Oil Springs collected on May 31 (Appendix 8). Site visit water temperature range was 18.9-20.1°C (Table 2). However, this species is not known from this location and was detected at very low read count (see Gallage (2020) and Gallage et al (2023) for details). The Lake Chubsucker detection is reported here, but is not considered a strong positive result. No other SARA-listed species were detected in the larval sampling program.

ACKNOWLEDGEMENTS

We acknowledge the many dedicated individuals who contributed to this project. For their contribution to field collections and primary sorting of samples, we thank: Carly White, Kirsten Wuerch, Jacob Burbank, Alison Drake, Courtney Leermakers, Megan Hutchings, Kurtis Smith, Alex Leclair, and Alex Verkuyl. For secondary processing of samples, we thank: Matthew Belanger, David Scott, Sarah Smith, Noel Soogrim, Alex Verkuyl, Dustin Boczek, and Olivia Sroka. For additional data management support, we thank Julia Colm, Davjit Sidhu, and Eric Smyth. We also thank Amy Boyko and Lianna Lopez for their helpful reviews. Funding for this project was provided by DFO's Species at Risk and Aquatic Invasive Species programs.

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TABLES AND FIGURES

Table 1. Summary of drift net sampling effort, variables used to calculate Catch Per Unit Effort (i.e. depth, velocity, water volume sampled), and Catch Per Unit Effort (CPUE) values at each index station in the Sydenham River, Ontario, 2017.

Index station	Alvinston	Oil Springs	Florence	Overall
Pilot Day(s)	April 19	April 20	April 19 & 20	April 19 & 20
First sampling day	May 17	May 18	May 19	May 17
Last sampling day	August 2	July 19	August 1	August 2
Total half-day sampling events	24	20	25	69
Total drift net sets	587	486	597	1,670
Total sampling minutes	17,611	14,580	17,910	50,101
Mean water depth (m)	0.31	0.64	0.48	0.48
Mean water velocity (m)	0.59	0.37	0.57	0.52
Total water volume sampled (m ³)	74,819.0	44,139.9	80,667.5	199,626.4
Average water volume per net (m ³)	127.5	90.8	135.1	119.5
Total larval fishes	3,494	1,860	13,064	18,418
Larvae freq. of occurrence (% nets)	389 (66%)	395 (81%)	552 (92%)	1,336 (80%)
Total CPUE (larvae/100 m ³)	4.7	4.2	16.2	1.1
Total eggs	6,572	116	859	7,547
Egg freq. of occurrence (% nets)	133 (23%)	34 (7%)	173 (29%)	340 (20%)
Total CPUE (eggs/100 m ³)	8.8	0.3	1.1	3.8

Table 2. Monthly average and range of daily mean water temperature (°C) from water temperature data logger readings at 30-minute intervals from March 24th to August 31st, 2017. Two temperature data loggers were placed at each index station (a total of six temperature loggers).

Month	Average [range] daily mean water temperature (°C)			
	Alvinston	Oil Springs	Florence	ALL
March (24-31)	6.8 [4.5-8.3]	6.9 [4.5-8.3]	6.5 [4.9-7.8]	6.7 [4.6-8.1]
April	11.1 [5.3-16.3]	11.1 [5.3-16.5]	10.9 [5.1-15.9]	11.0 [5.2-16.2]
May	14.5 [8.3-20.2]	14.6 [8.3-20.4]	14.5 [8.6-19.8]	14.5 [8.4-20.1]
June	21.4 [18.5-25.7]	21.5 [18.5-25.8]	21.3 [18.7-25.0]	21.4 [18.6-25.5]
July	23.4 [21.9-26.0]	23.5 [21.3-26.0]	23.6 [22.1-25.7]	23.5 [21.8-25.9]
August	21.7 [18.2-25.0]	21.8 [18.0-25.2]	22.5 [19.4-25.5]	22.0 [18.6-25.1]
Overall	16.5 [4.5-26.0]	16.6 [4.5-26.0]	16.6 [4.9-25.7]	16.5 [4.6-25.9]

Table 3. Species present in drift net samples by life stage and sampling location, based on bulk and individual-level genetic processing. Total species count includes only fishes identified to species (genus- and family-level identification not included). Invasive species (I) and SARA-listed species are identified by status (EN – Endangered, TH – Threatened, SC – Special Concern). Metabarcoding failed to differentiate between Ghost Shiner and Mimic Shiner, closely related species in the genus *Paranotropis*; both species were detected in samples identified by using barcoding. A value of 1 indicates the species was detected.

Family	Common name	Scientific name	Stage			Location			TOTAL
			Egg	Larval	Non-larval	Alvinston	Oil Springs	Florence	
Catostomidae	Sucker family	<i>Catostomidae</i>	0	0	1	0	0	1	0
	Quillback	<i>Cariodes cyprinus</i>	1	1	0	1	1	1	1
	White Sucker	<i>Catostomus commersonii</i>	1	1	1	1	1	1	1
	Lake Chubsucker (EN)	<i>Erimyzon sucetta</i>	0	1	0	0	1	0	1
	Northern Hogsucker	<i>Hypentelium nigricans</i>	1	1	0	1	1	0	1
	Buffalo sp. (unknown)	<i>Ictiobus</i> sp.	1	0	0	1	0	0	1
	Spotted Sucker (SC)	<i>Minytrema melanops</i>	1	1	0	1	1	1	1
	Silver Redhorse	<i>Moxostoma anisurum</i>	1	1	0	1	1	1	1
	Golden Redhorse	<i>Moxostoma erythrurum</i>	1	1	0	1	1	1	1
	Shorthead Redhorse	<i>Moxostoma macrolepidotum</i>	1	1	0	1	1	1	1
	Greater Redhorse	<i>Moxostoma valenciennesi</i>	0	1	0	1	0	1	1
	Rock Bass	<i>Ambloplites rupestris</i>	1	1	0	0	1	1	1
	Green Sunfish	<i>Lepomis cyanellus</i>	0	1	0	0	0	1	1
	Bluegill	<i>Lepomis macrochirus</i>	1	1	0	1	1	1	1
	Northern Sunfish (SC)	<i>Lepomis peltastes</i>	0	1	0	1	1	1	1
	Smallmouth Bass	<i>Micropterus dolomieu</i>	0	1	0	0	1	1	1
	White Crappie	<i>Pomoxis annularis</i>	0	1	0	0	0	1	1
Clupeidae	Gizzard Shad	<i>Dorosoma cepedianum</i>	0	1	0	0	0	1	1
Cyprinidae	Common Carp (I)	<i>Cyprinus carpio</i>	1	1	0	1	1	1	1
Esocidae	Northern Pike	<i>Esox lucius</i>	0	0	1	1	1	0	1
Gasterosteidae	Brook Stickleback	<i>Culaea inconstans</i>	0	0	1	1	1	1	1
Gobiidae	Round Goby (I)	<i>Neogobius melanostomus</i>	1	1	1	1	1	1	1
Hiodontidae	Mooneye	<i>Hiodon tergisus</i>	1	1	0	0	1	1	1
Ictaluridae	Channel Catfish	<i>Ictalurus punctatus</i>	0	1	1	0	0	1	1
	Stonecat	<i>Noturus flavus</i>	0	1	1	1	1	0	1
	Brindled Madtom	<i>Noturus miurus</i>	0	1	0	0	0	1	1
Lepisosteidae	Longnose Gar	<i>Lepisosteus osseus</i>	0	1	0	1	0	1	1
Leuciscidae	Shiner/Minnow family	<i>Leuciscidae</i>	0	0	1	0	0	1	0
	Spotfin Shiner	<i>Cyprinella spiloptera</i>	1	1	1	1	1	1	1
	Spottail Shiner	<i>Hudsonius hudsonius</i>	0	1	1	0	0	1	1
	Striped Shiner	<i>Luxilus chrysocephalus</i>	0	1	1	1	0	1	1
	Redfin Shiner	<i>Lythrurus umbratilis</i>	0	1	1	1	1	1	1
	Emerald Shiner	<i>Notropis atherinoides</i>	0	1	0	0	0	1	1
	Ghost/Mimic Shiner	<i>Paranotropis</i> spp.	1	1	0	1	1	1	0
	Ghost Shiner	<i>Paranotropis buchani</i>	1	1	1	1	1	1	1
	Mimic Shiner	<i>Paranotropis volucellus</i>	1	1	0	0	0	1	1
	Bluntnose Minnow	<i>Pimephales notatus</i>	1	1	1	1	1	1	1
	Fathead Minnow	<i>Pimephales promelas</i>	0	0	1	1	0	0	1
	Creek Chub	<i>Semotilus atromaculatus</i>	1	1	1	0	1	1	1
Percidae	Eastern Sand Darter (TH)	<i>Ammocrypta pellucida</i>	1	1	0	1	1	1	1
	Greenside Darter	<i>Etheostoma blennioides</i>	1	1	1	1	1	1	1
	Fantail Darter	<i>Etheostoma flabellare</i>	1	1	0	1	1	1	1
	Least Darter	<i>Etheostoma microperca</i>	0	1	0	0	0	1	1
	Johnny Darter	<i>Etheostoma nigrum</i>	1	1	1	1	1	1	1
	Logperch	<i>Percina caprodes</i>	1	1	0	1	1	1	1

Family	Common name	Scientific name	Stage			Location			TOTAL
			Egg	Larval	Non-larval	Alvinston	Oil Springs	Florence	
	Blackside Darter	<i>Percina maculata</i>	1	1	0	1	1	1	1
	Walleye	<i>Sander vitreus</i>	0	1	0	0	0	1	1
Percopsidae	Trout-perch	<i>Percopsis omiscomaycus</i>	0	1	0	0	0	1	1
Petromyzontidae	Sea Lamprey (I)	<i>Petromyzon marinus</i>	1	0	0	1	0	0	1
Salmonidae	Lake Trout	<i>Salvelinus namaycush</i>	0	1	0	0	1	0	1
Sciaenidae	Freshwater Drum	<i>Aplodinotus grunniens</i>	1	1	0	1	1	1	1
Unidentified	Unidentified	Unidentified	1	1	0	1	1	1	1
Species count			26	43	16	32	32	40	48

Table 4. Summary of individually identified fishes collected at Florence in the East Sydenham River, 2017. Fishes are ordered by detection date within families. Individuals were classified as larval or non-larval based on total length and maximum larval total length from the literature.

