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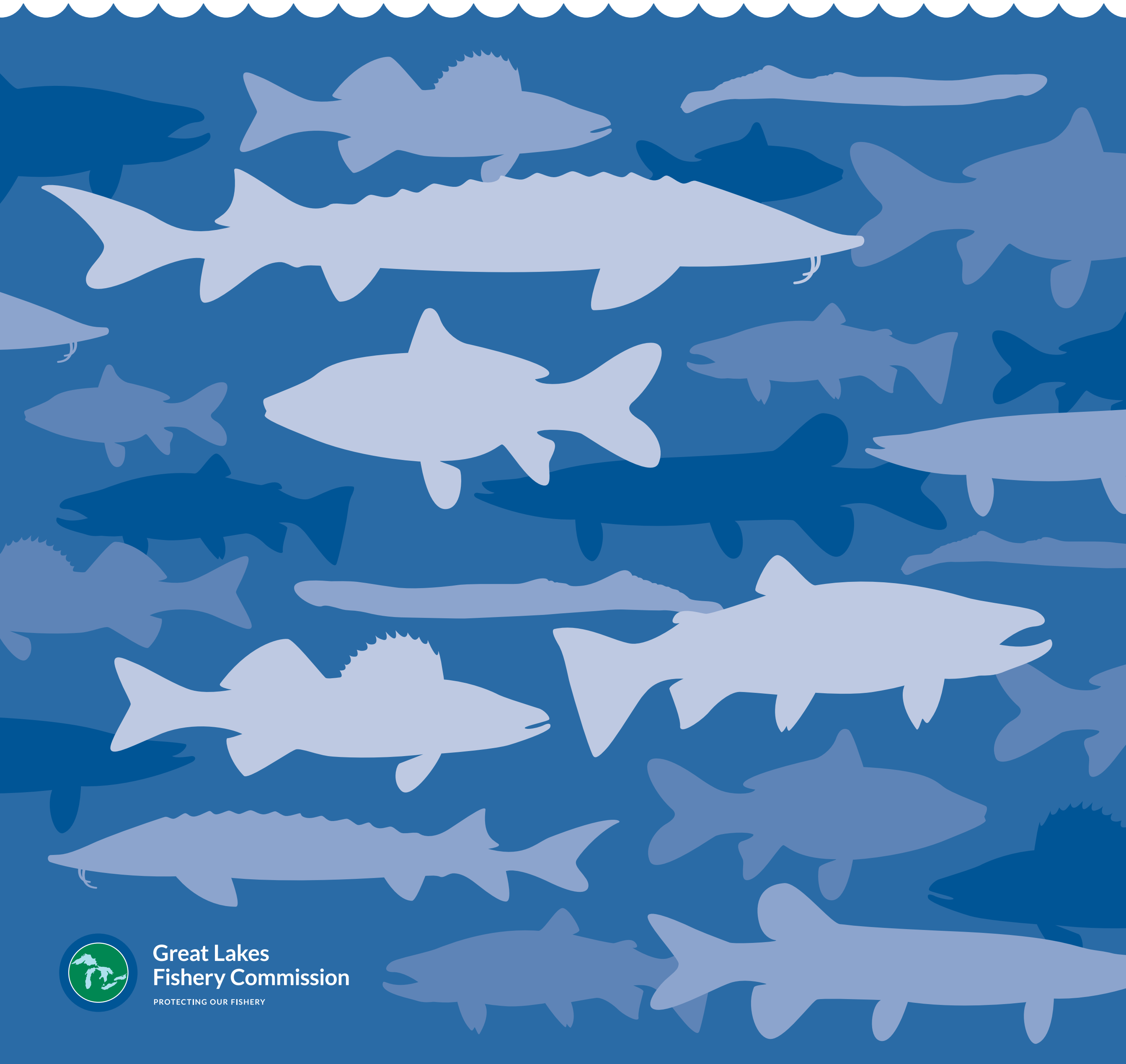

Great Lakes
Fishery Commission
PROTECTING OUR FISHERY

The Great Lakes Fishery Commission (Commission) was established by the Convention on Great Lakes Fisheries between Canada and the United States, which was ratified on October 11, 1955. The Commission was organized in April 1956 and assumed its duties as set forth in the Convention on July 1, 1956 (glfc.org/pubs/conv.pdf). The Commission has two major responsibilities: first, develop coordinated programs of research in the Great Lakes, and, on the basis of the findings, recommend measures that will permit the maximum sustained productivity of stocks of fish of common concern; second, formulate and implement a program to eradicate or minimize Sea Lamprey populations in the Great Lakes. The Commission is also required to publish or authorize the publication of scientific or other information obtained in the performance of its duties.

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Scope

Launched in 2022, *Laurentian* replaces three historically separate, irregularly published Commission journals: *Technical Report, Special Publication, and Miscellaneous Publication*. *Laurentian* will continue to serve as an outlet for publication of interdisciplinary review and synthesis papers; narrowly focused material with special relevance to a single but important aspect of the Commission's mandate under the Convention; and scientific reports from committees that work under the umbrella of the Commission. In addition, relevant papers that do not fit the format of mainstream journals owing, for instance, to length, extensive datasets, or nature of the material and its presentation, will be considered. For further clarification, authors are encouraged to review recent papers published under the three former titles, all available on the Commission's website (www.glfc.org).

Editorial Process

All accepted submissions to *Laurentian* will be citation indexed by ProQuest®. In continuing with this scholarly process, all submissions will be reviewed by external experts, freelance editors, or staff editors as indicated by the nature of the material. Manuscripts should be submitted to the Commission's Managing Editor (randye@glfc.org) to begin the editorial process. The editor may also be consulted in advance of submission, if authors are unsure regarding whether a proposed paper is suitable for *Laurentian*. After a submission is determined to be suitable for *Laurentian*, the Managing Editor will forward it to one or more freelance Technical Editors, who will arrange for peer review, as needed based on subject matter. Reviews by Technical Editors and the Managing Editor may satisfy the requirement for review, or additional reviews may be sought by a freelance editor. The Managing Editor will decide on acceptance and requirements for revision based on recommendations from technical editor(s) and the Managing Editor's own review.

Style

The style guide of the American Fisheries Society (A Guide to AFS Publications Style) has been adopted for *Laurentian* (https://fisheries.org/books-journals/writing-tools/style-guide/).

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Archive of Morphological Data for the *Coregonus artedi* Species Complex of the Great Lakes, Lake Nipigon, and Great Slave Lake

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ABSTRACT

Online Information

https://glfc.org/laurentian2025-01-1.php

This publication is a user guide for an archive of morphological data recorded by various authors from North American ciscoes of the *Coregonus artedi* species complex (subfamily *Coregoninae*). The archive is accessible from the Great Lakes Fishery Commission's (GLFC) server, is open access, and contains data for the Laurentian Great Lakes; Lake Nipigon, Ontario; and Great Slave Lake, Northwest Territories. The archive comprises morphometrics and meristics (together metrics) for 6,700 individual Cisco of which 1,400 are accompanied by images. In addition, the archive contains metrics presented as arrays by W. N. Koelz, Coregonid fishes of the Great Lakes, Bulletin of the U.S. Bureau of Fisheries 43(2):297-643, which were based on 10,000 individuals. Spreadsheets in the Metrics folder of the archive are divided broadly into Contemporary and Historical subfolders and the Contemporary subfolder is further divided into Cisco Monograph and Extra Monograph subfolders to encourage statistical assessment of findings in GLFC Miscellaneous Publication 2023. The Images folder is organized into subfolders by lake. Tables in this user guide allow for quick determination of the availability of data by lake, subspecies, author, and year.

INTRODUCTION

This publication explains how to access archival morphological data for the *Coregonus artedi* species complex collected from the Laurentian Great Lakes; Lake Nipigon, Ontario; and Great Slave Lake, Northwest Territories. Data were recorded historically (1917–1925) by Koelz (1929); contemporaneously (1961–2015) by Nicholas Mandrak (University of Toronto), Andrew Muir (Great Lakes Fishery Commission, GLFC), Chris Olds (USDA Forest Service), Thomas Pratt (Ontario Ministry of Natural Resources and Forestry, OMNRF), Scott Reid (OMNRF), Paul Vecsei (Tlicho Government), and Thomas Todd (U.S. Geological Survey, USGS, retired); and by Thomas Todd from the historical collections of Walter N. Koelz archived at the University of Michigan Museum of Zoology. Here *Coregonus artedi* refers to a species complex encompassing all of the forms (here subspecies) described in Koelz (1929) and several described in Eshenroder et al. (2021a, b; 2023 [revision of 2016 original]). Naming of subspecies is as per Eshenroder and Jacobson (2020), who used Koelz's (1929) specific and subspecific names for historically described forms and common names for those more recently described. This taxonomy differs from that of Page et al. (2023), who assigned species rank to all seven of Koelz's (1929) deepwater species, recognized the previously synonymized *C. nipigon* as a species, and placed all shallow-water forms in *C. artedi*, excepting *C. nipigon*.

Digital images of lateral profiles, where available, are included in the archive and can be cross-referenced by image number to the corresponding morphometric and meristic data (together metrics). Most of the morphological data comprise eight linear measurements and one meristic (gill raker number) used in Eshenroder et al. (2023), i.e., the "Cisco Monograph", but other metrics are provided based on availability and are defined in metadata tabs. Koelz's (1929) data, comprising 9,700 individuals, include summaries in the form of arrays from his Tables 6–11 and his individual "Representative Fish", which were digitized under the supervision of author D. L. Yule. In addition, the archive includes metrics for 650 of Koelz's museum specimens reanalyzed by T. Todd. Contemporary data comprising 2,400 individuals from Eshenroder et al. (2023) have been separated out from other contemporary data to allow for statistical analysis of results presented in the Cisco Monograph. All data are provided as open access spreadsheet files on the GLFC's server. Permission is not required for publication with the proviso that this publication and the contributing author are acknowledged.

ARCHIVAL STRUCTURE

The structure of the archive is shown as a tree diagram (Figure 1) with boxes representing folders/subfolders containing data. Metrics and images are the two major folders in the tree. The roots of the tree (subfolders) are individual authors who provided the data, although morphological spreadsheets may contain data from unnamed multiple authors. Hence, metrics for more than one lake and author are stored in the same subfolder. Subfolders under Metrics go first to the Contemporary and Historical subfolders. The Historical subfolder branches to the W. Koelz and T. Todd subfolders. Subfolders immediately under Contemporary go to the Cisco Monograph and Extra Monograph subfolders. The Extra Monograph subfolder contains collections made available to the GLFC that were not analyzed in the Cisco Monograph.

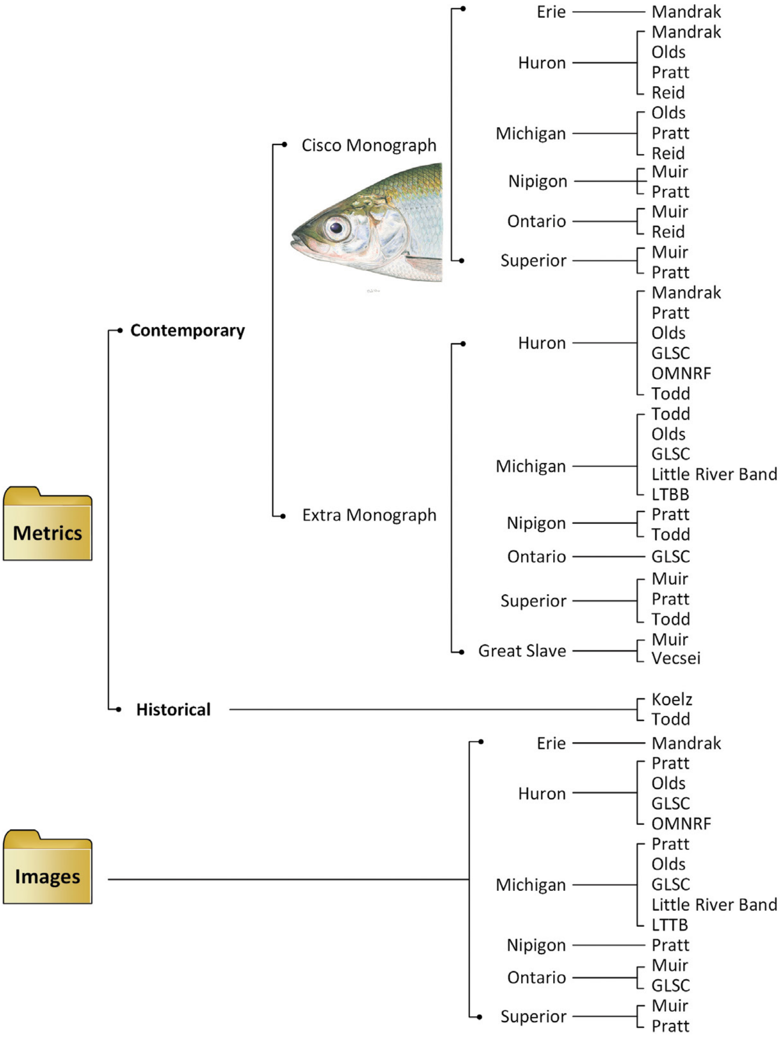

FIGURE 1. Tree diagram showing the hierarchical structure of the Archive of Cisco Metrics and Images (see text for relationships). GLSC = Great Lakes Science Center; OMNRF = Ontario Ministry of Natural Resources and Forestry; Little River Band = Little River Band of Ottawa Indians; LTBB = Little Traverse Bay Bands of Odawa Indians.

An inventory of the metrics available in spreadsheets is provided in the Appendix, Tables A1-A6:

- Table A1, Contemporary Cisco Monograph data
- Table A2, Contemporary Extra Monograph data
- Table A3, Historical data compiled by T. Todd from museum collections
- Table A4, W. Koelz's (1929) metrics as displayed in arrays and as representative fish
- Table A5, Number of monograph samples with images by lake, subspecies, author, and year
- Table A6, Number of extra monograph samples with images by lake, subspecies, author, and year

Using these tables, users can determine if the data they seek are available without having to search spreadsheets. They allow for a quick appraisal of what types of comparisons are possible among these metrics or among other datasets. To access metrics and images go to (https://glfc.box.com/s/dljglh1tzy0gfmjptsq0629kench06ll), which will take you to the archive.

Notes

The form (taxonomic) assignments made by authors are retained. Assignments differ among datasets and may range from visual identification to statistically supported assignments based on metrics. We strongly recommend that where users combine datasets, they apply their own standardized quantitative approach to assigning taxonomy based on the objectives of the study. Predicted assignments, however, have been made for Lake Huron ciscoes collected by N. Mandrak, T. Pratt, and S. Reid. The assignments made by these authors preceded Eshenroder et al. (2023), who hypothesized that five historical subspecies of deepwater Cisco had introgressed into a hybrid swarm, which they named hybrida. Eshenroder et al. (2021a, b), however, determined that all of the shallow-water Cisco likely encountered in Lake Huron by these authors would have been various types of shorthead cisco, as the more-fusiform, historically dominant shallow-water cisco (typical *artedi*) had been extirpated. Therefore, using the taxonomy of Eshenroder et al. (2021a, b), these three authors were likely to have collected only two subspecies of Cisco in Lake Huron—deepwater hybrida and shallow-water shorthead cisco.

We used a linear discriminant analysis (LDA; Johnson and Wichern 1998) to assign the specimens collected from Lake Huron by N. Mandrak (2005-2006), T. Pratt (2005-2007), and S. Reid (2012) into either of two groups: shorthead cisco or hybrida. The LDA model was fitted to data collected in Lake Huron between 2015 and 2019, including 348 hybrida and 580 shorthead cisco that were classified based on morphology. These data were collected by multiple agencies and are maintained in a GLFC database maintained at the Great Lakes Science Center. As several linear measurements (e.g., body depth and maxillary length) were not available for the majority of the collections made by these three authors, only four predictor variables were included in the LDA model. The predictor variables were standard length, standard length to head length ratio, head length to snout length ratio, and total gill rakers. This set comprises a measure of size, two measures of shape, and a meristic all of which have been used by field biologists to distinguish hybrida and shorthead cisco (Eshenroder et al. 2023; Martin et al. 2023). Data for these four variables were available for 97% of the specimens collected by the three authors. The LDA model fitting and prediction were carried out in R (R Core Team 2021) and the package "MASS" (Venables and Ripley 2002).

Referring to the Contemporary subfolder, Cisco Monograph and Extra Monograph spreadsheets, the LDA (predicted) assignments for Lake Huron are in Column Z in the Cisco Monograph and Extra Monograph spreadsheets. Note that predicted assignments could not be made for about 3% of the specimens due to missing data, and these are listed as “None.” Because T. Pratt’s collection was accompanied by images, a priori (visual) assignments are provided in Column Y for his Cisco Monograph spreadsheet and in Column AB for his Extra Monograph spreadsheet. Out of 125 individuals with a priori assignments in the Cisco Monograph spreadsheet, 10 individuals classified as shorthead cisco in the LDA (column Z) were visually (a priori) determined to be hybrida, and 7 individuals classified as shorthead cisco in the LDA were visually determined to be shorthead cisco. Out of 981 individuals with a priori assignments in the Cisco Monograph spreadsheet, 4 individuals classified as shorthead cisco in the LDA were visually classified as hybrida, and 1 individual classified as a shorthead cisco in the LDA was visually classified as Lake Whitefish *C. clupeaformis* x Cisco. In addition, 2 individuals classified a priori as Lake Whitefish x Cisco were classified in the LDA as shorthead cisco, and 1 individual classified a priori as a Lake Whitefish was classified in the LDA as shorthead cisco. The Extra Monograph spreadsheet LDA had no data for hybrids or Lake Whitefish so these discrepancies are not remarkable and are provided for completeness. The Extra Monograph spreadsheet LDA assignments of shorthead cisco appear to be 100% correct whereas the LDA assignments of hybrida had an error rate of 9%.

As demonstrated above in our comparison of LDA and a priori assignments, lateral profile images allow for a level of quality control, including reassignment, not otherwise possible. Moreover, we encourage field biologists to learn how to make a priori assignments just as they would with fishes that look less alike. Not all samples are statistically processed each year and those that are may be several years old. Recognition of unexpected morphologies in the field also allows for resampling while individuals are more likely to remain available. We note that most of A. Muir’s and T. Pratt’s contemporary samples (Appendix Tables A5 and A6) are cross-referenced to images. As depicted in the Cisco Monograph, the photographic setup used by T. Pratt differs from that used by A. Muir, which can affect comparison of post facto measurements. Images, too, can be helpful in dealing with outliers in morphometric data (Eshenroder et al. 2021a). As a matter of routine, any statistical comparisons of morphology should be preceded by an outlier analysis to minimize measurement error. The data presented here have not been subjected to quality control. We recommend that users undertake outlier identification and treatment before employing these data in an analysis. We assume that elimination of outlier individuals will tend to eliminate misclassifications. See Eshenroder et al. (2021a) for a method of identifying and treating outliers.

Other Data

Stanford Smith’s morphological data on Cisco tabulated in Eshenroder et al. (2023) have been digitized and published https://www.sciencebase.gov/catalog/item/5f5a314182cefd9f20863b48 (Pollens-Dempsey et al. 2021). This publication provides data for an important middle period between Koelz (1929) and the contemporary collections in this archive. Likewise, morphological data used in recently completed and ongoing collaborative studies of Cisco involving the GLFC, but not included in Eshenroder et al. 2023, have been compiled into a database maintained at the Great Lakes Science Center. As of April 23, 2024, it comprised 2,283 individuals. Use is by request; contact research@glfc.org.

APPENDIX: INVENTORY OF DATA AVAILABLE IN THE ARCHIVE OF CISCO METRICS AND IMAGES

TABLE A1. Contemporary Cisco monograph subfolder: number (*N*) of contemporary samples by lake, subspecies, author (this publication), and year. See Tabular Data in Eshenroder et al. (2023) for additional information.

Lake	Subspecies	Author(s)	Year(s)	*N*
Erie	hybrida	Mandrak	2003–2015	19
Huron	*artedi*-like	Mandrak	2005–2006	10
Huron	*hoyi*-like	Mandrak	2005–2006	418
Huron	*zenithicus*-like	Mandrak	2005–2006	10
Huron	*artedi*-like	Pratt	2006–2007	60
Huron	*hoyi*-like	Pratt	2005–2007	58
Huron	*zenithicus*-like	Pratt	2005–2007	8
Huron	*artedi*-like	Reid	2012	74
Huron	*hoyi*-like	Reid	2012	109
Huron	*zenithicus*-like	Reid	2012	17
Huron	*albus*-like	Olds	2015	24
Michigan	*hoyi*	Pratt	2008	47
Michigan	*hoyi*	Reid	2011	113
Michigan	*albus*-like	Olds	2015	25
Nipigon	*artedi*	Muir	2013	4
Nipigon	*nigripinnis*	Muir	2013	16
Nipigon	*zenithicus*	Muir	2013	9
Nipigon	unknown	Muir	2013	2
Nipigon	*artedi*	Pratt	2008	68

TABLE A1. Continued.

Lake	Subspecies	Author(s)	Year(s)	*N*
Nipigon	*hoyi*	Pratt	2008	124
Nipigon	*nigripinnis*	Pratt	2008	191
Nipigon	*zenithicus*	Pratt	2008	57
Ontario	*artedi*	Reid	2011	106
Ontario	*artedi*	Muir	2013	41
Superior	*artedi*	Pratt	2004–2008	199
Superior	*hoyi*	Pratt	2004–2008	179
Superior	*kiyi*	Pratt	2004–2008	61
Superior	*zenithicus*	Pratt	2004–2008	167
Superior	*artedi*	Muir	2009–2010	45
Superior	*hoyi*	Muir	2009–2010	81
Superior	*kiyi*	Muir	2009–2010	30
Superior	*zenithicus*	Muir	2009–2010	22

TABLE A2. Contemporary extra monograph subfolder: number (*N*) of contemporary samples by lake, subspecies, author (this publication), and year. OMNRF = Ontario Ministry of Natural Resources and Forestry; USGS = U.S. Geological Survey; LRB = Little River Band of Ottawa Indians; LTBB = Little Traverse Bay Bands of Odawa Indians.

Lake	Subspecies	Author(s)	Year(s)	*N*
Huron	Bloater X Lake herring	Mandrak	2005–2006	10
Huron	Unknown	Mandrak	2005–2006	261
Huron	*hoyi*	Olds	2015	49
Huron	Bloater X Lake herring	Pratt	2006–2007	25
Huron	Bloater X Shortjaw	Pratt	2005–2006	23
Huron	*Kiyi* X Lake herring	Pratt	2007	2
Huron	Lake herring X Shortjaw	Pratt	2005–2007	7
Huron	*artedi*	Olds	2015–2018	407
Huron	Unknown	Olds	2015	2
Huron	*artedi*	GLSC	2016–2018	58
Huron	*hoyi*	GLSC	2016	349
Huron	Unknown	GLSC	2017	26
Huron	*artedi*	OMNRF	2017–2018	150
Huron	*artedi*	Todd	1974	5
Huron	*artedi*	Todd	1995	20
Huron	*hoyi*	Todd	1974–1979	4
Huron	*zenithicus*	Todd	1979	1
Michigan	*artedi*	Olds	2017–2018	12
Michigan	*hoyi*	Olds	2016–2017	25
Michigan	*hoyi*	GLSC	2018	20
Michigan	*artedi*	Olds	2017–2018	43

TABLE A2. Continued.

Lake	Subspecies	Author(s)	Year(s)	*N*
Michigan	*artedi*	Olds	2018	22
Michigan	*alpenae*	Todd	1961	1
Michigan	*hoyi*	Todd	1973–1974	173
Michigan	*reighardi*	Todd	1972	2
Michigan	*artedi*	LTBB	2018	22
Michigan	*artedi*	LRB	2018	23
Nipigon	Blackfin X Bloater	Pratt	2008	6
Nipigon	Blackfin X Lake herring	Pratt	2008	4
Nipigon	Blackfin X Shortjaw	Pratt	2008	7
Nipigon	Bloater X Lake herring	Pratt	2008	3
Nipigon	Bloater X Shortjaw	Pratt	2008	8
Nipigon	Lake herring X Shortjaw	Pratt	2008	8
Nipigon	*artedi*	Todd	1973	9
Nipigon	*hoyi*	Todd	1973	7
Nipigon	*nigripinnis*	Todd	1973	9
Nipigon	*reighardi*	Todd	1973	7
Nipigon	*zenithicus*	Todd	1973	6
Ontario	*artedi*	GLSC	2018	24
Superior	*reighardi*	Pratt	2006	8
Superior	Bloater X *Kiyi*	Pratt	2006–2007	5
Superior	Bloater X Lake herring	Pratt	2006–2007	8
Superior	Bloater X Shortjaw	Pratt	2006–2007	15
Superior	*Kiyi* X Lake herring	Pratt	2006–2008	14

TABLE A2. Continued.