Common name	Larval					Juvenile & non-larval					Total count
	Count	TL (mm)		Date		Count	TL (mm)		Date		
		Mean	Range	First	Last		Mean	Range	First	Last	
Walleye	5	5.5	(4.5-6.6)	19-Apr	20-Apr	0	*	*	*	*	5
Logperch	1014	5.7	(1.2-14.4)	20-Apr	15-Jun	1	21.4	*	29-Jun	29-Jun	1015
Common Carp (I)	4	4.9	(4.2-5.5)	19-May	22-May	0	*	*	*	*	4
Trout-perch	6	5.7	(5-6.3)	19-May	24-May	0	*	*	*	*	6
Quillback	36	8.5	(5.8-9.9)	19-May	01-Jun	0	*	*	*	*	36
Mooneye	74	10.3	(5.6-14.9)	19-May	06-Jun	0	*	*	*	*	74
Blackside Darter	99	7.5	(4.3-16.8)	19-May	15-Jun	13	19.1	(17.1-23.6)	13-Jun	06-Jul	112
White Sucker	42	15.1	(7.9-23.4)	19-May	22-Jun	10	25.9	(24.2-28.2)	19-Jun	27-Jun	52
Greenside Darter	463	9.1	(1.4-15)	19-May	18-Jul	69	16.1	(15-22)	06-Jun	20-Jul	532
Shorthead Redhorse	8125	14.8	(3.2-19)	19-May	18-Jul	118	20.3	(16.2-28.4)	08-Jun	20-Jul	8243
Johnny Darter	48	5.1	(2.4-7.2)	22-May	06-Jul	2	21	(15-27)	11-Jul	13-Jul	50
Spottail Shiner	1	5.2	*	24-May	24-May	3	25.7	(25-26)	06-Jul	20-Jul	4
Least Darter	1	4.4	*	30-May	30-May	0	*	*	*	*	1
Striped Shiner	2	7.6	(7.3-7.8)	30-May	01-Jun	3	24.3	(23-26)	18-Jul	18-Jul	5
Fantail Darter	5	9	(5.2-14.5)	30-May	27-Jun	0	*	*	*	*	5
Spotted Sucker (SC)	228	12.4	(6-23.8)	30-May	25-Jul	0	*	*	*	*	228
Bluntnose Minnow	117	5.8	(3.3-19.9)	30-May	01-Aug	7	48.4	(37-60)	19-May	13-Jul	124
Longnose Gar	21	25.5	(18.5-33.8)	06-Jun	15-Jun	0	*	*	*	*	21
Silver Redhorse	3	18.3	(15.7-19.7)	06-Jun	22-Jun	0	*	*	*	*	3
Golden Redhorse	52	15.7	(8.7-23.8)	06-Jun	18-Jul	3	29.1	(28.3-29.6)	18-Jul	18-Jul	55
Ghost Shiner	783	4.7	(0.8-9)	06-Jun	01-Aug	105	34.9	(10.1-56)	19-May	31-Jul	888
Greater Redhorse	7	14.6	(13.6-15.4)	08-Jun	15-Jun	0	*	*	*	*	7
Eastern Sand Darter (TH)	15	7.4	(4.1-17.2)	08-Jun	26-Jul	1	19.1	*	13-Jul	13-Jul	16
Spotfin Shiner	839	4.9	(1.7-11.2)	08-Jun	01-Aug	13	35.2	(17.1-44)	08-Jun	25-Jul	852
Mimic Shiner	22	4.8	(3.4-6.9)	08-Jun	01-Aug	0	*	*	*	*	22
Smallmouth Bass	2	8.8	(8.7-8.9)	15-Jun	19-Jun	0	*	*	*	*	2
Round Goby (I)	14	7.3	(4.6-10.7)	15-Jun	18-Jul	2	21	(20-22)	13-Jul	20-Jul	16
White Crappie	30	4.2	(1.9-11.7)	15-Jun	01-Aug	0	*	*	*	*	30
Northern Sunfish (SC)	68	6.3	(4.2-7.9)	15-Jun	01-Aug	0	*	*	*	*	68
Rock Bass	7	7.5	(6.7-8.6)	22-Jun	13-Jul	0	*	*	*	*	7
Green Sunfish	5	3.9	(2.2-4.5)	22-Jun	25-Jul	0	*	*	*	*	5
Redfin Shiner	7	4.9	(4.4-5.4)	22-Jun	26-Jul	3	25	(24-26)	13-Jun	13-Jun	10

Common name	Larval					Juvenile & non-larval					Total count
	Count	TL (mm)		Date		Count	TL (mm)		Date		
		Mean	Range	First	Last		Mean	Range	First	Last	
Bluegill	20	5.5	(3.8-14.4)	22-Jun	26-Jul	0	*	*	*	*	20
Creek Chub	1	11.6	*	27-Jun	27-Jun	0	*	*	*	*	1
Channel Catfish	3	10.8	(6.3-14.3)	04-Jul	04-Jul	17	17.7	(15.1-21)	29-Jun	13-Jul	20
Emerald Shiner	18	5.6	(4-7.1)	11-Jul	01-Aug	0	*	*	*	*	18
Gizzard Shad	1	5.9	*	01-Aug	01-Aug	0	*	*	*	*	1
Brook Stickleback	0	*	*	*	*	2	23	(21-25)	27-Jun	18-Jul	2
Brindled Madtom	0	*	*	*	*	1	15.7	*	26-Jul	26-Jul	1
Unidentified	595	10.8	(1.1-27.1)	19-Apr	01-Aug	2	25.5	(22-29)	15-Jun	19-Jun	597
Grand Total	12783	12	(0.8-33.8)	19-Apr	01-Aug	375	24.9	(10.1-60)	19-May	31-Jul	13158

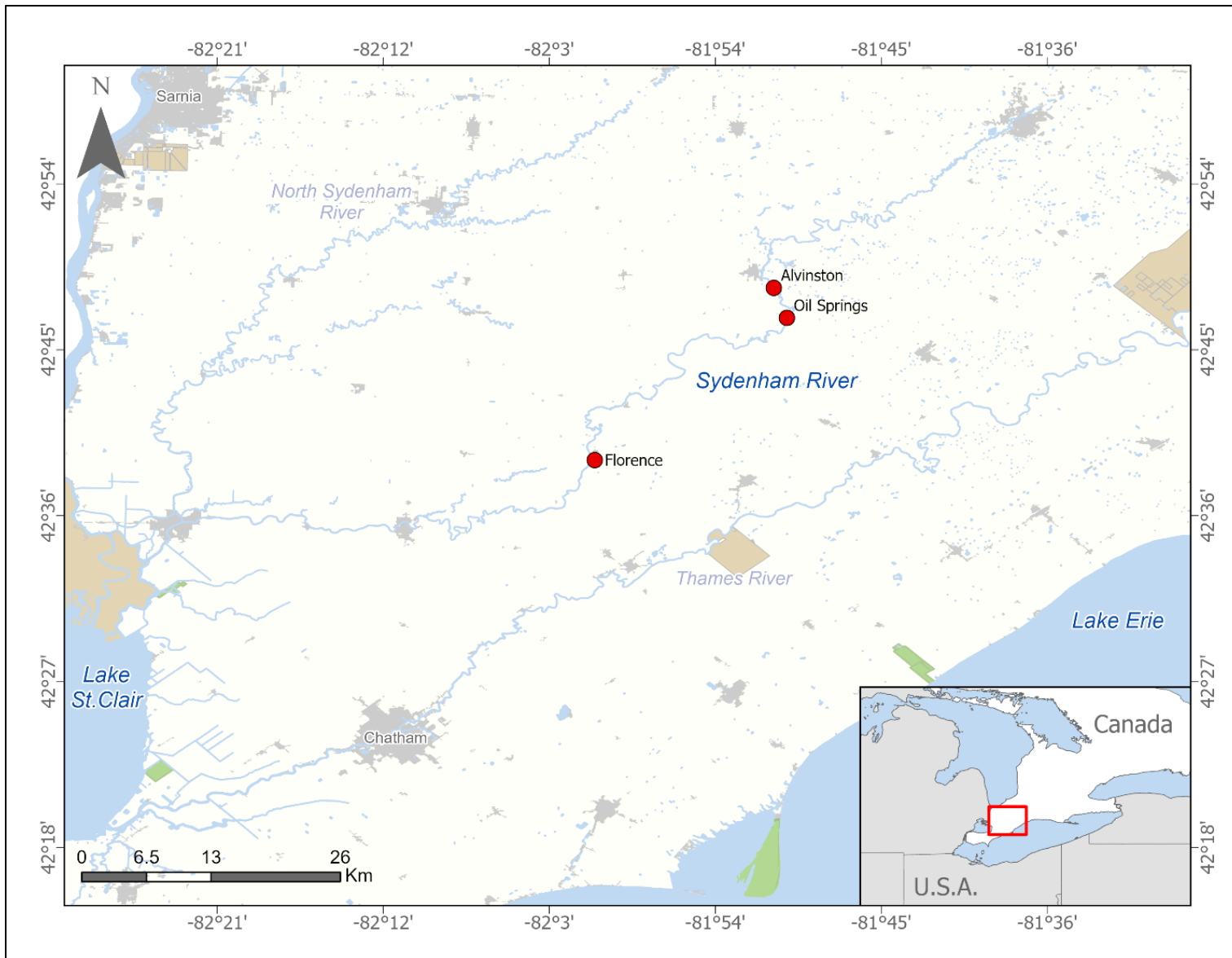
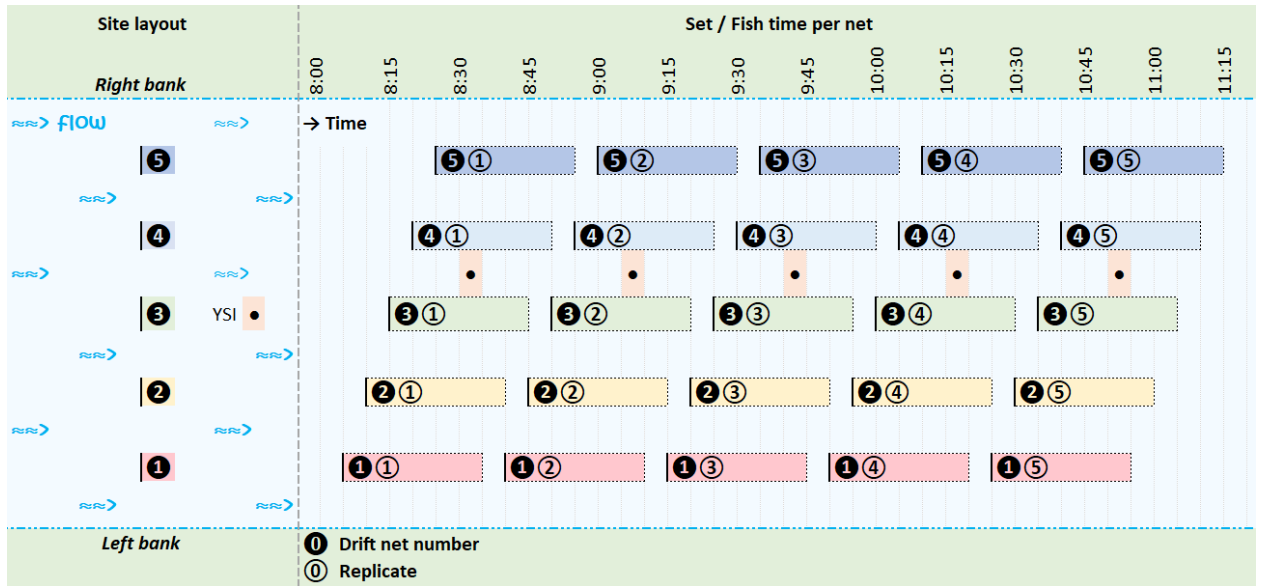


Figure 1. Map of larval drift station locations sampled in the Sydenham River, Ontario, in 2017.



a) site map diagram and example sampling event timeline



b) FLO-20170726



c) FLO-20170615

Figure 2. Site map diagram indicating (a) net placement along the transect, and example site visit timeline indicating the set time and fish time for each net and the timing of water quality measurements using the YSI multiparameter sonde during each site visit. Site photos of Florence station showing the sampling position of five drift nets across the river. Photos show the situation where drift nets were partially submerged and close to the road crossing in low-water conditions (b, FLO-20170726) as well as fully submerged and further downstream from the road crossing in high-water conditions (c, FLO-20170615).