Lake	Subspecies	Author(s)	Year(s)	*N*
Superior	*Kiyi* X Shortjaw	Pratt	2006	6
Superior	Lake herring X Shortjaw	Pratt	2006–2008	7
Superior	Unknown	Pratt	2006	1
Superior	Bloater X Lake herring	Muir	2009	9
Superior	Bloater X Shortjaw	Muir	2009	3
Superior	*artedi*	Todd	1973–1974	114
Superior	*artedi-zenithicus*	Todd	1974	1
Superior	*hoyi*	Todd	1973–1974	77
Superior	*hoyi-artedi*	Todd	1974	1
Superior	*hoyi-zenithicus*	Todd	1974	1
Superior	*kiyi*	Todd	1973–1974	70
Superior	*kiyi-zenithicus*	Todd	1974	1
Superior	*reighardi*	Todd	1973	24
Superior	*zenithicus*	Todd	1973–1974	257
Superior	*zenithicus-hoyi*	Todd	1974	1
Great Slave	*artedi*-lacustrine	Muir	2008	8
Great Slave	*zenithicus*	Muir	2008	10
Great Slave	*artedi*-lacustrine	Muir	2008–2009	171
Great Slave	*artedi*-riverine	Muir	2008	150
Great Slave	*zenithicus*	Muir	2008–2009	69
Great Slave	*artedi*-lacustrine	Vecsei	2008	53
Great Slave	*artedi*-riverine	Vecsei	2008	82
Great Slave	zenithicus	Vecsei	2008	53

TABLE A3. Historical subfolder compiled by T. Todd from museum collections (number (*N*) of historical samples by lake, subspecies, and years).

Lake	Subspecies	Year(s)	*N*
Erie	*artedi*	1920–1929	5
Huron	*alpenae*	1917–1919	16
Huron	*artedi*	1919	6
Huron	*hoyi*	1919–1923	20
Huron	*johannae*	1917–1923	27
Huron	*kiyi*	1917–1923	14
Huron	*reighardi*	1919	1
Huron	*zenithicus*	1917–1919	30
Huron	*zenithicus*	1931	1
Michigan	*alpenae*	1920–1924	34
Michigan	*artedi*	1921	5
Michigan	*hoyi*	1921	2
Michigan	*johannae*	1906	8
Michigan	*johannae*	1920	10
Michigan	*nigripinnis*	1920	3
Michigan	*reighardi*	1920–1923	24
Michigan	*zenithicus*	1920–1924	30
Nipigon	*artedi*	1922–1923	9
Nipigon	*hoyi*	1922	26
Nipigon	*nipigon*	1922	7
Nipigon	*reighardi dymondi*	1922	32
Nipigon	*zenithicus*	1922	22

TABLE A3. Continued.

Lake	Subspecies	Year(s)	*N*
Ontario	*artedi*	1921	5
Ontario	*hoyi*	1923	10
Ontario	*kiyi*	1921	7
Ontario	*reighardi*	1921	20
Superior	*artedi*	1923	8
Superior	*hoyi*	1921–1922	13
Superior	*kiyi*	1921–1922	21
Superior	*nigripinnis*	1921	2
Superior	*nigripinnis cyanopterus*	1917	26
Superior	*reighardi dymondi*	1921–1923	57
Superior	*zenithicus*	1921–1923	196

TABLE A4. W. Koelz’s (1929) historical subfolder (displayed in arrays and as representative fish); number (*N*) of samples by lake, subspecies, and years.

Lake	Subspecies	Year(s)	*N*
Erie	*artedi*	1920–1924	750
Huron	*johannae*	1917–1923	441
Huron	*alpenae*	1917–1925	387
Huron	*zenithicus*	1917–1923	162
Huron	*nigripinnis*	1917–1923	130
Huron	*kiyi*	1917–1923	212
Huron	*hoyi*	1917–1923	873
Huron	*artedi*	1917–1924	340
Michigan	*johannae*	1919–1923	122
Michigan	*alpenae*	1919–1924	383
Michigan	*zenithicus*	1919–1923	140
Michigan	*reighardi*	1919–1924	406
Michigan	*nigripinnis*	1919–1923	53
Michigan	*kiyi*	1919–1923	212
Michigan	*hoyi*	1919–1923	1,149
Michigan	*artedi*	1919–1923	391
Nipigon	*zenithicus*	1922	160
Nipigon	*reighardi*	1922	97
Nipigon	*nigripinnis*	1922	230
Nipigon	*hoyi*	1922	174
Nipigon	*artedi*	1922	82
Nipigon	*nipigon*	1922	43

TABLE A4. Continued.

Lake	Subspecies	Year(s)	*N*
Ontario	*reighardi*	1921–1923	76
Ontario	*kiyi*	1921–1923	132
Ontario	*hoyi*	1917–1923	255
Ontario	*artedi*	1917–1923	266
Superior	*zenithicus*	1917–1925	956
Superior	*reighardi*	1921–1923	234
Superior	*nigripinnis*	1917–1923	162
Superior	*kiyi*	1917–1925	79
Superior	*hoyi*	1921–1923	333
Superior	*artedi*	1917–1925	254

TABLE A5. Number (*N*) of monograph samples with images by lake, subspecies, author (this publication), and year (may include multiple images of same fish and images of anatomical features).

Lake	Subspecies	Author(s)	Year(s)	*N*
Erie	swarm cisco	Mandrak	2003–2015	19
Huron	*artedi*	Olds	2015	93
Michigan	*hoyi*	Pratt	2008	82
Nipigon	*artedi*	Pratt	2008	67[13]
Nipigon	*hoyi*	Pratt	2008	124[1]
Nipigon	*nigripinnis*	Pratt	2008	188[1]
Nipigon	*zenithicus*	Pratt	2008	56[1]
Ontario	*artedi*	Muir	2013	41
Superior	*artedi*	Pratt	2004–2008	190[14]
Superior	*hoyi*	Pratt	2004–2008	177[2]
Superior	*kiyi*	Pratt	2004–2008	60[2]
Superior	*zenithicus*	Pratt	2004–2008	164[2]
Superior	*artedi*	Muir	2009–2010	45[15]
Superior	*hoyi*	Muir	2009–2010	71[3]
Superior	*kiyi*	Muir	2009–2010	29[3]
Superior	*zenithicus*	Muir	2009–2010	19[3]

[13] Individual fish, all images combined = 1,406.

[14] Individual fish, all images combined = 1,255.

[15] Individual fish, all images combined = 342.

TABLE A6. Number (*N*) of extra monograph samples with images by lake, subspecies, author (this publication), and year (may include multiple images of same fish and images of anatomical features); GLSC = Great Lakes Science Center; OMNRF = Ontario Ministry of Natural Resources and Forestry; LRB = Little River Band of Ottawa Indians; LTBB = Little Traverse Bay Bands of Odawa Indians.

Lake	Subspecies	Author(s)	Year(s)	*N*
Huron	Hybrida	Pratt	2005-2006	199
Huron	Hybrida	Pratt	2007	338
Huron	Bloater X Lake herring	Pratt	2006–2007	25
Huron	Bloater X Shortjaw	Pratt	2005–2006	21
Huron	*Kiyi* X Lake herring	Pratt	2007	2
Huron	Lake herring X Shortjaw	Pratt	2005–2007	7
Huron	*artedi*	Olds	2016	811
Huron	*artedi*	Olds	2018	144
Huron	*artedi*	GLSC	2016	21
Huron	*artedi*	GLSC	2017	32
Huron	*artedi* (21), hybrida (5)	GLSC	2018	26
Huron	*artedi*	OMNRF	2017	144
Huron	*artedi*	OMNRF	2018	219
Michigan	*artedi* (14), hybrida (12)	Olds	2017–2018	26
Michigan	*hoyi*	GLSC	2018	20
Michigan	*hoyi*	GLSC	2018	43
Michigan	*hoyi*	Pratt	2008	141
Michigan	*artedi*	LRB	2017–2018	43
Michigan	*artedi*	LTBB	2018	51
Nipigon	Blackfin X Bloater	Pratt	2008	6
Nipigon	Blackfin X Lake herring	Pratt	2008	3
Nipigon	Blackfin X Shortjaw	Pratt	2008	7

TABLE A6. Continued.

Lake	Subspecies	Author(s)	Year(s)	*N*
Nipigon	Bloater X Lake herring	Pratt	2008	3
Nipigon	Bloater X Shortjaw	Pratt	2008	7
Nipigon	Lake herring X Shortjaw	Pratt	2008	8
Ontario	*artedi*	GLSC	2018	24
Superior	*reighardi*	Pratt	2006	8
Superior	Bloater X *Kiyi*	Pratt	2006–2007	5
Superior	Bloater X Lake herring	Pratt	2006–2007	8
Superior	Bloater X Shortjaw	Pratt	2006–2007	14
Superior	*Kiyi* X Lake herring	Pratt	2006–2008	14
Superior	*Kiyi* X Shortjaw	Pratt	2006	5
Superior	Lake herring X Shortjaw	Pratt	2006–2008	7
Superior	Unknown	Pratt	2006	1
Superior	Bloater X Lake herring	Muir	2009	9
Superior	Bloater X Shortjaw	Muir	2009	3

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Fish-Assemblage Evaluation in the Lower Sandusky River, Ohio, Following Dam Removal

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ABSTRACT

Online Information

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The Sandusky River, Ohio, USA, has experienced more than a century of alterations, including dam implementation and removal, causing a cascade of habitat changes. The physical changes in the river led to establishment of several invasive species. Ten hoop-net sampling sites, spaced about 500 m apart were established in the river to monitor fish assemblage and their habitat preferences. Four 10-d sampling events were completed from April through October 2021. Ordination analyses were used to assess fish-assemblage structure seasonably, species-habitat relationships, and life-history strategies of 31 species. Generalized linear mixed-effects models were used to assess temporal factors that may drive diversity and community assemblage. Models indicated increased species richness after removal of the dam. Presence and proportion of catch data were compared to Ohio Environmental Protection Agency 2009 pre-dam-removal data to further assess changes in fish assemblage. Several species, especially catostomids, have begun to use the habitat downstream of the former dam, altering fish assemblage throughout the river. We expect shifts in assemblage structure to persist, making continued monitoring essential for understanding how non-native and recreationally important species continue to respond to dam removal.

INTRODUCTION

Tributary condition and functionality are vital to the success of resident species in the Laurentian Great Lakes. The Sandusky River, a tributary of Lake Erie, has functioned historically as a nursery (Becher and Gottgen 2012) and spawning ground for native fish, including White Bass *Morone chrysops* (Hayden et al. 2011) and Walleye *Sander vitreus* (Trautman 1981; DuFour et al. 2015; Zimmerman and Rice 2019; Myers et al. 2024). Creel records from the Sandusky River suggest the Walleye population has declined in recent years, which is hypothesized to be from a lack of quality spawning habitat in the river (Cheng et al. 2006). Cumulative impacts of dam construction and habitat degradation are also likely culprits for decreased sportfish abundance throughout the Great Lakes (Fielder et al. 2007).

Depending on a dam's function and location along a river course, a dam can have large effects on available habitat and flow regime (Ward and Stanford 1983). In addition to altering abiotic components of a river, dams can fragment river connectivity, block fish migrations, and limit spawning habitat, which can alter the composition of native fish communities (Catalano et al. 2007; Acre et al. 2021). Historically, four dams were placed across the Sandusky River. The largest of the dams, Ballville Dam, was located 29 river kilometers (rkm) upstream of the Lake Erie confluence (Figure 1); it was built in 1911 to provide hydroelectric power to the city of Fremont, Ohio (USFWS 2016). Ballville Dam's presence on the Sandusky River resulted in an increase in sedimentation, causing a shift

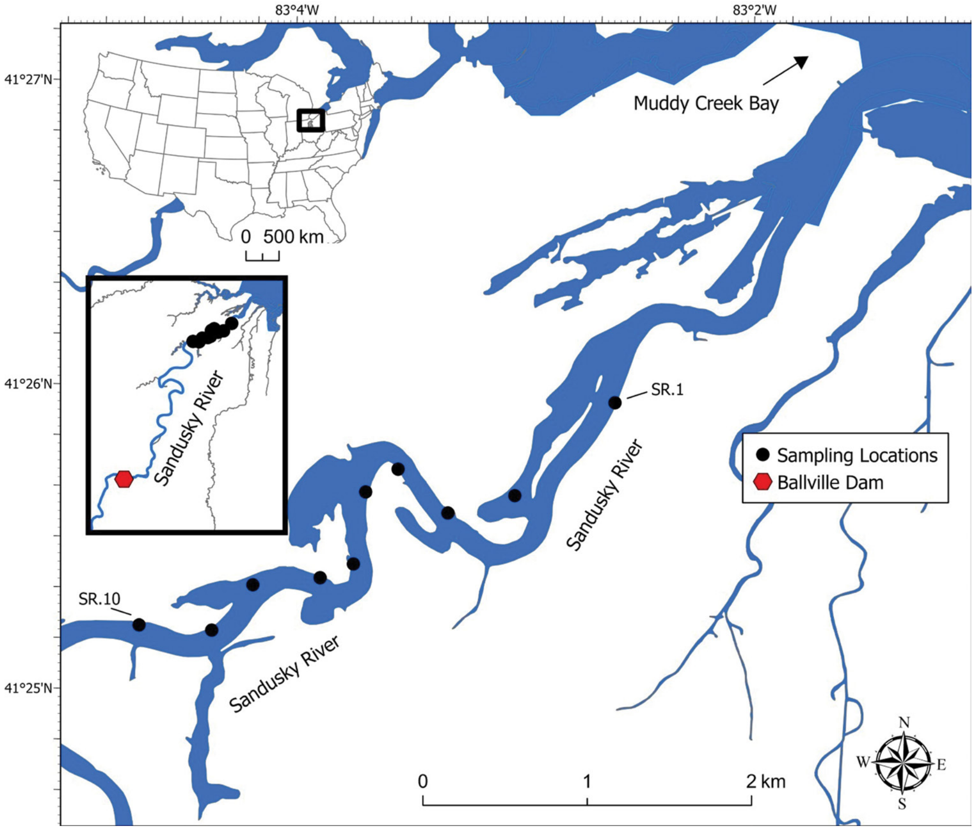

FIGURE 1. Location of hoop-net sampling sites in the Sandusky River, Ohio, in 2021. Sites were sequentially numbered from SR.1 to SR.10. At SR. 1, SR. 3–SR. 6, and SR. 8–SR. 10, a hoop net was deployed on the upstream and downstream sides of a platform depicted together with dots, including in the legend. At control sites (SR. 2 and SR. 7), a platform was not present, and 2 hoop nets were placed on either side of a buoy.

from gravel to sand substrates in portions of the river (Sanderson 2009). Alterations in streamflow and sedimentation create ideal conditions for invasions by non-native species (Murphy et al. 2007) and often negatively affect native fish diversity and species richness (Sanderson 2009). For example, Ballville Dam restricted Walleye upstream passage, confining spawners downstream to one tenth of the suitable spawning habitat (Cheng et al. 2006).

Ballville Dam removal began in 2016 and was completed in 2018, opening 36 rkm of the upper Sandusky River for fish passage from Lake Erie to historically available habitat (Cheng et al. 2006; Gillenwater et al. 2006; USFWS 2016). The dam removal was hypothesized to spatially shift the availability of previously known spawning habitats, benefiting Lake Erie fish populations over time (Myers et al. 2024). A study by Sasak (2021) concluded removal of the Ballville Dam increased the amount of Walleye and White Bass preferred habitat by 21.9 hectares. Two years after dam removal, Walleye was found upstream of the old dam site; however, catch per unit effort of White Bass and Walleye was lower after dam removal than before (Sasak 2021). Further, removal of the dam increased river flow and exposed large amounts of sediment that likely increased spawning habitat for invasive species (Bellmore et al. 2017).

Since the 1950s, multiple invasive species have expanded their range into the Lake Erie basin and use the Sandusky River as spawning and feeding grounds. White Perch *Morone americana* was first reported in Lake Erie in 1953 (Larsen 1954) and has been collected consistently throughout the Sandusky River for decades (Schaeffer and Margraf 1987). Several invasive cyprinids and xenocyprinida are also present in the river, most notably a population of Grass Carp *Ctenopharyngodon idella* (DuFour et al. 2021). It was believed that no self-sustaining populations of Grass Carp had been found in a Lake Erie tributary (Kocovsky et al. 2012) until Embke et al. (2016) discovered evidence of its reproduction in the Sandusky River.

A self-sustaining population of Grass Carp in the Laurentian Great Lakes could reduce aquatic macrophytes, resulting in an ecological cascade of potentially deleterious effects (Cudmore et al. 2017; Gertzan et al. 2017). The primary effect of a decrease in macrophytes is changed water quality and deterioration of critical spawning and nursery habitat, causing a reduction in native fish recruitment (Chapman et al. 2013; Wilson et al. 2014). Reduced recruitment along with egg predation by White Perch could reduce the recruitment of native sport fish to Lake Erie (Schaeffer and Margraf 1987; Fielder 2002). Therefore, detection and management of non-native species in the Lake Erie basin are necessary to mitigate alteration of native fish communities.

The proximity of our sampling locations to Lake Erie allowed us to observe fish movements into the Sandusky River and back into the lake. Most migratory species, such as Walleye, White Bass, and White Perch, move into the river in early April or when temperatures are typically between 15 and 18°C (Schaeffer and Margraf 1987). However, it is largely unknown how the introduction of other invasive species combined with the removal of the Ballville Dam have impacted fish migration.

The objectives of this study were to establish baseline fish-assemblage diversity metrics and distinguish how invasive species affect them; determine fish-assemblage shifts on a pre/post-dam-removal temporal scale; and compare presence of native and non-native species in relation to habitat characteristics. We hypothesized that transient species would increase diversity downstream near Lake Erie, but that increased diversity would be limited by the presence of invasive species. We further hypothesized an increase in species richness, influenced by non-native species following dam removal. The results of this study will serve as a reference for future fish-assemblage research in the Sandusky River and Lake Erie basin.

METHODS

Study Area

The Sandusky River is 207 rkm long with a drainage of approximately 4,700 kilometers^2 located in northwest Ohio, USA (Harris et al. 2021). The river flows through Muddy Creek Bay and Sandusky Bay before entering the western basin of Lake Erie (Figure 1). The lower Sandusky River watershed had good overall habitat quality. Historically, free-flowing portions of the river were predominated by limestone bedrock and gravel substrates, but most of the lotic sites over time were covered by fine sand and silt (OEPA 2011). Prior to 2018, the reach of the Sandusky River impounded by the Ballville Dam had bottom substrates predominated by muck and silt (OEPA 2011). Sandusky Bay is negatively influenced by the surrounding agricultural landscape and poor sewage treatment (OEPA 2010).

Study Design

Our study took place in the Sandusky River near its confluence with Muddy Creek Bay. Ten fixed sampling locations were distributed throughout the study area at a minimum of 500 meters (m) apart (Figure 1). Eight sites were marked with a platform (deployed as part of another project) to standardize hoop-net placement, and two sites were marked with a buoy to assess any bias the platform may have introduced to our fish catches. Four trials, each 10 days (d) in duration, were completed from April through October 2021.

Fish Collection

Two hoop nets were set daily at each of the ten sites. One hoop net was set immediately upstream of each platform or buoy, and one was set directly downstream, approximately 25 m apart. Landmarks were used to standardize the distance from the platform for each set. Each hoop net was 3.7 m long with an initial hoop diameter of 0.9 m and 3.8-cm square-mesh netting. A 3.1-m wing was attached to each side of a hoop net with a 7.6-m lead anchoring the net to the river bottom, like a traditional fyke net. The wings were set at an approximately 60° angle from the lead. Each net contained seven hoops and two throats attached to the second and fourth hoops. Nets were set perpendicular to the river flow, opening toward the shoreline. When appropriate, hoop nets were also secured with a safety rope connected to a tree on the bank. Nets were fished for approximately 24 hours (h). Catch rates for each site were defined as the number of fish caught in the two hoop nets per 24-h sampling period. Fish were identified to species, their total length was measured to the nearest millimeter, and they were then released.

Predictor Variables

Water-quality information was collected near the same time of day when hoop nets were monitored during each 10-d sampling period. A YSI Exo2 multiparameter sonde (Xylem®; Yellow Springs, Ohio) was used to measure dissolved oxygen (mg/L), specific conductivity (µS/cm), turbidity (FNU), pH, and chlorophyll (µg/L). Daily minimum and maximum depths (m) and water temperature (°C) were calculated from data recorded using an Onset HOBO® Water Level Data Logger (Onset Computer®; Borne, Massachusetts) that were attached to two of the floating platforms approximately 0.5 m above the bottom substrate, and recorded data at 15-minute intervals throughout each 10-d trial. Water temperature and mean depth were used as measures of the change in river depth, a proxy for river discharge, throughout the study and were linked to fish-capture data at the date and time hoop nets were lifted. The distance of each site from the confluence of Muddy Creek Bay (m) was estimated from the midpoint of a site using ArcGIS® Pro 2.7.1 (Esri®; Redlands, California).

Physical-habitat data were recorded at the start of each of the four 10-d trials. Vegetation coverage was characterized by type of vegetation (submerged, emerged, or floating) and genus (Phragmites (*Phragmites* spp.), Duckweed (*Lemna* spp.), Lotus (*Nelumbo* spp.)). Large wood debris (trees, logs, branches, roots) and river-bottom substrate (silt, sand, clay, rock) were also categorized at each site. Physical habitat was defined and recorded as the percentage of area within 50 m of a platform having a habitat feature present. Physical-habitat features were considered variable, and coverage was estimated at time of data collection to account for temporal changes.

Data Analysis

Differences in fish assemblages among trials were visualized using nonmetric multidimensional scaling (NMDS; *vegan* package version 2.5.7; R Program© version 4.1.2; Oksanen et al. 2019). First, Bray-Curtis similarity coefficients were calculated based on joint occurrence and catch of fish species. Because our objective was to determine temporal shifts in fish assemblages, data were averaged per trial. These data were $\log(x + 1)$ transformed prior to calculating the similarity coefficient to reduce the weight of the dominant species. An analysis of similarity (ANOSIM) determined the statistical differences among trial groups (function 'anosim'; *vegan* package version 2.5.7; R Program© version 4.1.2). A similarity of percentage analysis (SIMPER) was then used to identify the fish species that drove dissimilarities among groups in trials, which helped to quantify how each species contributed to the assemblage differences among trial groups (function 'simper', *vegan* package version 2.5.7; R Program© version 4.1.2). Values from the analysis were generated per species. A significant P-value suggested a contribution of species to the dissimilarity of fish assemblage among trials.

Species-habitat and site-habitat associations were identified with a canonical correspondence analysis (CCA). The log-transformed fish abundance for all species (n = 31) was compared to the habitat data set across the ten sites (*vegan* package version 2.5.7; R Program© version 4.1.2). The habitat data set included environmental variables, physical habitat, and mean temperature and water depth. For visualization purposes, habitat variables with CCA1 or CCA2 absolute eigenvalues of 1.5 or less were removed from the plot (n = 11). A permutational analysis of variance (PERMANOVA) test (function anova.cca; *vegan* package version 2.5.7; R Program© version 4.1.2) verified the significance of the model. A Shannon-Wiener Diversity index (H') was calculated for fish capture data per day. A random forest analysis (Breiman 2001) was used to evaluate the relative influence of the predictor variables mentioned above, with site and trial as factor variables on H' (function randomForest; package randomForest version 4.6.14; R Program© version 4.1.2; Liaw and Wiener 2002).

Pearson correlation coefficients were calculated for the predictor variables (n = 29), and highly correlated (≥0.7; Härdle and Simar 2019) or confounded variables were removed to avoid false-positive influence (function cor; package stats version 4.1.2; R Program© version 4.1.2). RandomForest functions build a selected number of regression trees (m) from a bootstrap sample of the original data set to allow for non-linear relationships between predictors and a response variable without making any parametric assumptions about the distribution of the response variable (Breiman 2001). To stabilize the mean squared error (MSE), we used 500 trees that diagnostics showed were adequate (function tuneRF; package randomForest version 4.7-1.1; R Program© version 4.1.2). For each regression tree, a set of predictors (*mtry*) was randomly selected from the original predictors at a given node. Using function tuneRF from the randomForest package, the optimal value (with respect to out-of-bag error estimate) of *mtry* was found to be seven.

The percentage increase in the MSE (%IncMSE), when variables were randomly permuted, and the total decrease in node “impurity” from splitting on a given descriptor (IncNodePurity) were averaged over all generated trees to show the importance of variables in the randomForest analysis. The importance score reflects the loss of prediction accuracy associated with omitting, in turn, each predictor variable. We present the top ten variables through partial dependence plots (PDPs) that demonstrate the marginal effect of a selected variable on the response variable, in the order of importance, or greatest MSE. This provides insight to the directionality of the effect for a given predictor (function partial; package pdp version 0.7.0; R Program© version 4.1.2; Greenwell et al. 2018).

The fitdistrplus function (package MASS version 7.3-58.1; R Program© version 4.1.2; Delignette-Muller and Dutang 2015) determined a gamma error distribution provided the best fit of the model. We then fit a generalized linear mixed-effects model (GLMER) with life-history strategy (equilibrium, periodic, and opportunistic), as defined by Winemiller and Rose (1992) and Miyazono et al. (2010), time of year (Julian date), and distance to the bay (m) as predictor variables, with site as a random effect (function glmer; package *lme4* version 1.1-27.1; Bates et al. 2021). Candidate models were generated with combinations of fixed effects using the dredge function (package MuMIn version 1.46.0, R Program© version 4.1.2; Burnham and Anderson 2002), and Akaike’s information criterion (Akaike 1973) was used to determine the best-fit model. The predicted response values were plotted with ggpredict (package ggeffects version 1.1.1; R Program© version 4.1.2; Lüdecke 2018). Each variable was back transformed to make predictions at the population level.