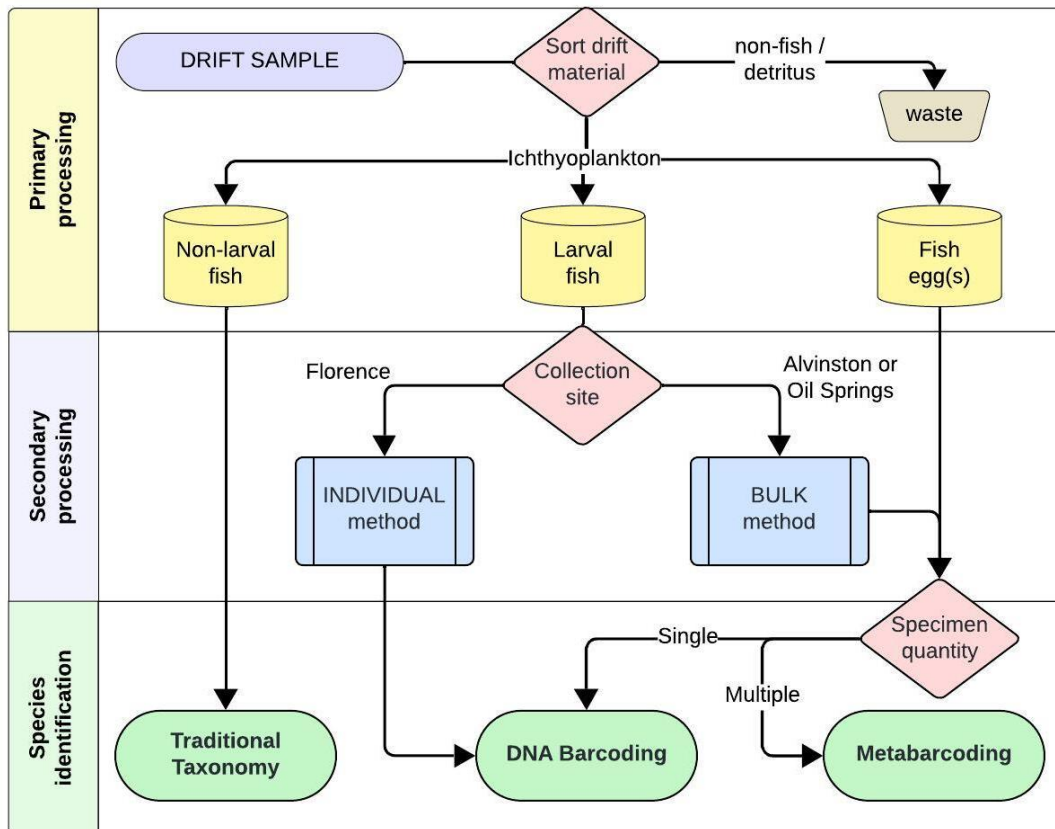


Figure 3. Flowchart depicting sample processing by sub-sample type (non-larval fish, larval fish, or fish eggs), secondary processing method by collection site, and subsequent species identification methods. DNA barcoding was conducted by the Canadian Centre for DNA Barcoding (CCDB) laboratory at the University of Guelph, and metabarcoding was conducted by University of Toronto Scarborough; all other sample processing was conducted by DFO’s Fish Species at Risk laboratory.

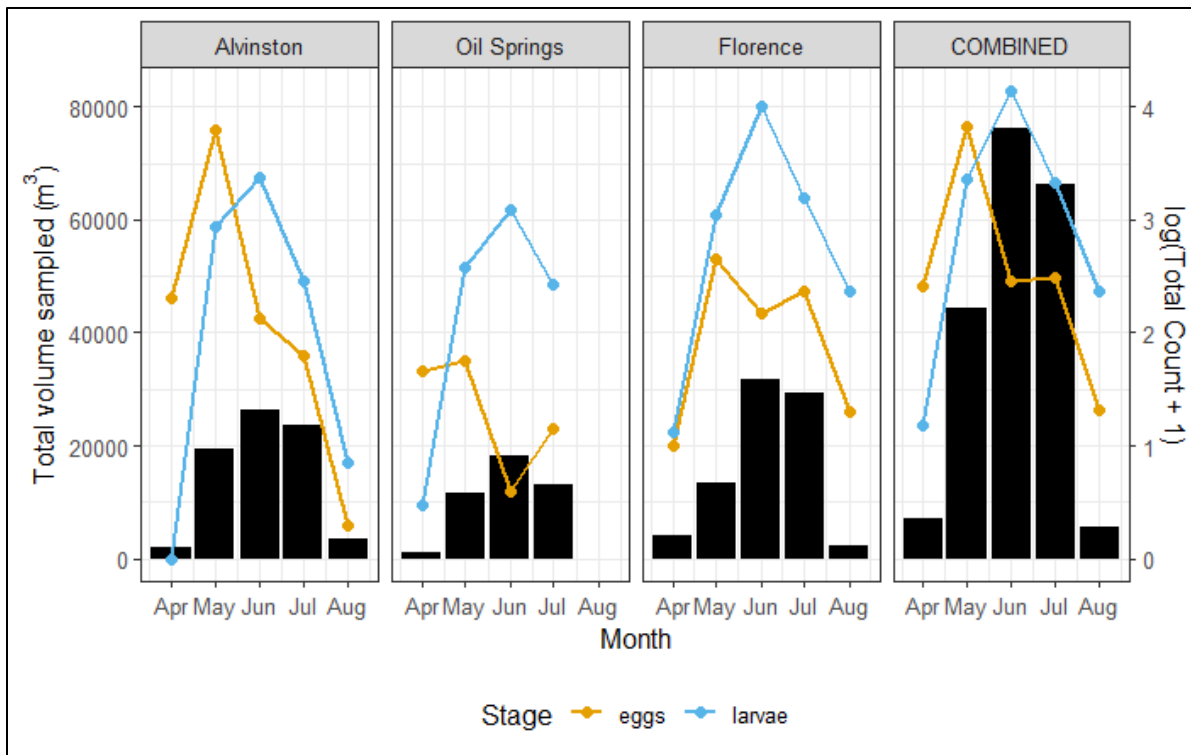


Figure 4. Monthly total effort, represented by total water volume sampled (m^3) and monthly total counts of larval fishes and eggs (displayed as $\log(\text{total count} + 1)$) by collection site and combined across all three sampling locations.

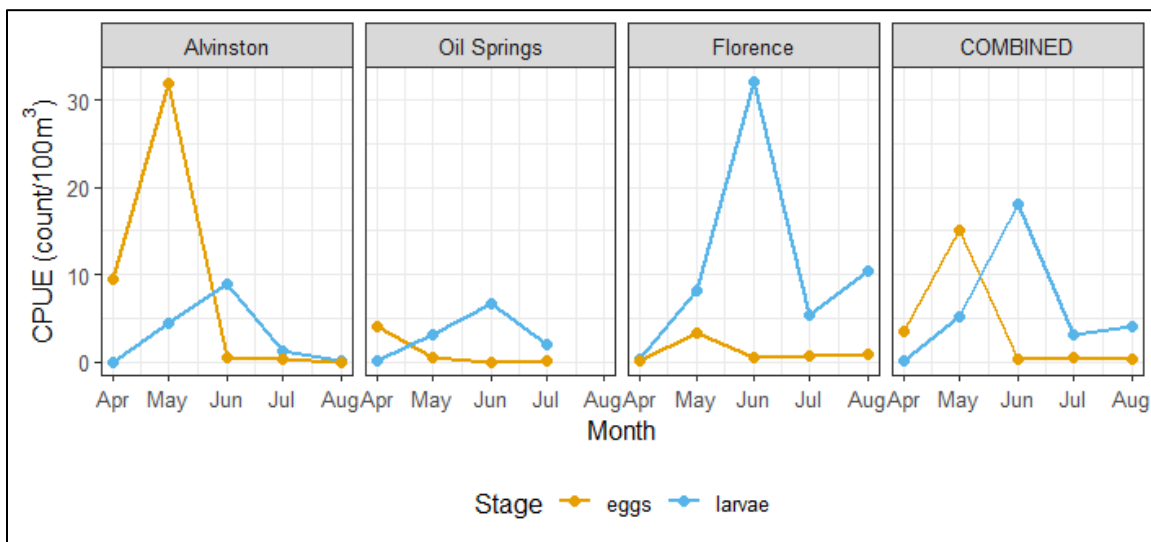


Figure 5. Monthly catch per unit effort (CPUE) of larval fishes and eggs, where effort is the total water volume sampled. Data are summarized individually per location and cumulatively across all three sampling locations.