The discussion of our results was aided by a comparison of fish-assemblage data collected throughout the Sandusky River (0.8 rkm to 29 rkm) by the Ohio Environmental Protection Agency (OEPA) in 2009 (OEPA 2010). The OEPA sampled fish using electrofishing from July through September 2009. It is widely accepted that electrofishing is more efficient at detecting fish species in comparison to hoop nets (Pugh and Schramm 1998; Smith et al. 2015). Therefore, only presence/absence data, species richness, and species proportion of catch were compared between the two data sets. For comparison, the OEPA data set was reduced to fish captured within our sampling area (with a degree of estimation). Additionally, small-bodied fish and hybrids, which could not be captured by our hoop nets, were removed from the OEPA data set prior to comparisons. The species removed included: Emerald Shiner *Notropis atherinoides*, Spottail Shiner *Notropis hudsonius*, Spotfin Shiner *Cyprinella spiloptera*, Fathead Minnow *Pimephales promelas*, Common Carp *Cyrpinus carpio* x Goldfish *Carassius auratus*, Blackstripe Topminnow *Fundulus notatus*, Brook Silverside *Labidesthes sicculus*, Logperch *Percina caprodes*, Mimic Shiner *Notropis volucellus*, Bluntnose Minnow *Pimephales notatus*, and Ghost Shiner *Notropis buchanani*. The OEPA identified bullheads *Ameiurus* spp. to species, whereas we identified them to genus. Therefore, all OEPA bullhead data were added together for consistency between the two data sets.

Results

Over four trials, 8,875 fish were captured in 880 hoop-net sets with approximately 21,013 h of soak time. The total catch comprised 31 species representing 11 families (Table 1). Non-native species (Grass Carp, Common Carp, Goldfish, White Perch) comprised 13% of the total catch. Twelve species were present in ≤5% of our daily catches (*n* = 440) and considered rare. Fish relative abundance and species richness decreased sequentially across trials (Table 2).

Seventy-one percent of all fish were caught in Trial 1, 15% in Trial 2, and 7% in Trials 3 and 4. Eighty percent of non-native fishes and 87% of recreationally/commercially important species were captured between April and June 2021 (Trials 1 and 2). Important sportfish species, including Smallmouth Bass *Micropterus dolomieu*, Rock Bass *Ambloplites rupestris*, Walleye, and Yellow Perch *Perca flavescens*, were only captured in Trials 1 and 2.

TABLE 1. Total catch of each fish species in hoop nets from the Sandusky River, Ohio, during 2021. The corresponding ID number is used throughout the document to identify the species. **Bold ID numbers represent non-native species.**

ID	Family	Species	Common name	Life-history strategy	Catch
5	Amiidae	*Amia calva*	Bowfin	Equilibrium	123
1	Catostomidae	*Ictiobus cyprinellus*	Bigmouth Buffalo	Periodic	111
3	Catostomidae	*Moxostoma duquesnei*	Black Redhorse	Periodic	3
17	Catostomidae	*Carpiodes cyprinus*	Quillback	Periodic	243
19	Catostomidae	*Moxostoma carinatum*	River Redhorse	Periodic	5
21	Catostomidae	*Moxostoma macrolepidotum*	Shorthead Redhorse	Periodic	6
22	Catostomidae	*Moxostoma anisurum*	Silver Redhorse	Periodic	3
24	Catostomidae	*Ictiobus bubalus*	Smallmouth Buffalo	Periodic	900
25	Catostomidae	*Minytrema melanops*	Spotted Sucker	Periodic	24
30	Catostomidae	*Catostomus commersonii*	White Sucker	Periodic	8
2	Centrarchidae	*Pomoxis nigromaculatus*	Black Crappie	Equilibrium	277
4	Centrarchidae	*Lepomis macrochirus*	Bluegill	Equilibrium	1,019
14	Centrarchidae	*Micropterus salmoides*	Largemouth Bass	Equilibrium	130

TABLE 1. Continued.

ID	Family	Species	Common name	Life-history strategy	Catch
18	Centrarchidae	*Lepomis humilis*	Orangespotted Sunfish	Equilibrium	1
20	Centrarchidae	*Ambloplites rupestris*	Rock Bass	Equilibrium	5
23	Centrarchidae	*Micropterus dolomieu*	Smallmouth Bass	Equilibrium	2
28	Centrarchidae	*Pomoxis annularis*	White Crappie	Equilibrium	233
11	Clupeidae	*Dorosoma cepedianum*	Gizzard Shad	Periodic	131
8	Cyprinidae	*Cyprinus carpio*	Common Carp	Periodic	444
12	Cyprinidae	*Carassius auratus*	Goldfish	Periodic	159
16	Esocidae	*Esox lucius*	Northern Pike	Equilibrium	114
6	Ictaluridae	*Ameiurus* spp.	Bullhead	Equilibrium	3,303
7	Ictaluridae	*Ictalurus punctatus*	Channel Catfish	Equilibrium	626
9	Ictaluridae	*Pylodictis olivaris*	Flathead Catfish	Equilibrium	183
15	Lepisosteidae	*Lepisosteus osseus*	Longnose Gar	Periodic	53
27	Moronidae	*Morone chrysops*	White Bass	Periodic	167
29	Moronidae	*Morone americana*	White Perch	Periodic	511
26	Percidae	*Sander vitreus*	Walleye	Periodic	3
31	Percidae	*Perca flavescens*	Yellow Perch	Periodic	1
10	Sciaenidae	*Aplodinotus grunniens*	Freshwater Drum	Periodic	83
13	Xenocyprididae	*Ctenopharyngodon idella*	Grass Carp	Periodic	4

TABLE 2. Summary of sampling dates and fish catches made in hoop nets on the Sandusky River, Ohio, in 2021. Species captured are represented by their numeric ID.

Trial	Sampling dates	Total catch	Number of species	Species captured
1	April 29–May 9	6,322	28	1–12, 14–18, 20–30
2	July 1–July 11	1,347	26	1, 2, 4–17, 19, 21, 23–29, 31
3	August 19–August 29	626	22	1, 2, 4–10, 12–17, 21, 22, 24, 25, 27–29
4	October 6–October 16	580	21	1, 2, 4–10, 12, 14–17, 19, 22, 24, 25, 27–29

The NMDS analysis (stress = 0.15) revealed temporal changes in fish assemblages (ANOSIM, r = 0.70, P < 0.001; Figure 2). Trial 1 was isolated in ordination space due to a unique composition characterized by a high catch of bullheads *Ameiurus* spp. (ID 6, numbers refer to species in Figures 1, 2), White Perch (ID 29), Quillback *Carpiodes cyprinus* (ID 17), White Bass (ID 27), Gizzard Shad *Dorosoma cepedianum* (ID 11), and rare species, such as Rock Bass (ID 20), White Sucker *Catostomus commersonii* (ID 30), Black Redhorse *Moxostoma duquesnei* (ID 4), and Orangespotted Sunfish *Lepomis humilis* (ID 18) (Figure 2). Trial 2 was isolated in ordination space due to a high abundance of Channel Catfish *Ictalurus punctatus* (ID 7), the presence of rarely caught Yellow Perch (ID 31), and the highest relative abundance of rarely captured River Redhorse *Moxostoma carinatum* (ID 19). Trial 2 was close in ordination space to Trial 3 due to a relatively equal and high catch of Flathead Catfish *Pylodictis olivaris* (ID 9) and rarely captured Grass Carp (ID 13). Trial 4 was primarily categorized by presence of Silver Redhorse *Moxostoma anisurum* (ID 22). A relatively high catch of Largemouth Bass *Micropterus salmoides* (ID 14) was made during Trials 1 and 4 compared to Trials 2 and 3 (Figure 2). Due to changes in fish assemblage among trials, the trials illustrated in Figure 2 occurred sequentially in ordination space in a counterclockwise pattern representing a distinct directional shift in fish assemblage over time.

There was a significant association between habitat features and species' catches (PERMANOVA, F = 2.975, df = 31, P = 0.001; Figure 3). Temperature, specific conductivity, turbidity, and chlorophyll explained most of the variation in assemblage structure. Substrate influenced assemblage structure more than aquatic vegetation. Most species were associated with a silt substrate and greater point velocity, but several other species appear to have specific habitat requirements. Ictalurids were strongly connected to varying habitat features. Flathead Catfish (ID 9) were captured in areas with higher temperatures and vegetation (*Phragmites* sp.), while Channel Catfish (ID 7) were linked to sites with increased minimum depth and duckweed blooms. Bullhead species (ID 6) had an affinity for shorelines with high turbidity and silt.

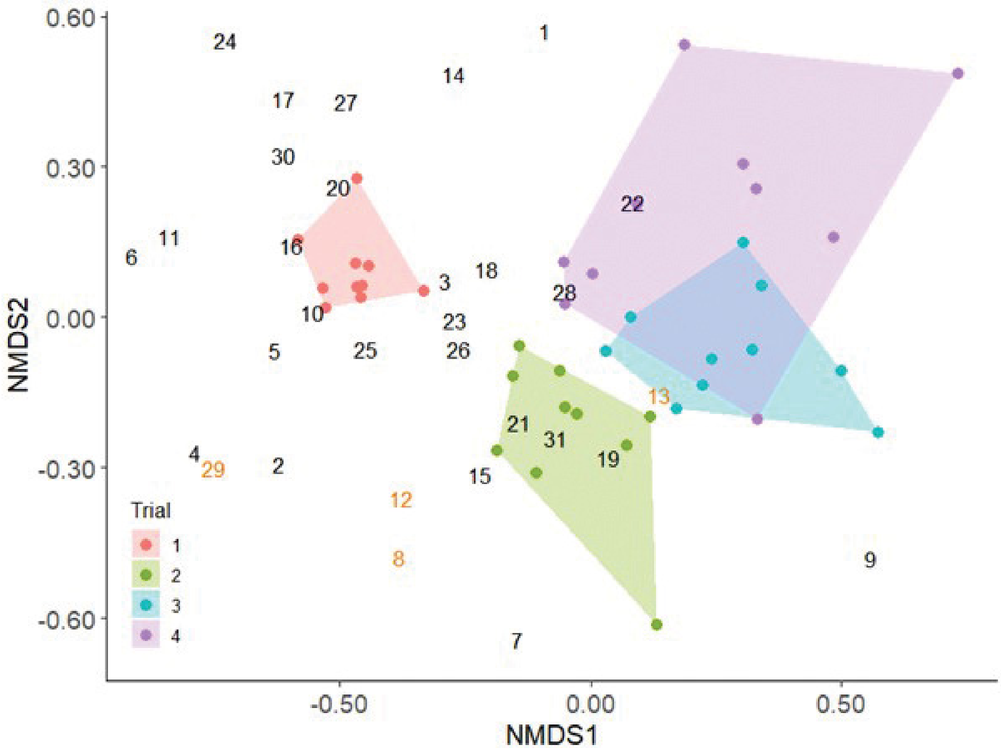

FIGURE 2. Nonmetric multidimensional scaling (NMDS) ordination of fish species collected across four sampling trials in the Sandusky River, Ohio, in 2021. Species are plotted based on their scores for each axis (stress = 0.15). Each point corresponds to a site and is colored by trial number. Convex hull polygons are colored similarly and represent the assemblage space across each site by trial. Numbers correspond to species ID (see Table 1) where black numbers are native species and orange are non-native species.

Notably, non-native Common Carp (ID 8), Goldfish (ID 12), and Grass Carp (ID 13) had habitat preferences similar to Channel Catfish, but they were more closely associated with sites characterized by shallow depths and sporadic duckweed blooms. Longnose Gar *Lepisosteus osseus* (ID15), Smallmouth Buffalo *Ictiobus bubalus* (ID 24), and White Sucker (ID 30) appeared at the center of the ordination indicating general habitat associations.

When comparing community assemblage by trial (Figure 2) and species composition by habitat (Figure 3), the relationship suggests environmental and habitat features may influence when some species are present in a river and for how long. This theory is supported by data on White Sucker (ID 30), Quillback (ID 17), and Northern Pike *Esox lucius* (ID 16), habitat generalists that were not strongly associated with any trial in the NMDS.

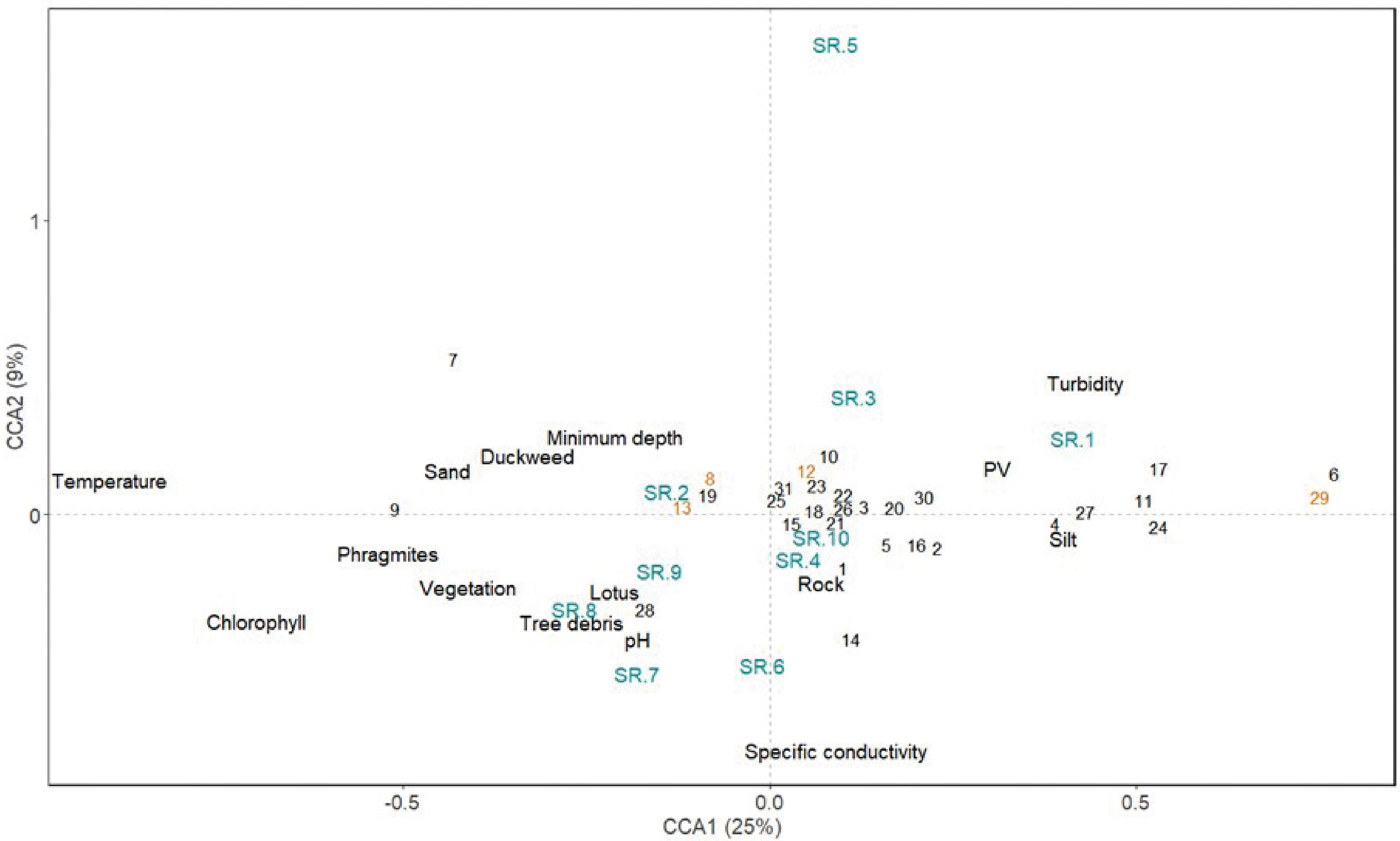

FIGURE 3. Canonical correspondence analysis (CCA) diagram representing the relationship of $\log_{10}$ transformed fish catch (n = 31) to habitat variables for eigenvalues greater than 1.5 in the lower Sandusky River, Ohio, in 2021. Sites are represented by green text, and species are separated into native (black) and non-native species (orange). PV = point velocity.

The random forest model, which included nine water-quality variables, 11 physical-habitat features, and three sampling specific factors, accurately classified 49% of the variation in daily fish diversity (*n* = 440). Variables with a %MSE of 10% or higher were considered important and interpreted further (Figure 4). Site, trial, and distance to bay contributed strongly to the model (Figure 5). Low fish diversity at site SR.2 can likely be attributed to its location. It was the only site located mid-channel away from either bank and near a shoal. Shoreline species diversity is known to be higher than mid-channel diversity (Wolter and Bischoff 2001). As with our catch, diversity was primarily affected by water-quality variables. Wood debris and log debris were the only physical-habitat features that significantly influenced the model and, therefore, fish diversity. Fish diversity was inversely related to temperature, pH, specific conductivity, water depth, log debris, and distance from the bay (Figure 5 A, B, C, E, F, H), while it was positively related to % wood debris (Figure 5 D).

We hypothesized that our data would characterize fish-assemblage shifts as fish moved upriver for spawning or for feeding in the warmer months or large-scale downstream movements to the bay in accordance with overwintering behaviors (Hayden et al. 2014; Childress and McIntyre 2015). The GLMER revealed significant associations between the predictor variables and fish catch (intercept coefficient = 5.02, SE = 0.25, *P* <0.00). Diversity was higher closest to the bay; decreased and remained relatively constant after site SR.1 (Figure 5h). This pattern indicates a community-wide preference to remain near the bay. However, as a main effect in the GLMER analysis, distance to bay was not a significant predictor of fish catch (coefficient = 0.18, SE = 0.24, *P* = 0.46). Distance to bay and sampling date interacted significantly to create temporal-specific patterns in fish catch within the sampled area (coefficient = 0.26, SE = 0.07, *P* <0.01). Our fish catch was higher in the spring throughout the sampling area, but particularly higher further from the bay. Sampling date (coefficient = 0.18, SE = 0.24, *P* <0.01) and life-history strategy were also significant to the model (coefficient = 0.26, SE = 0.07, *P* <0.01), as fish with an equilibrium life-history strategy accounted for much of the catch.

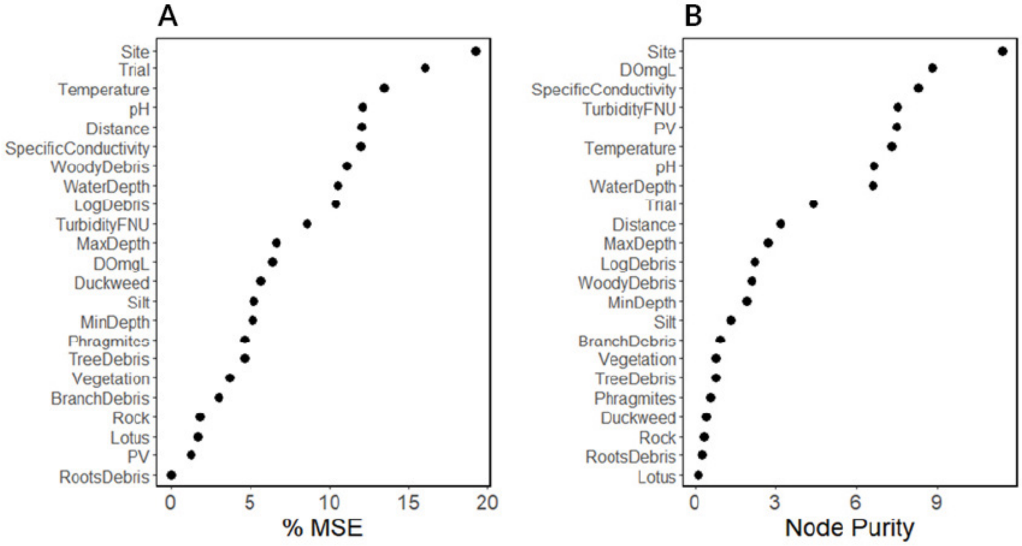

FIGURE 4. The % mean squared error (MSE) and node purity of the Random Forest model for predicting habitat variables most important to fish assemblage in the Sandusky River during 2021. The % MSE helped determine which habitat features contributed most to the model. PV = point velocity; DO = dissolved oxygen; FNU = Formazin Nephelometric Units.

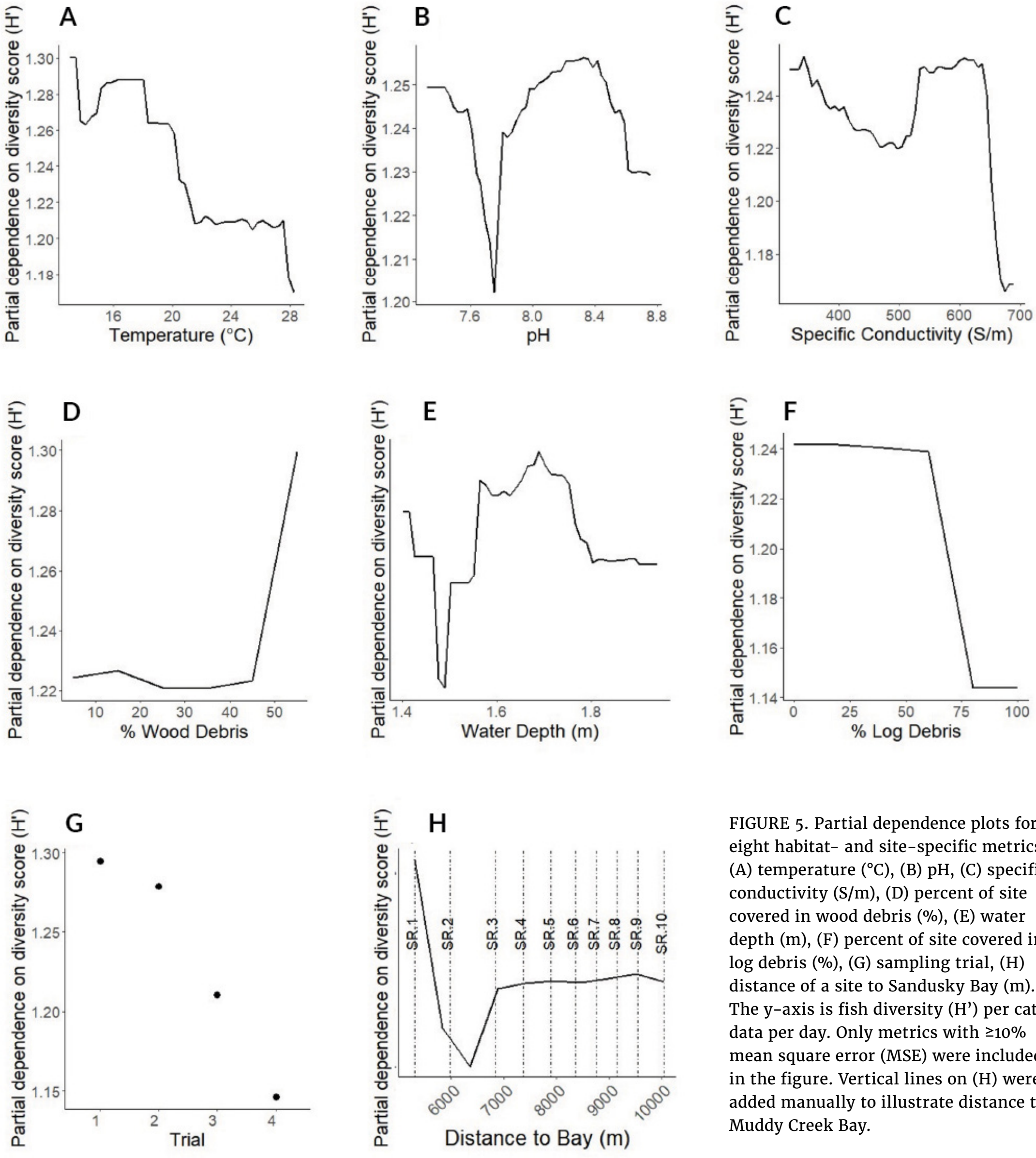

FIGURE 5. Partial dependence plots for eight habitat- and site-specific metrics: (A) temperature (°C), (B) pH, (C) specific conductivity (S/m), (D) percent of site covered in wood debris (%), (E) water depth (m), (F) percent of site covered in log debris (%), (G) sampling trial, (H) distance of a site to Sandusky Bay (m). The y-axis is fish diversity (H') per catch data per day. Only metrics with ≥10% mean square error (MSE) were included in the figure. Vertical lines on (H) were added manually to illustrate distance to Muddy Creek Bay.