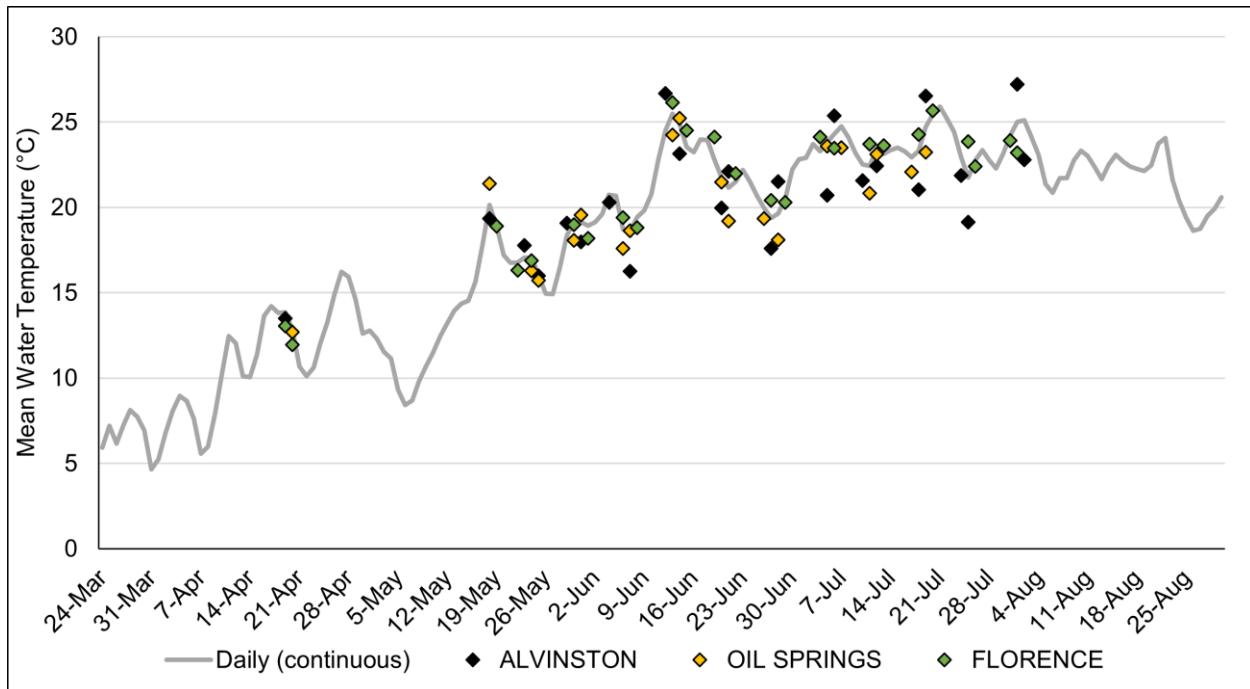


Figure 6. Instantaneous mean water temperature (°C; points) measured using a multiparameter sonde, up to five times per sampling event at each station. Daily mean water temperature (grey line) estimated as the mean temperature from six in-stream temperature data loggers (one pair per site), continuously logging water temperature semi-hourly from March 24 to August 31, 2017, in the Sydenham River.



Figure 7. Frequency of occurrence of each species by life stage, as total count of drift net sets with positive detection, across three sampling stations in the East Sydenham River, 2017.

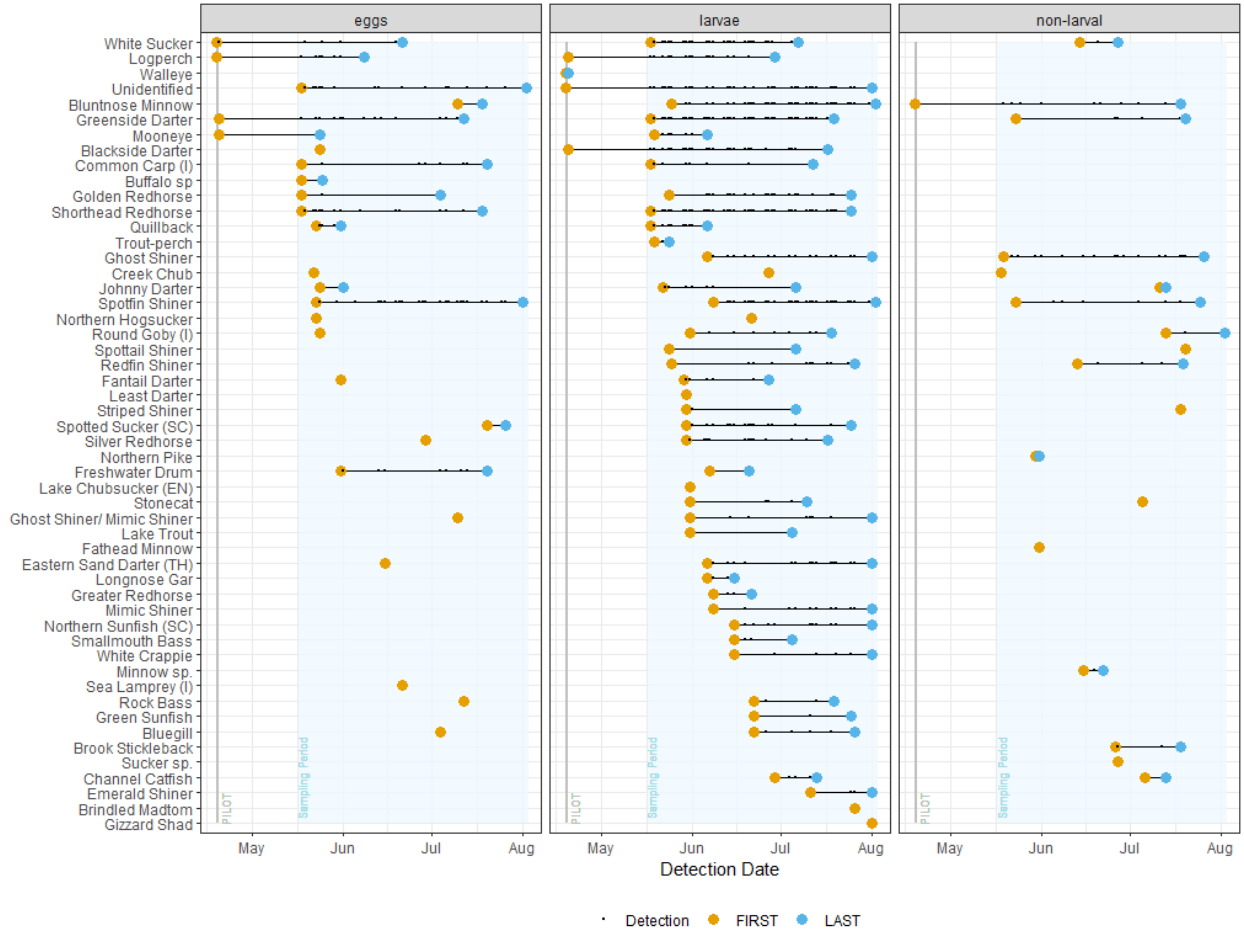


Figure 8. Dumbbell plot of species-specific detection date range per life stage, detected in drift nets in the East Sydenham River (orange = first detection, blue = last detection, black ticks = remaining detections). Sampling periods are indicated by shaded areas, where pilot sampling (grey) occurred on April 19-20, and the sustained weekly sampling period (light blue) occurred on May 18-August 2, 2017.

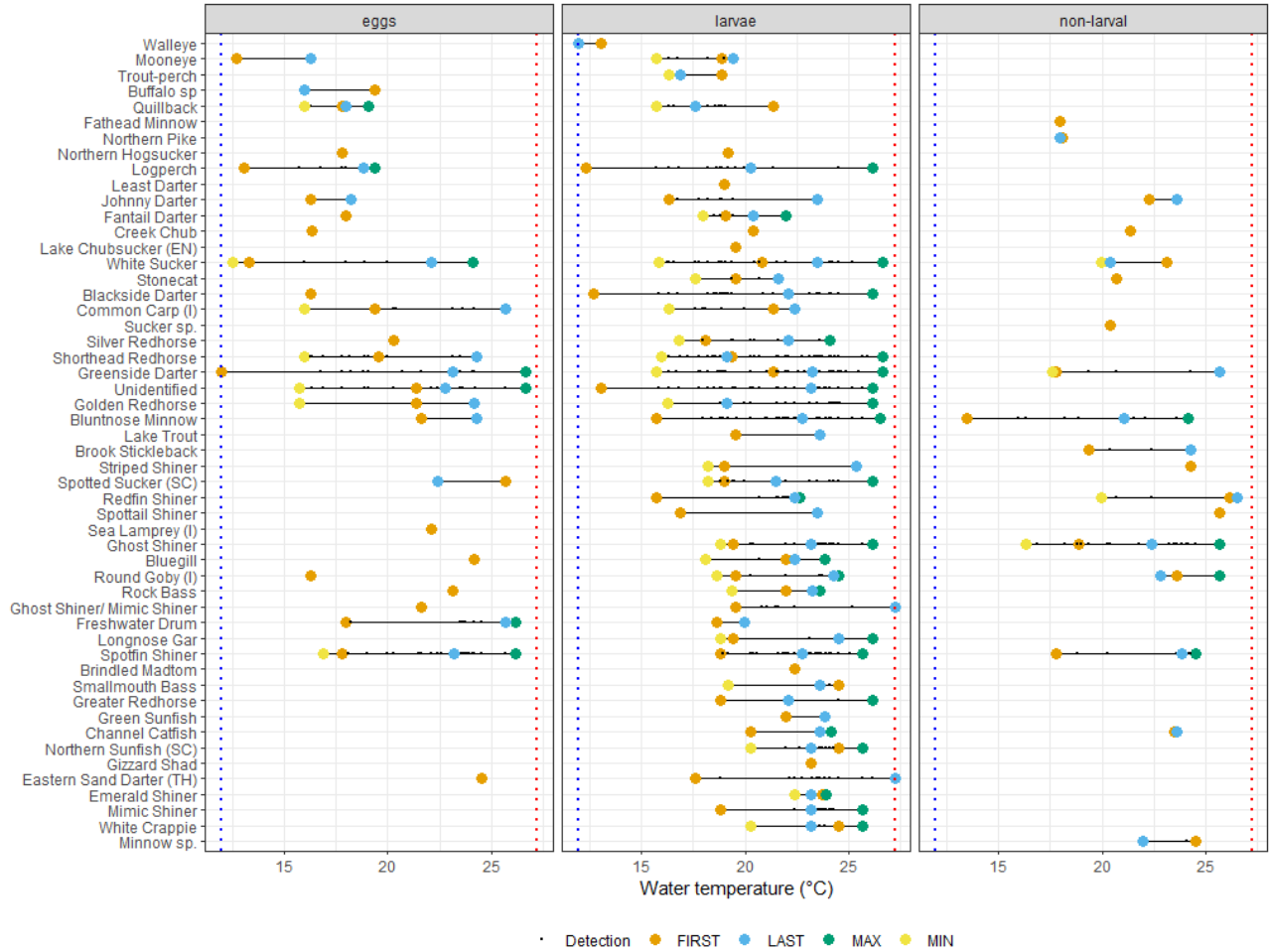


Figure 9. Dumbbell plot of mean water temperature for each site visit at detection per species by life stage. Dots on each dumbbell represent the temperature on the date of first and last detection, as well as the minimum and maximum temperature at which each species was detected. Vertical dashed lines at 11.96°C (blue) and 27.22°C (red) indicate the minimum and maximum mean site visit temperature across all sampling.

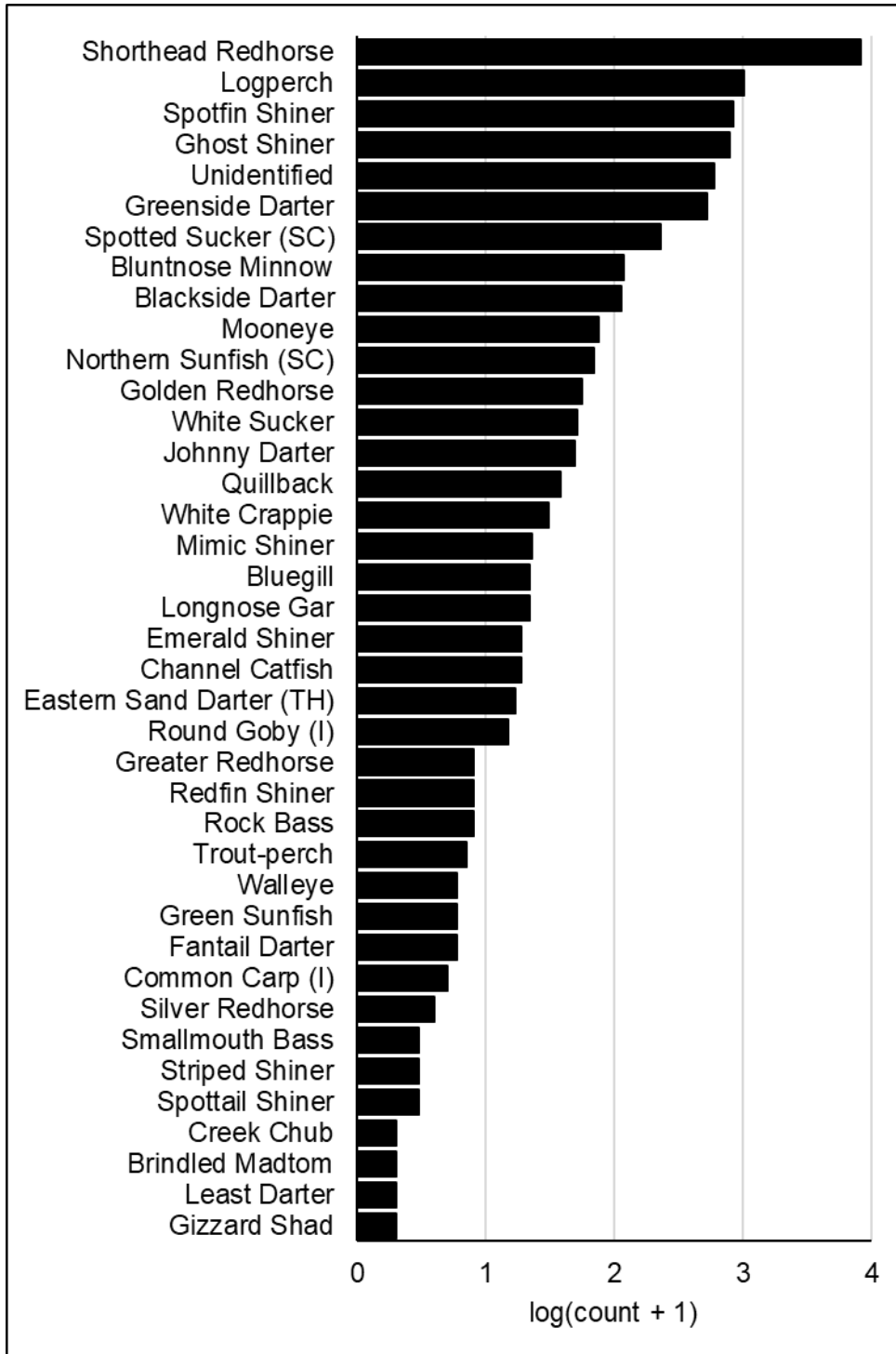


Figure 10. Rank-abundance of larvae by species for the individually identified fishes collected in drift nets in the East Sydenham River at Florence in 2017.

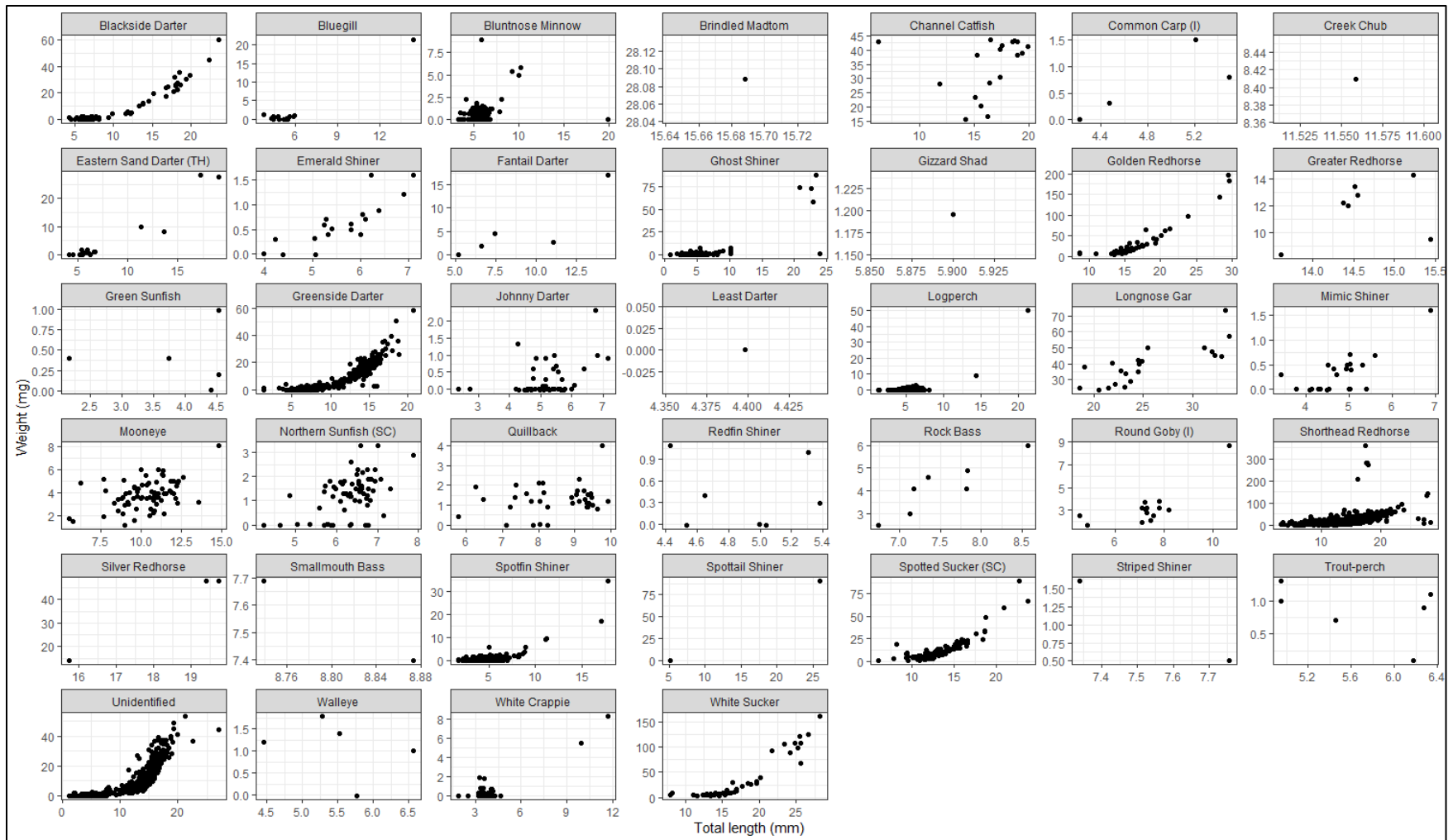


Figure 11. Length-weight relationship for fishes collected at Florence in the East Sydenham River, 2017 based on individual total length, weight, and genetic identification.



*Eastern Sand Darter, 4.5 mm
Florence, July 6, 2017*



*Bulk sample containing Eastern Sand Darter
and Greenside Darter,
Oil Springs, June 6, 2017*



*Eastern Sand Darter, 6.6 mm
Florence, July 26, 2017*



*Eastern Sand Darter, 19.1 mm
Florence, July 13, 2017*

Figure 12. Select microscope images of Eastern Sand Darter detected in drift nets in the East Sydenham River in 2017.



Spotted Sucker, 17.6 mm
Florence, June 22, 2017



Spotted Sucker, 12.1 mm
Florence, June 6, 2017



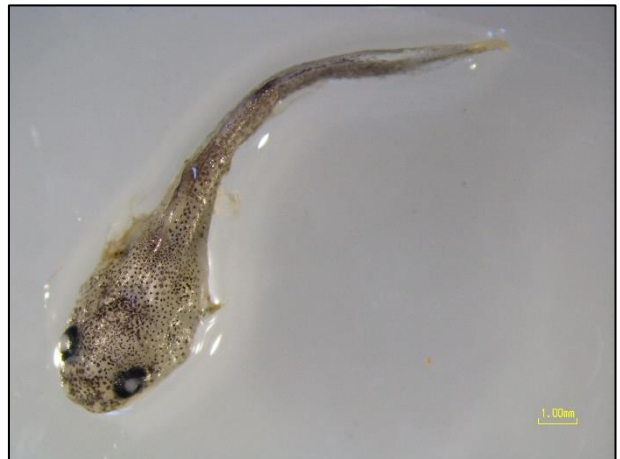
Northern Sunfish, 5.8 mm
Florence, June 19, 2017



Northern Sunfish, 6.2 mm
Florence, July 31, 2017



Stonecat, 16 mm
Alvinston, July 5, 2017



Brindled Madtom, 15.7 mm
Florence, July 26, 2017

Figure 13. Microscope images of Spotted Sucker, Northern Sunfish, and madtoms from the East Sydenham River in 2017 identified using DNA barcoding or metabarcoding techniques.

APPENDICES

Appendix 1. Location of index stations in the Sydenham River, Ontario, 2017. Coordinates indicate the right bank at the index station. The location of the drift net transect varied slightly at each visit in response to changes in water levels across sampling events.