Species richness was high in 2021 compared to 2009 (Table 3). Catostomids made up a higher percent of total catch in 2021 than in 2009, and two new sucker species were present in 2021—Black Redhorse and White Sucker. Differences in the catch of important sportfish varied between the OEPA and our data set, with the most intriguing result being the presence of Walleye and Northern Pike in 2021, as both species were absent in 2009. We also saw an increase in percentages for Black Crappie *Pomoxis nigromaculatus*, White Crappie *Pomoxis annularis*, and Channel Catfish (Table 3) and a decrease in sunfish species between 2009 and 2021. Changes in invasive-species presence and occurrence were variable as Common Carp and Goldfish were a higher percentage of total catch in 2009 than in 2021, but White Perch was much lower in 2021. Grass Carp was not caught in 2009 but was a small percentage of the total catch in 2021 (Table 3).

TABLE 3. A comparison of Ohio Environmental Protection Agency (OEPA) electrofishing catches in 2009 with the present study in a similar section of the Sandusky River (rkm 1 to rkm 8). Small-bodied fish were removed from the OEPA data set.

Species	OEPA study (2009)		This study (2021)	
	Catch	%	Catch	%
Bigmouth Buffalo	7	0.66	111	1.27
Black Crappie	1	0.09	277	3.18
Black Redhorse	0	0.00	3	0.03
Bluegill	47	4.43	1,019	11.69
Bowfin	0	0.00	123	1.41
Bullhead spp.	3	0.28	3,303	37.90
Channel Catfish	8	0.75	626	7.18
Common Carp	93	8.77	444	5.09
Flathead Catfish	1	0.09	183	2.10
Freshwater Drum	21	1.98	83	0.95
Gizzard Shad	651	61.36	131	1.50
Golden Redhorse *Moxostoma erythrurum*	32	3.02	0	0.00
Goldfish	41	3.86	159	1.82
Grass Carp	0	0.00	4	0.05
Green Sunfish	6	0.57	0	0.00
Largemouth Bass	17	1.60	130	1.49
Longnose Gar	3	0.28	53	0.61
Northern Pike	0	0.00	114	1.31
Orangespotted Sunfish	8	0.75	1	0.01
Pumpkinseed *Lepomis gibbosus*	17	1.60	0	0.00
Quillback	0	0.00	243	2.79
River Redhorse	0	0.00	5	0.06

TABLE 3. Continued.

Species	OEPA study (2009)		This study (2021)	
	Catch	%	Catch	%
Rock Bass	1	0.09	5	0.06
Shorthead Redhorse	9	0.85	6	0.07
Silver Redhorse	0	0.00	3	0.03
Smallmouth Bass	1	0.09	2	0.02
Smallmouth Buffalo	41	3.86	900	10.33
Spotted Sucker	4	0.38	24	0.28
Walleye	0	0.00	3	0.03
White Bass	14	1.32	167	1.92
White Crappie	5	0.47	233	2.67
White Perch	7	0.66	352	4.04
White Sucker	0	0.00	8	0.09
Yellow Perch	23	2.17	1	0.01
Total Catch	1,061		8,716	
Total Number of Species	25		31	

DISCUSSION

Often, large-scale anthropogenic disruptions to river systems alter natural patterns of flow (Poff and Hart 2002) and reduce available native fish habitat (Nunes et al. 2015). After decades of declining water quality and reduced habitat connectivity, the Ballville Dam was removed from the Sandusky River in 2018 (USFWS 2016). The dam removal caused a temporary influx of fine sediment downstream of the dam, covering coarse substrates and filling deep pools (Evans et al. 2007; Murphy et al. 2007) that native species, such as Walleye (Thompson 2009) and several catostomids (Bowman 1970; Jenkins 1980; Reid 2006), require for spawning habitat (Thompson 2009). The effect correlates with the serial discontinuity concept, which predicts that natural and anthropogenic disruptions will lead to downstream altered states at the population, community, and even ecosystem level (Ward and Stanford 1983, 1995). As the quality of fish habitat often declines in the immediate years after dam removal because large amounts of fine sediments are exposed (Bellmore et al., 2017), the resident fish community may change and decrease in species richness (Poff et al. 2007; Kornis et al. 2015; Sasak 2021). It can take years for rivers to recover from impoundment effects as the physical habitat stabilizes and native species begin to disperse into newly restored segments of the river (Sasak 2021).

Important native sportfish like Walleye and White Bass have not yet been reported in the restored upstream spawning habitat (Sasak 2021), but we documented that the spatial organization of the Sandusky River fish community has started to change downstream. All catostomids besides Shorthead Redhorse *Moxostoma macrolepidotum* had an increased presence in the Sandusky River since the collection of the OEPA 2009 data. *Moxostoma* species, Smallmouth Buffalo and Spotted Sucker *Minytrema melanops* were present upstream of the dam site in 2020 (Sasak 2021). These suckers prefer riffle/run habitat with deeper pools nearby, habitat features often found near dams (Reid 2006, 2008). Therefore, dam removal likely reconnected ideal spawning and feeding habitat for catostomids to the downstream section of the river, contributing to the increase in their presence and to species richness. This theory is further supported by the presence of White Sucker in 2021, which was not caught in the main stem of the Sandusky River in 2009 (OEPA 2010) or upstream of the dam site in 2020 (Sasak 2021). Common Carp, Goldfish, and White Perch are also utilizing the restored habitat upstream of the former dam site, but how they alter the fish assemblage in that section of the river is unknown (Sasak 2021). We found that Common Carp, Grass Carp, and Goldfish were most often captured in deep water with submerged or floating vegetation and/or wood debris, whereas White Perch were caught in silt substrates and relatively faster-moving water. These habitat features increased downstream post dam removal (Lisius et al. 2018), which could, in part, have contributed to the introduction of Grass Carp to the Sandusky River. However, as invasive species continue to establish in the restored upstream portion of the river, they may compete with native fish for complex habitats, such as the riffle/pool sections preferred by *Moxostoma*.

The presence of some fish species in the lower Sandusky River was not affected by the removal of Ballville Dam. Instead, other habitat features were the primary drivers of their presence. There is not a lot of information on how the Sandusky River has changed since 2018, which makes it difficult to discern what changes in the community assemblage between 2009 and 2021 are a direct result of dam removal. However, our CCA analysis showed several species grouping closely in ordination space, suggesting similar habitat usage. Those same species were also often collected during the same trial. This indicates that, for species such as Channel and Flathead Catfish (ID 7, 9), Bullhead (ID 6), Gizzard Shad (ID 11), Bigmouth Buffalo *Ictiobus cyprinellus* (ID 1), and Largemouth Bass (ID 14), habitat and environmental variables may strongly influence fish presence and temporal migrations in the Sandusky River, regardless of changes from dam removal.

Life-history strategy may also affect fish movements and drive assemblage distribution in the river. For example, several of the rarely captured catostomids and moronids with a periodic life-history strategy were nearly exclusive to the spring sampling event, consistent with their spawning behavior (Winemiller and Rose 1992; Miyazono et al. 2010). Reid (2006) documented spawning for six redhorse species occurring between May and June, but individuals were captured at spawning habitats several weeks prior to spawning, which correlates to our findings. If fish were actively migrating downstream to the bay following spring and early-summer spawning, we would expect to see an increase in catches closer to the bay in mid to late summer, which was not the case for our study. This observation, as well as what we know about migratory behavior in freshwater fish, suggests that these species are remaining upstream instead of returning to the bay, but continued surveys further upstream of our sampling sites would be needed to confirm this hypothesis.

CCA results suggest most species prefer shallow depths and habitat complexity, such as branch debris, rock substrate, or submerged aquatic vegetation, a finding supported by the PDP. Downstream habitat alteration in the Sandusky River resulting from the removal of the Ballville Dam may have increased suitable habitat for several species that were not previously present in the river. Northern Pike, a species that prefers shallow depths and emergent and submergent vegetation (Diana et al. 1977), and Bowfin *Amia calva*, a species that resides in shallow, nearshore areas often with structural complexity (Patterson and Longbottom 1989; Midwood et al. 2016), were not present in the OEPA 2009 catch but were present in our 2021 catches. However, neither species was present upstream of the former dam in 2020 (Sasak 2021). In this instance, the often temporary but negative changes downstream of dam removal provided suitable habitat for some species, which increased species richness in the river. Despite the evident changes in habitat, it is not clear what specific changes in the Sandusky River promoted the establishment of Bowfin and Northern Pike.

Catostomids appear to have benefited most from dam removal, but sportfish, such as Walleye and Northern Pike, are beginning to migrate into the Sandusky River. The fish assemblage is expected to continue to change in the upcoming years. Short-term changes are detectable within five years, but it can take decades for a system to reach equilibrium after a large disturbance (Shafroth et al. 2002). The intermediate disturbance hypothesis suggests that the fish community may never reach equilibrium depending on the type and intensity of the disturbance (Connell 1978). Therefore, future studies should continue to assess diversity metrics and fish community dynamics upstream and downstream of the former dam. Although we were not able to compare habitat and environmental features pre- and post-dam removal, our results can be used to access changes in habitat throughout the system. Continued monitoring is particularly important in the Sandusky River, as removal of the dam could increase the abundance of recreationally and economically important species in Lake Erie as well as influence the spread of non-native species in the Great Lakes.

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Author contributions include: conceptualization: MRA, AMT, JTJW, RDC; developing methods: KS, MRA, ATM, JJW, DB, RDC; data analysis: KS, MRA; preparation of figures and tables: KS, MRA; conducting the research, data interpretation, and writing: KS, MRA, ATM, JJW, DB, TMH, TMW, RLM, JJA, RDC.

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Optimization of Methods for the Collection of Larval Sea Lamprey Environmental DNA (eDNA) from Great Lakes Tributaries

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ABSTRACT

Online Information

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Background: Environmental DNA (eDNA) sampling and analysis have the potential to revolutionize species monitoring, but the effective implementation in field conditions remains uncertain. The current study addresses this knowledge gap by developing a robust eDNA sampling protocol for the detection of larval Sea Lamprey *Petromyzon marinus* in Great Lakes tributaries.

Methods: Three experiments were conducted to optimize eDNA field sample collection. The first experiment compared the performance of 0.45-μm, 1.2-μm, 5.0-μm cellulose nitrate (CN) filters and a 1.5-μm glass-fiber filter to determine which filter consistently yielded the highest median DNA copy number. The second experiment evaluated the performance of two filtration devices for eDNA sample collection and filtration, an autosampler (Halltech OSMOS aquatic eDNA sampler, Halltech Environmental and Aquatic Research, Guelph, Ontario, Canada) and a handheld peristaltic pump. In the third experiment, a biweekly eDNA survey was conducted to investigate the temporal dynamics of spawning Sea Lamprey eDNA to determine at what point during the season only larval lamprey eDNA is detected.

Results: Our findings indicate that CN filters with a pore size of 1.2 μm or 5.0 μm captured consistently the highest amount of eDNA, but the 5.0-μm CN filter was selected for routine use due to its superior performance and reduced risk of clogging. We found no significant performance differences between the OSMOS aquatic eDNA sampler and the peristaltic pump across three response variables (frequency of contaminated field negative controls, PCR inhibition, and positive detections), suggesting both devices can reliably be used. Moreover, our study found that the spawning Sea Lamprey eDNA signal attenuates approximately 4–6 weeks after the last adult Sea Lamprey capture, which is consistent with previous research.

Discussion: By synthesizing the results, we provide a streamlined eDNA sampling protocol for larval Sea Lamprey monitoring. We recommend beginning eDNA sampling at least six weeks after the end of the estimated regional spawning period and using a 5.0-μm CN filter in combination with the OSMOS aquatic eDNA sampler, with the handheld peristaltic pump serving as a backup. This optimized approach improves the efficacy and reliability of eDNA-based monitoring.

INTRODUCTION

The invasive Sea Lamprey *Petromyzon marinus*, which contributed to the collapse of the Great Lakes fishery (Lawrie 1970), remains a persistent threat to this vital ecosystem and industry (McLaughlin et al. 2021). Since the initial application of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) in the latter half of the 20th century (Schnick 1972; Smith and Tibbles 1980), TFM has been critical for disrupting the Sea Lamprey life cycle in Great Lakes tributaries by killing larvae prior to their transformation into parasitic juveniles (Wilkie et al. 2019); TFM continues to be used today as the primary means to control Sea Lamprey (Marsden and Siefkes 2019; Wilkie et al. 2019). Electrofishing serves as the main method for assessing larval Sea Lamprey presence, abundance, and size distribution (Slade et al. 2003). These data, when inputted into the Empiric Stream Treatment Ranking system (Christie et al. 2003; Hansen and Jones 2008), guide the selection of tributaries with the most favorable cost-to-kill ratio for TFM treatment. In the historical record, more than 500 out of 5,311 (9.4%) Great Lakes tributaries have faced Sea Lamprey infestations (Barber and Steeves 2020). Yet, constraints in the Great Lakes Fishery Commission's (GLFC) Sea Lamprey Control Program's budget and staffing allow for treatment of only one quarter of infested streams each year (Jubar et al. 2021). Also, electrofishing surveys have limitations. Steeves et al. (2003) demonstrated that, even at medium to high larval Sea Lamprey densities, the probability of detection through electrofishing was only 0.48; in cases of low larval Sea Lamprey densities, electrofishing performance was even less effective. Additionally, electrofishing cannot usually be conducted from late October to May nor in areas with water too deep to wade for backpack electrofishing or impassable for boat-based electrofishing. Given the number and extent of tributaries in the Great Lakes basin, an exploration of alternative methods to supplement electrofishing could greatly enhance Sea Lamprey surveillance.