Index station name	Narrative locality	Latitude	Longitude
Alvinston	Approximately 20-30 m downstream of Courtright Line	42.80606	-81.84691
Oil Springs	Approximately 12-45 m downstream of Pratt Siding Road	42.77879	-81.83522
Florence	Approximately 3-40 m downstream of Lambton Line	42.65041	-82.00876

Appendix 2. Summary of drift net sampling effort during each sampling event. Note: sampling effort on April 19-20 is inconsistent with the remainder of the sampling as these were pilot sampling days to test equipment and refine the sampling protocol.

a) *Alvinston* (n = 24 visits)

Sampling date	Visit ID	Time of day	Arrival time	Departure time	Wetted width (m)	Mean depth (m)	Mean water velocity (m/s)	Nets	Reps	Total nets	Total effort time (min)	Total volume (m ³)
2017-04-19	ALV-20170419	PM	11:49	13:57	22.0	0.40	0.70	6	2	12	361	2093.1
2017-05-18	ALV-20170518	AM	8:45	12:10	21.0	0.38	0.64	5	5	25	750	3972.6
2017-05-23	ALV-20170523	PM	11:46	16:00	18.0	0.59	0.62	5	5	25	750	3854.8
2017-05-25	ALV-20170525	PM	13:00	16:50	21.0	0.38	0.58	5	5	25	750	3499.0
2017-05-29	ALV-20170529	PM	11:10	14:40	21.0	0.56	0.64	5	5	25	750	3947.1
2017-05-31	ALV-20170531	AM	8:00	11:40	20.0	0.42	0.65	5	5	25	750	4053.1
2017-06-04	ALV-20170605	PM	10:50	14:28	19.9	0.32	0.52	5	5	25	750	3086.5
2017-06-07	ALV-20170607	AM	8:05	11:40	20.3	0.30	0.60	5	5	25	750	3508.9
2017-06-12	ALV-20170612	PM	12:15	16:05	20.1	0.26	0.53	5	5	25	750	2686.5
2017-06-14	ALV-20170614	AM	8:20	11:45	20.0	0.24	0.56	5	5	25	750	2704.5
2017-06-20	ALV-20170620	AM	8:13	11:45	20.4	0.31	0.64	5	5	25	750	3721.0
2017-06-21	ALV-20170621	PM	12:25	16:00	20.5	0.40	0.67	5	5	25	750	4136.9
2017-06-27	ALV-20170627	AM	8:00	11:50	20.4	0.35	0.42	5	5	25	750	2513.5
2017-06-28	ALV-20170628	PM	12:30	16:15	20.2	0.32	0.65	5	5	25	750	3872.5
2017-07-05	ALV-20170705	AM	8:00	11:45	19.8	0.23	0.74	5	5	25	750	3471.7
2017-07-06	ALV-20170706	PM	12:00	15:40	19.5	0.23	0.56	5	5	25	750	2679.9
2017-07-10	ALV-20170710	PM	13:20	17:00	20.0	0.32	0.55	5	5	25	750	2633.0
2017-07-12	ALV-20170712	AM	8:00	11:45	20.3	0.28	0.68	5	5	25	750	3815.5
2017-07-18	ALV-20170718	AM	8:00	11:45	20.1	0.24	0.65	5	5	25	750	3255.5

Sampling date	Visit ID	Time of day	Arrival time	Departure time	Wetted width (m)	Mean depth (m)	Mean water velocity (m/s)	Nets	Reps	Total nets	Total effort time (min)	Total volume (m ³)	
2017-07-19	ALV-20170719	PM	12:20	16:00	20.4	0.24	0.62	5	5	25	750	3080.7	
2017-07-24	ALV-20170724	PM	10:45	14:30	19.9	0.21	0.48	5	5	25	750	2093.1	
2017-07-25	ALV-20170725	AM	8:00	11:45	20.2	0.22	0.57	5	5	25	750	2612.6	
2017-08-01	ALV-20170801	PM	12:20	16:00	20.3	0.18	0.47	5	5	25	750	1751.1	
2017-08-02	ALV-20170802	AM	8:00	11:45	20.0	0.19	0.45	5	5	25	750	1776.2	
					mean:	20.2	0.32	0.59	sum:		587	17611	74819.1

b) *Oil Springs (n = 20 visits)*

Sampling date	Visit ID	Time of day	Arrival time	Departure time	Wetted width (m)	Mean depth (m)	Mean water velocity (m/s)	Nets	Reps	Total nets	Total effort time (min)	Total volume (m ³)	
2017-04-20	OIL-20170420	AM	9:45	12:00	20.0	0.74	0.40	6*	2	11	330	1083.0	
2017-05-18	OIL-20170518	PM	13:13	16:30	20.0	0.62	0.43	5	5	25	750	2680.2	
2017-05-24	OIL-20170524	AM	8:20	11:50	20.0	0.70	0.35	5	5	25	750	2156.1	
2017-05-25	OIL-20170525	AM	8:20	12:20	20.2	0.63	0.36	5	5	25	750	2208.3	
2017-05-30	OIL-20170530	AM	8:15	11:45	21.1	0.82	0.39	5	5	25	750	2402.0	
2017-05-31	OIL-20170531	PM	12:30	16:05	20.2	0.68	0.34	5	5	25	750	2116.4	
2017-06-06	OIL-20170606	AM	8:05	11:50	19.7	0.54	0.32	5	5	25	750	2017.0	
2017-06-07	OIL-20170607	PM	12:20	16:00	19.8	0.58	0.35	5	5	25	750	2161.1	
2017-06-13	OIL-20170613	AM	8:10	11:45	19.1	0.47	0.25	5	5	25	750	1556.6	
2017-06-14	OIL-20170614	PM	12:45	16:15	19.1	0.49	0.26	5	5	25	750	1604.7	
2017-06-20	OIL-20170620	PM	12:30	16:30	18.7	0.57	0.40	5	5	25	750	2513.8	
2017-06-21	OIL-20170621	AM	8:05	11:45	19.1	0.71	0.46	5	5	25	750	2866.5	
2017-06-26	OIL-20170626	PM	11:00	14:40	19.2	0.65	0.46	5	5	25	750	2836.7	
2017-06-28	OIL-20170628	AM	8:00	11:50	19.2	0.63	0.44	5	5	25	750	2739.9	
2017-07-05	OIL-20170705	PM	12:30	16:05	19.5	0.45	0.32	5	5	25	750	1974.8	
2017-07-07	OIL-20170707	AM	8:15	12:00	19.2	0.71	0.37	5	5	25	750	2290.3	
2017-07-11	OIL-20170711	AM	8:10	12:00	18.0	0.70	0.34	5	5	25	750	2136.2	
2017-07-12	OIL-20170712	PM	12:15	16:15	17.9	0.77	0.29	5	5	25	750	1778.5	
2017-07-17	OIL-20170717	PM	10:45	14:15	17.6	0.81	0.44	5	5	25	750	2754.8	
2017-07-19	OIL-20170719	AM	8:00	11:45	17.6	0.61	0.36	5	5	25	750	2262.9	
					mean:	19.3	0.64	0.37	sum:		486	14580	44139.9

c) Florence (n = 25 visits)

Sampling date	Visit ID	Time of day	Arrival time	Departure time	Wetted width (m)	Mean depth (m)	Mean water velocity (m/s)	Nets	Reps	Total nets	Total effort time (min)	Total volume (m ³)
2017-04-19	FLO-20170419	AM	8:10	11:00	19.0	0.85	0.65	6	2	12	360	1930.1
2017-04-20	FLO-20170420	PM	13:23	15:45	20.5	0.88	0.86	5	2	10	300	2123.8
2017-05-19	FLO-20170519	AM	8:25	12:00	18.2	0.67	0.46	5	5	25	750	2864.1
2017-05-22	FLO-20170522	PM	12:00	16:20	18.3	0.70	0.60	5	5	25	750	3726.0
2017-05-24	FLO-20170524	PM	12:50	16:30	19.3	0.81	0.67	5	5	25	750	4148.3
2017-05-30	FLO-20170530	PM	13:00	16:50	17.6	0.89	0.43	5	5	25	750	2695.1
2017-06-01	FLO-20170601	AM	8:00	11:30	16.4	0.71	0.68	5	5	25	750	4205.4
2017-06-06	FLO-20170606	PM	13:00	16:50	15.9	0.52	0.61	5	5	25	750	3758.3
2017-06-08	FLO-20170608	AM	7:50	11:45	15.0	0.51	0.57	5	5	25	750	3559.6
2017-06-13	FLO-20170613	PM	12:40	16:15	14.9	0.41	0.46	5	5	25	750	2826.8
2017-06-15	FLO-20170615	AM	8:00	11:35	15.1	0.38	0.45	5	5	25	750	2806.9
2017-06-19	FLO-20170619	PM	11:30	15:10	19.5	0.57	0.52	5	5	25	750	3231.7
2017-06-22	FLO-20170622	AM	7:50	11:40	15.8	0.57	0.60	5	5	25	750	3738.4
2017-06-27	FLO-20170627	PM	12:50	16:20	14.9	0.60	0.60	5	5	25	750	3713.6
2017-06-29	FLO-20170629	AM	8:00	11:40	15.3	0.48	0.62	5	5	25	750	3827.8
2017-07-04	FLO-20170704	PM	11:10	14:45	16.5	0.37	0.62	5	5	25	750	3831.7
2017-07-06	FLO-20170706	AM	7:50	11:30	15.8	0.27	0.60	5	5	25	750	3279.7
2017-07-11	FLO-20170711	PM	12:30	16:15	16.6	0.32	0.62	5	5	25	750	3714.8
2017-07-13	FLO-20170713	AM	8:00	11:40	17.7	0.47	0.57	5	5	25	750	3519.8
2017-07-18	FLO-20170718	PM	12:15	16:00	16.0	0.34	0.59	5	5	25	750	3650.0
2017-07-20	FLO-20170720	AM	7:45	11:30	18.2	0.33	0.55	5	5	25	750	3312.9
2017-07-25	FLO-20170725	PM	12:30	16:00	15.9	0.24	0.58	5	5	25	750	3040.9
2017-07-26	FLO-20170726	AM	7:45	11:30	16.0	0.22	0.58	5	5	25	750	2756.9
2017-07-31	FLO-20170731	PM	10:45	14:25	15.7	0.20	0.48	5	5	25	750	2207.7
2017-08-01	FLO-20170801	AM	7:45	11:30	16.7	0.24	0.44	5	5	25	750	2197.1
mean:					16.8	0.50	0.58	sum:		597	17910	80667.5

Appendix 3. Summary of mean sampling effort at each index station and resulting ichthyoplankton catch, frequency of occurrence (FOO), and catch per unit effort (CPUE) by month.

a) *Alvinston*

Month	Sampling events	Drift nets set	Mean depth (m)	Mean water velocity (m/s)	Total volume (m ³)	Total larvae	FOO larvae (% nets)	Mean larvae CPUE (fish/m ³)	Total eggs	FOO eggs (% nets)	Mean egg CPUE (egg/m ³)
April	1	12	0.40	0.70	2,093.1	0	0	0.000	199	92	0.094
May	5	125	0.47	0.63	19,326.4	850	74	0.047	6,175	69	0.307
June	8	200	0.31	0.57	26,230.2	2,354	90	0.083	135	10	0.004
July	8	200	0.25	0.61	23,642.0	284	57	0.011	62	7	0.002
August	2	50	0.19	0.46	3,527.4	6	10	0.002	1	2	0.000
Overall	24	587	0.31	0.59	74,819.0	3,494	66	0.042	6,572	22	0.070

b) *Oil Springs*

Month	Sampling events	Drift nets set	Mean depth (m)	Mean water velocity (m/s)	Total volume (m ³)	Total larvae	FOO larvae (% nets)	Mean larvae CPUE (fish/m ³)	Total eggs	FOO eggs (% nets)	Mean egg CPUE (egg/m ³)
April	1	11	0.74	0.40	1,083.0	2	18	0.003	45	36	0.024
May	5	125	0.69	0.37	11,563.0	372	78	0.032	55	18	0.003
June	8	200	0.58	0.37	18,296.3	1,221	91	0.062	3	1	0.000
July	6	150	0.67	0.35	13,197.5	265	75	0.021	13	4	0.001
Overall	20	486	0.64	0.37	44,139.9	1,860	81	0.040	116	7	0.002

c) *Florence*

Month	Sampling events	Drift nets set	Mean depth (m)	Mean water velocity (m/s)	Total volume (m ³)	Total larvae	FOO larvae (% nets)	Mean larvae CPUE (fish/m ³)	Total eggs	FOO eggs (% nets)	Mean egg CPUE (egg/m ³)
April	2	22	0.86	0.74	4,053.9	12	45	0.003	9	27	0.002
May	4	100	0.77	0.54	13,433.5	1,088	81	0.070	448	38	0.027
June	9	225	0.53	0.57	31,668.5	10,165	100	0.330	148	13	0.004
July	9	225	0.31	0.58	29,314.5	1,571	94	0.054	235	40	0.008
August	1	25	0.24	0.44	2,197.1	228	100	0.097	19	36	0.010
Overall	25	597	0.48	0.57	80,667.5	13,064	92	0.161	859	29	0.009

Appendix 4. Mean air temperature and water chemistry measured during each sampling event at each index station summarized by month.

a) Alvinston

Month	Air temp. (°C)	Water temp. (°C)	Conductivity (µS/cm)	DO (mg/L)	Turbidity (NTU)	pH	Water clarity (m)
April	18.6	13.50	506.50	9.30	33.00	8.25	0.24
May	21.8	18.03	545.69	9.40	42.65	8.18	0.28
June	22.6	20.94	584.25	9.05	35.04	8.25	0.25
July	23.8	22.34	601.23	8.68	38.03	8.32	0.19
August	26.9	25.00	630.90	9.96	20.97	8.46	0.29
Overall	23.32	21.08	585.14	9.08	36.48	8.28	0.24

b) Oil Springs

Month	Air temp. (°C)	Water temp. (°C)	Conductivity (µS/cm)	DO (mg/L)	Turbidity (NTU)	pH	Water clarity (m)
April	13.9	12.68	493.00	8.89	31.17	8.24	0.20
May	19.3	18.20	555.94	8.94	26.55	8.16	0.29
June	21.9	20.47	579.95	8.03	34.47	8.19	0.25
July	23.4	22.72	607.85	7.35	49.76	8.26	0.15
Overall	21.70	20.50	581.51	8.06	37.15	8.21	0.23

c) Florence

Month	Air temp. (°C)	Water temp. (°C)	Conductivity (µS/cm)	DO (mg/L)	Turbidity (NTU)	pH	Water clarity (m)
April	14.8	12.51	462.10	9.13	26.71	8.22	0.22
May	18.8	17.78	511.48	8.83	19.98	8.16	0.30
June	23.1	21.54	585.44	7.06	38.95	8.11	0.20
July	28.1	23.89	593.84	7.76	43.16	8.23	0.20
August	22.8	23.21	601.12	7.53	23.06	8.22	0.24
Overall	23.90	21.72	574.59	7.68	36.44	8.17	0.22

Appendix 5. Aquatic and riparian vegetation type by percent composition within and immediately adjacent to the sampled transect, averaged across site visits by sampling location.

a) *Alvinston*

Month	Deciduous	Coniferous	Herbaceous	Shrubs	None	Dominant type	Emergent	Submerged	Floating	Open water	Dominant type
April	30	0	30	10	30	Herbaceous	0	0	0	100	Open water
May	20	0	50	30	0	Herbaceous	0	0	0	100	Open water
June	19	0	45	36	0	Herbaceous	0	0	0	100	Open water
July	20	0	49	31	0	Herbaceous	0	0	0	100	Open water
August	20	0	50	30	0	Herbaceous	0	0	0	100	Open water
Overall	20	0	47	32	1	Herbaceous	0	0	0	100	Open water

b) *Oil Springs*

Month	Deciduous	Coniferous	Herbaceous	Shrubs	None	Dominant type	Emergent	Submerged	Floating	Open water	Dominant type
April	80	0	10	10	0	Deciduous	0	0	0	100	Open water
May	20	0	52	28	0	Herbaceous	0	0	0	100	Open water
June	21	0	38	41	0	Shrubs	0	0	0	100	Open water
July	10	0	60	30	0	Herbaceous	0	0	0	100	Open water
Overall	21	0	47	33	0	Herbaceous	0	0	0	100	Open water

c) *Florence*

Month	Deciduous	Coniferous	Herbaceous	Shrubs	None	Dominant type	Emergent	Submerged	Floating	Open water	Dominant type
April	20	0	50	10	20	Herbaceous	0	0	0	100	Open water
May	33	0	50	18	0	Herbaceous	0	0	0	100	Open water
June	20	0	46	34	0	Herbaceous	4	0	0	96	Open water
July	13	0	57	30	0	Herbaceous	5	0	0	95	Open water
August	20	0	50	30	0	Herbaceous	5	0	0	95	Open water
Overall	20	0	51	28	2	Herbaceous	3	0	0	97	Open water

Appendix 6. Substrate type by percent composition within and immediately adjacent to the sampled transect, averaged across site visits by sampling location.

a) *Alvinston*

Month	Organic	Clay	Silt	Sand	Gravel	Cobble	Boulder	Hardpan	Bedrock	Concrete	Rubble	Dominant type
April	0	0	0	0	30	50	20	0	0	0	0	Cobble
May	0	0	0	0	38	44	18	0	0	0	0	Cobble
June	0	0	0	5	31	37	28	0	0	0	0	Cobble
July	0	0	0	5	19	42	34	0	0	0	0	Cobble
August	0	0	0	0	20	50	30	0	0	0	0	Cobble
Overall	0	0	0	3	27	42	28	0	0	0	0	Cobble

b) *Oil Springs*

Month	Organic	Clay	Silt	Sand	Gravel	Cobble	Boulder	Hardpan	Bedrock	Concrete	Rubble	Dominant type
April	0	0	0	40	40	20	0	0	0	0	0	Gravel
May	10	0	0	16	46	19	9	0	0	0	0	Gravel
June	1	0	0	9	40	38	12	0	0	0	0	Gravel
July	0	0	0	7	28	45	20	0	0	0	0	Cobble
Overall	3	0	0	12	38	35	13	0	0	0	0	Gravel

c) *Florence*

Month	Organic	Clay	Silt	Sand	Gravel	Cobble	Boulder	Hardpan	Bedrock	Concrete	Rubble	Dominant type
April	0	0	0	0	30	50	20	0	0	0	0	Cobble
May	0	0	0	1	48	26	25	0	0	0	0	Gravel
June	0	0	0	7	24	29	39	0	0	0	0	Boulder
July	0	0	0	8	17	30	46	0	0	0	0	Boulder
August	0	0	0	10	10	30	50	0	0	0	0	Boulder
Overall	0	0	0	6	25	31	38	0	0	0	0	Boulder

Appendix 7. Species detection by life stage, station, and identification method (Trad. Taxonomy = traditional taxonomy). A value of 1 indicates that the species was detected.

Scientific name	Common name	Eggs				Larvae				Non-larval				Method		
		Alvinston	Oil Springs	Florence	Total	Alvinston	Oil Springs	Florence	Total	Alvinston	Oil Springs	Florence	Total	Metabarcoding	DNA Barcoding	Trad. Taxonomy
<i>Ambloplites rupestris</i>	Rock Bass	0	1	0	1	0	1	1	1	0	0	0	0	1	1	0
<i>Ammocrypta pellucida</i>	Eastern Sand Darter (TH)	0	0	1	1	1	1	1	1	0	0	0	0	1	1	0
<i>Aplodinotus grunniens</i>	Freshwater Drum	1	0	1	1	1	1	0	1	0	0	0	0	1	0	0
<i>Carpiodes cyprinus</i>	Quillback	1	1	0	1	1	1	1	1	0	0	0	0	1	1	0
<i>Catostomidae</i>	Sucker family	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1
<i>Catostomus commersonii</i>	White Sucker	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
<i>Culaea inconstans</i>	Brook Stickleback	0	0	0	0	0	0	0	0	1	1	1	1	0	0	1
<i>Cyprinella spiloptera</i>	Spotfin Shiner	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
<i>Cyprinus carpio</i>	Common Carp (I)	1	1	1	1	1	1	1	1	0	0	0	0	1	1	0
<i>Dorosoma cepedianum</i>	Gizzard Shad	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0
<i>Erimyzon sucetta</i>	Lake Chubsucker (EN)	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0
<i>Esox lucius</i>	Northern Pike	0	0	0	0	0	0	0	0	1	1	0	1	0	0	1
<i>Etheostoma blennioides</i>	Greenside Darter	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Etheostoma flabellare</i>	Fantail Darter	1	0	0	1	1	1	1	1	0	0	0	0	0	1	0
<i>Etheostoma microperca</i>	Least Darter	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0
<i>Etheostoma nigrum</i>	Johnny Darter	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1
<i>Hiodon tergisus</i>	Mooneye	0	1	0	1	0	1	1	1	0	0	0	0	1	1	0
<i>Hudsonius hudsonius</i>	Spottail Shiner	0	0	0	0	0	0	1	1	0	0	1	1	0	1	1
<i>Hypentelium nigricans</i>	Northern Hogsucker	1	0	0	1	0	1	0	1	0	0	0	0	1	0	0
<i>Ictalurus punctatus</i>	Channel Catfish	0	0	0	0	0	0	1	1	0	0	1	1	0	1	1
<i>Ictiobus</i> sp.	Buffalo sp.	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0
<i>Lepisosteus osseus</i>	Longnose Gar	0	0	0	0	1	0	1	1	0	0	0	0	1	1	0
<i>Lepomis cyanellus</i>	Green Sunfish	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0
<i>Lepomis macrochirus</i>	Bluegill	0	0	1	1	1	1	1	1	0	0	0	0	1	1	0
<i>Lepomis peltastes</i>	Northern Sunfish (SC)	0	0	0	0	1	1	1	1	0	0	0	0	1	1	0
<i>Leuciscidae</i>	Minnnow/Shiner family	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1
<i>Luxilus chrysocephalus</i>	Striped Shiner	0	0	0	0	1	0	1	1	0	0	1	1	1	1	1
<i>Lythrurus umbratilis</i>	Redfin Shiner	0	0	0	0	1	1	1	1	1	0	1	1	1	1	1
<i>Micropterus dolomieu</i>	Smallmouth Bass	0	0	0	0	0	1	1	1	0	0	0	0	1	1	0

Scientific name	Common name	Eggs				Larvae				Non-larval				Method		
		Alvinston	Oil Springs	Florence	Total	Alvinston	Oil Springs	Florence	Total	Alvinston	Oil Springs	Florence	Total	Metabarcoding	DNA Barcoding	Trad. Taxonomy
<i>Minytrema melanops</i>	Spotted Sucker (SC)	0	0	1	1	1	1	1	1	0	0	0	0	1	1	0
<i>Moxostoma anisurum</i>	Silver Redhorse	0	0	1	1	1	1	1	1	0	0	0	0	1	1	0
<i>Moxostoma erythrum</i>	Golden Redhorse	0	1	1	1	1	1	1	1	0	0	0	0	1	1	0
<i>Moxostoma macrolepidotum</i>	Shorthead Redhorse	1	1	1	1	1	1	1	1	0	0	0	0	1	1	0
<i>Moxostoma valenciennesi</i>	Greater Redhorse	0	0	0	0	1	0	1	1	0	0	0	0	1	1	0
<i>Neogobius melanostomus</i>	Round Goby (I)	0	1	0	1	0	1	1	1	1	0	1	1	1	1	1
<i>Notropis atherinoides</i>	Emerald Shiner	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0
<i>Noturus flavus</i>	Stonecat	0	0	0	0	1	1	0	1	1	0	0	1	1	0	1
<i>Noturus miurus</i>	Brindled Madtom	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0
<i>Paranotropis buchanani</i>	Ghost Shiner	0	0	0	0	0	0	1	1	1	1	1	1	0	1	1
<i>Paranotropis spp.</i>	Ghost/Mimic Shiner	1	0	0	1	1	1	0	1	0	0	0	0	1	0	0
<i>Paranotropis volucellus</i>	Mimic Shiner	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0
<i>Percina caprodes</i>	Logperch	1	1	1	1	1	1	1	1	0	0	0	0	1	1	0
<i>Percina maculata</i>	Blackside Darter	0	1	0	1	1	1	1	1	0	0	0	0	1	1	0
<i>Percopsis omiscomaycus</i>	Trout-perch	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0
<i>Petromyzon marinus</i>	Sea Lamprey	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0
<i>Pimephales notatus</i>	Bluntnose Minnow	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Pimephales promelas</i>	Fathead Minnow	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1
<i>Pomoxis annularis</i>	White Crappie	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0
<i>Salvelinus namaycush</i>	Lake Trout	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0
<i>Sander vitreus</i>	Walleye	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0
<i>Semotilus atromaculatus</i>	Creek Chub	0	0	1	1	0	0	1	1	0	1	0	1	1	1	1
Unidentified	Unidentified	1	1	1	1	1	1	1	1	0	0	0	0	1	1	0

Appendix 8. Positive detection summary by species and life stage, pooled across all net sets at all locations per day. Total sampling days (TSD) is the number of days (out of 47) for which species were detected; the date range of detections; and the water temperature (point-measurement during sampling) range.

Scientific name	Common name	Larvae			Eggs		
		TSD	Date	Water temp. (°C)	TSD	Date	Water temp. (°C)
<i>Carpiodes cyprinus</i>	Quillback	10	May 18 ² - Jun 6	15.1 - 22.2	5	May 23 - May 31	15.9 - 19.8
<i>Catostomus commersonii</i>	White Sucker	26	May 18 ² - Jul 7	15.1 - 27.7	8	Apr 19 ¹ - Jun 21	12.0 - 24.4
<i>Erimyzon sucetta</i>	Lake Chubsucker (EN)	1	May 31 - May 31	18.9 - 20.1	-	-	-
<i>Hypentelium nigricans</i>	Northern Hogsucker	1	Jun 21 - Jun 21	19.1 - 19.4	1	May 23 - May 23	17.0 - 18.4
<i>Ictiobus</i>	Buffalo sp.	-	-	-	2	May 18 ² - May 25	15.9 - 20.3
<i>Minytrema melanops</i>	Spotted Sucker (SC)	16	May 30 - Jul 25	17.3 - 26.6	2	Jul 20 - Jul 26	21.4 - 25.9
<i>Moxostoma anisurum</i>	Silver Redhorse	13	May 30 - Jul 17	15.7 - 24.4	1	Jun 29 - Jun 29	20.2 - 20.4
<i>Moxostoma erythrurum</i>	Golden Redhorse	20	May 24 - Jul 25	15.7 - 27.8	3	May 18 ² - Jul 4	15.1 - 24.5
<i>Moxostoma macrolepidotum</i>	Shorthead Redhorse	39	May 18 ² - Jul 31	15.1 - 27.8	15	May 18 ² - Jul 18	15.7 - 24.7
<i>Moxostoma valenciennesi</i>	Greater Redhorse	4	Jun 8 - Jun 21	18.1 - 26.6	-	-	-
<i>Ambloplites rupestris</i>	Rock Bass	5	Jun 22 - Jul 19	19.0 - 23.8	1	Jul 12 - Jul 12	23.0 - 23.2
<i>Lepomis cyanellus</i>	Green Sunfish	3	Jun 22 - Jul 25	21.9 - 24.4	-	-	-
<i>Lepomis macrochirus</i>	Bluegill	7	Jun 22 - Jul 26	17.9 - 24.7	1	Jul 4 - Jul 4	23.6 - 24.5
<i>Lepomis peltastes</i>	Northern Sunfish (SC)	12	Jun 15 - Aug 1 ³	20.2 - 25.9	-	-	-
<i>Micropterus dolomieu</i>	Smallmouth Bass	4	Jun 15 - Jul 5	19.1 - 24.7	-	-	-
<i>Pomoxis annularis</i>	White Crappie	6	Jun 15 - Aug 1 ³	20.2 - 25.9	-	-	-
<i>Dorosoma cepedianum</i>	Gizzard Shad	1	Aug 1 - Aug 1 ³	22.5 - 23.8	-	-	-
<i>Cyprinus carpio</i>	Common Carp (I)	8	May 18 ² - Jul 12	16.0 - 22.7	8	May 18 ² - Jul 20	15.9 - 25.9
<i>Neogobius melanostomus</i>	Round Goby (I)	10	May 31 - Jul 18	17.8 - 24.7	1	May 24 - May 24	16.2 - 16.4
<i>Hiodon tergisus</i>	Mooneye	7	May 19 ² - Jun 6	15.1 - 19.7	2	Apr 20 ¹ - May 24	12.7 - 16.4
<i>Ictalurus punctatus</i>	Channel Catfish	5	Jun 29 - Jul 13	20.1 - 24.5	-	-	-
<i>Noturus flavus</i>	Stonecat	5	May 31 - Jul 10	17.3 - 21.8	-	-	-
<i>Noturus miurus</i>	Brindled Madtom	1	Jul 26 - Jul 26	21.4 - 23.2	-	-	-
<i>Lepisosteus osseus</i>	Longnose Gar	5	Jun 6 - Jun 15	18.1 - 26.6	-	-	-
<i>Cyprinella spiloptera</i>	Spotfin Shiner	29	Jun 8 - Aug 2 ³	17.9 - 27.8	26	May 23 - Aug 1 ³	16.8 - 26.6
<i>Hudsonius hudsonius</i>	Spottail Shiner	2	May 24 - Jul 6	16.8 - 23.9	-	-	-
<i>Luxilus chrysocephalus</i>	Striped Shiner	3	May 30 - Jul 6	17.6 - 26.9	-	-	-
<i>Lythrurus umbratilis</i>	Redfin Shiner	12	May 25 - Jul 26	15.1 - 24.7	-	-	-
<i>Notropis atherinoides</i>	Emerald Shiner	5	Jul 11 - Aug 1 ³	21.4 - 25.1	-	-	-
<i>Paranotropis</i> spp.	Ghost Shiner/ Mimic Shiner	8	May 31 - Aug 1 ³	18.9 - 28.6	1	Jul 10 - Jul 10	21.5 - 21.8
<i>Paranotropis buchmanani</i>	Ghost Shiner	19	Jun 6 - Aug 1 ³	18.1 - 26.6	-	-	-
<i>Paranotropis volucellus</i>	Mimic Shiner	12	Jun 8 - Aug 1 ³	18.1 - 25.9	-	-	-
<i>Pimephales notatus</i>	Bluntnose Minnow	33	May 25 - Aug 2 ³	15.1 - 28.6	2	Jul 10 - Jul 18	21.5 - 24.7

Scientific name	Common name	Larvae			Eggs		
		TSD	Date	Water temp. (°C)	TSD	Date	Water temp. (°C)
<i>Semotilus atromaculatus</i>	Creek Chub	1	Jun 27 - Jun 27	20.3 - 20.5	1	May 22 - May 22	16.0 - 16.9
<i>Ammocrypta pellucida</i>	Eastern Sand Darter (TH)	16	Jun 6 - Aug 1 ³	17.5 - 28.6	1	Jun 15 - Jun 15	24.3 - 24.7
<i>Etheostoma blennioides</i>	Greenside Darter	37	May 18 ² - Jul 19	15.1 - 27.7	15	Apr 20 ¹ - Jul 12	12.0 - 27.7
<i>Etheostoma flabellare</i>	Fantail Darter	7	May 29 - Jun 27	17.7 - 22.1	1	May 31 - May 31	17.7 - 18.3
<i>Etheostoma microperca</i>	Least Darter	1	May 30 - May 30	18.2 - 19.7	-	-	-
<i>Etheostoma nigrum</i>	Johnny Darter	9	May 22 - Jul 31	16.0 - 23.9	2	May 24 - Jun 1	16.2 - 18.5
<i>Percina caprodes</i>	Logperch	17	Apr 20 ¹ - Jul 31	12.0 - 26.6	8	Apr 19 ¹ - Jun 8	13.1 - 20.3
<i>Percina maculata</i>	Blackside Darter	24	Apr 20 ¹ - Jul 17	12.7 - 26.6	1	May 24 - May 24	16.2 - 16.4
<i>Sander vitreus</i>	Walleye	2	Apr 19 ¹ - Apr 20 ¹	12.0 - 13.1	-	-	-
<i>Percopsis omiscomaycus</i>	Trout-perch	3	May 19 ² - May 24	16.0 - 19.1	-	-	-
<i>Petromyzon marinus</i>	Sea Lamprey (I)	-	-	-	1	Jun 21 - Jun 21	21.2 - 22.8
<i>Salvelinus namaycush</i>	Lake Trout	2	May 31 - Jul 5	18.9 - 24.2	-	-	-
<i>Aplodinotus grunniens</i>	Freshwater Drum	2	Jun 7 - Jun 20	17.8 - 20.5	9	May 31 - Jul 20	17.6 - 26.6
Unidentified	Unidentified	34	Apr 19 ¹ - Aug 1 ³	13.1 - 26.9	17	May 18 ² - Aug 2 ³	15.1 - 27.7

¹ Pilot sampling days: April 19, 20

² First full week of sampling (May 18, 19)

³ Last sampling days (Aug 1, 2)