Amid these challenges, the emergence of environmental DNA (eDNA) monitoring presents a possible supplement to electrofishing as a method of detecting larval Sea Lamprey infestations. Research has shown that for detecting low-density aquatic macroorganisms, eDNA can be more effective than electrofishing (Sigsgaard et al. 2015; McKelvey et al. 2016; Wilcox et al. 2016, 2018; Strickland and Roberts 2019), and it is a promising avenue for furthering Sea Lamprey control measures (Docker and Hume 2019). In addition, eDNA methods have been used to detect species in winter (Feng et al. 2020; Khalsa et al. 2020), which would extend the monitoring season for larval assessment. Thus, although follow-up electrofishing surveys would be required to determine abundance and larval size distribution, eDNA screening across large spatial scales could permit efficient detection of new Sea Lamprey infestations, monitor for reinfestation after TFM treatment, determine instream distribution prior to lampricide treatment, and extend the field season. A first pass using eDNA monitoring could permit more efficient deployment of electrofishing crews.

Those in ecosystem management roles may find the burgeoning use of eDNA for monitoring aquatic species of management and conservation concern encouraging, prompting them to consider adopting eDNA methods for monitoring their species of interest. However, a multitude of eDNA methods are used to monitor aquatic macro-organisms (Tsuji et al. 2019), with many of these specialized methodologies being developed independently by different research groups (Goldberg et al. 2016). This proliferation of techniques, while exciting on one hand, has also complicated the selection of eDNA methods for novel applications, hindering the reproducibility of studies and adoption of eDNA methods for species detection (Tsuji et al. 2019). Ecosystem managers may face uncertainty when deciding which eDNA methods to use, as the methods used can affect the efficacy of eDNA collection and processing (Hinlo et al. 2017).

The extensive use of electrofishing in the GLFC's Sea Lamprey Control Program (Hansen and Jones 2008) presents an opportunity to evaluate the use of eDNA techniques for detecting larval Sea Lamprey. In previous studies (Gingera et al. 2016; Schloesser et al. 2018), using polymerase chain reaction (PCR) assays to detect Sea Lamprey eDNA has shown promise, both within controlled-density tanks and in Great Lakes tributaries. However, it is crucial to note that, prior to the present study, an optimized and thoroughly validated eDNA sampling protocol specific to larval Sea Lamprey within Great Lakes tributaries did not exist. This gap in knowledge is crucial because, without an optimized protocol, those in ecosystem management roles may hesitate to adopt eDNA methods (Loeza-Quintana et al. 2020).

The process of using targeted eDNA methods for aquatic species encompasses six primary stages: (1) collecting water samples, (2) filtering water samples, (3) preserving the eDNA and other non-aqueous materials on the filter, (4) isolating eDNA, (5) quantitative (qPCR) analysis, and (6) statistical analysis and data interpretation (Langlois et al. 2021). Steps 1–3 are typically conducted in the field (steps 1 and 2 are combined when using an automated eDNA sampler), and steps 4–6 are performed in the laboratory. Furthermore, the ecology of the target species should guide decisions on where and when to collect eDNA samples (Rees et al. 2014). By comparing different sample collection and filtration methods and analyzing the impact of the target species' ecology on eDNA detection, a unified sampling protocol can be developed for future efforts.

There are an almost infinite number of combinations of different parameters that have been used in eDNA studies, and the diverse applications of eDNA do not lend themselves to a one-size-fits-all protocol (Kumar et al. 2020). However, we identified parameters that were already deemed to be effective based on our previous experience (e.g., Gingera et al. 2016; Schloesser et al. 2018; Loeza-Quintana et al. 2020, 2021; Milián-García et al. 2021) and review of the literature versus parameters that required more thorough testing and optimization for our specific needs. For example, best practices dictate that water samples be filtered immediately on-site rather than transported back to the laboratory (Laramie et al. 2015). On-site water filtration systems used for eDNA typically include portable peristaltic pumps, automated eDNA samplers, and vacuum pumps with a filter funnel manifold. Because a vacuum pump would require access to a less portable power source (e.g., a streamside generator), we tested only the first two options. Similarly, although eDNA preservation can be achieved by storage in 95–100% ethanol, RNAlater, or Longmire's or ATL buffer, (Majaneva et al. 2018; Kumar et al. 2020), handling large volumes of ethanol or other liquid storage media (and needing to keep them on ice or at -20°C) is not practical for Sea Lamprey larval assessment teams, which are often away from laboratory or other sample storage locations for more than one week. Majaneva et al. (2018) concluded that silica gel desiccant improved species detection and resulted in lower variability relative to other filter storage media. Also, during subsequent processing, dried filters are easily broken into pieces in microcentrifuge tubes, reducing handling time and permitting sub-sampling (e.g., for archiving or method comparison).

Researchers should aim to maximize eDNA capture from water samples, and the choice of filter type is significant due to eDNA's varied forms in water. Smaller pores capture intracellular and smaller extracellular DNA fragments, but they can more easily be clogged by particles like clay and soil (Kumar et al. 2020). Conversely, filters with larger pores work well for capturing larger eDNA fragments and clog less than smaller-pored filters, but they may allow smaller fragments to pass through the filter (Turner et al. 2014). However, small filter pore sizes, as highlighted by Kumar et al. (2020), may pose a risk. Even filters with relatively large pores, such as 1.5 µm, can become clogged in turbid environments. This clogging can lead to inhibition and reduced sample volumes, potentially hampering detection. Thomas et al. (2018) recommended a 5.0-µm pore size due to its lower risk of clogging and higher DNA yield when filtering larger volumes. Alongside pore size, filter material is another factor to consider. In their guidance document, Vazquez et al. (2023) highlight that filter materials such as cellulose nitrate (CN), glass fiber (GF), and mixed cellulose ester are commonly employed in aquatic eDNA studies. Use of these materials is supported by findings in the research of Majaneva et al. (2018) and Muha et al. (2019). In prior research, 1.5-µm GF filters were used to collect larval Sea Lamprey eDNA (Gingera et al. 2016; Schloesser et al. 2018). However, a study by Hinlo et al. (2017) comparing filter types for a different aquatic invader, the Oriental Weatherloach *Misgurnus anguillicaudatus*, found that, when using the DNeasy® Blood & Tissue Kit (Qiagen, Venlo, The Netherlands) for extractions, 1.2-µm GF filters had a significantly lower DNA yield compared to 1.2-µm CN filters.

Laramie et al. (2015) and Nolan et al. (2023) highlight the significant impact the choice of water-sample filtration device can have on the effectiveness of eDNA studies. To establish an eDNA field methods protocol that ensures the production of dependable species-occurrence data, researchers should thoroughly compare filtration devices commonly used in eDNA research. This assessment involves evaluating various critical factors, including the potential for contaminated negative controls (resulting in false positives), the risk of PCR inhibition and the likelihood of missing the target organisms (leading to false negatives), and the effectiveness of detecting the target organism(s).

Determining the appropriate time window for sampling is a crucial aspect of an eDNA field methods protocol. The Sea Lamprey's life history complicates eDNA monitoring, as PCR analysis cannot differentiate between adult/spawner and larval Sea Lamprey eDNA, both of which occur within tributaries. In spring, adult Sea Lamprey in the Great Lakes migrate upstream to spawn (Manion and Hansen 1980), often guided by pheromones released by larval Sea Lamprey (Vrieze et al. 2010). After spawning, adult Sea Lamprey die, while the larvae remain burrowed in the sediment for typically 3–5 years until they metamorphose into parasitic juveniles and out-migrate to the lakes (Dawson et al. 2015). To accurately monitor larval Sea Lamprey without conflating detections with spawning adults or their decomposing remains, sampling must take place after spawner eDNA has dissipated from the tributary. Gingera et al. (2016) used conventional PCR (cPCR) to establish that the eDNA signal from spawners in the Little Thessalon River of northern Lake Huron diminished around July 22, approximately one month after the end of the spawning run. However, given the higher sensitivity of qPCR compared to cPCR (Xia et al. 2018), it is possible that spawner eDNA will be detected longer with qPCR; therefore, it is important to test spawner eDNA attenuation times with qPCR to determine the time after which eDNA detected in a stream can be assumed to originate exclusively from larvae. Additionally, as summarized by Rourke et al. (2022), a multitude of biotic factors influence eDNA concentrations and dispersion in aquatic systems. For example, the flow rate and hydrology of specific river systems affect eDNA dispersion (Harrison et al. 2019; Rourke et al. 2022) and influence how long eDNA remains in the aquatic environment after the target species has departed.

To test and refine eDNA field sampling methods for larval Sea Lamprey, we conducted three experiments. These methods were based on the species' ecology, and they were designed to be efficient and sensitive to its presence, while minimizing the risk of both false-positive and false-negative errors. The first experiment assessed various filter types to determine the most suitable material and pore size for our research scenario. In the second experiment, we compared two filtration devices: the OSMOS Aquatic eDNA sampler (Halltech Environmental and Aquatic Research, Guelph, Ontario, Canada; Nolan et al. 2023) and a handheld do-it-yourself peristaltic pump described in Gingera et al. (2016). In the third experiment, we conducted biweekly eDNA surveys in a Great Lakes tributary during July and August to determine when eDNA detections of adult Sea Lamprey stop after the spawning run. By synthesizing the results of our research, we aim to develop an efficient and sensitive eDNA sampling protocol for detecting the presence of larval Sea Lamprey. Our goal is to provide an effective supplement to electrofishing to enhance the surveillance of larval Sea Lamprey in Great Lakes tributaries.

METHODS

Experiment 1: Assessing Filter Material and Pore Size

Study Design and Site Selection

This experiment's objective was to identify the filter type that consistently collected the highest quantity of eDNA. At each location, one field negative control and four biological samples per filter type were collected and filtered; the negative control tested for possible contamination in the equipment or during collection and filtration. A total of 2 L of tap water was filtered for each negative control, with 10 L and 2 L of river water filtered for each biological sample in the first and second sampling activities, respectively, using the OSMOS aquatic eDNA sampler. Samples for each filter type, starting with the negative control, were consecutively filtered. As per the standard protocol, if a filter clogged before reaching the desired water volume, the filter was preserved, and the clogging event and filtered volume were noted. Filtration volume was decreased from 10 L to 2 L in the second experiment, because 2 L was the largest volume that could be consistently filtered at most locations. The field data sheet (see Supplementary Information S1: Environmental DNA Sampling Field Data Sheet in the online Supplement of this publication) used to record sampling metadata was a modified version of the GEN-FISH Lotic eDNA Collection Data Sheet (GEN-FISH 2022a). Upon returning from field collection, filters were kept at -20°C until DNA extraction occurred. Sterile techniques were used for the collection of all samples to minimize the risk of contamination that could lead to false positives. Briefly, these techniques included thorough decontamination of all non-single-use sampling supplies. For materials that came into direct contact with the samples (e.g., gloves, forceps, storage bags, filter canisters, tubing), items were either changed between sampling stations or thoroughly decontaminated to prevent cross-contamination between sampling stations. For the complete guide on eDNA sample collection, see Appendix.

We conducted two independent field trials to determine the optimal filter type only at locations that contained larval Sea Lamprey densities greater than 0.1 larvae/m^2. Low target-organism density can cause stochasticity in the quantity of eDNA captured across field sample replicates (Van Driessche et al. 2023); thus, to reduce the effect of this stochasticity, we restricted sampling to areas with relatively high larval densities (Sea Lamprey Control Centre (SLCC)), Fisheries and Oceans Canada (DFO), unpublished data). The first field trial took place in September 2021, at a sampling station on Big Otter Creek (42.854 405° N, -80.724 185° W) in the municipality of Tillsonburg, Ontario. The second field trial occurred in June 2022, at a sampling station on the Credit River (43.632 731 5° N, -79.759 016 8° W) in the municipality of Brampton, Ontario (Figure 1). In 2020, electrofishing surveys at the Big Otter Creek station reported an average larval density of 3.3 larvae/m², and, in 2021, electrofishing surveys at the Credit River station reported an average larval density of 0.5 larvae/m² (L. Sumner, DFO; unpublished data).

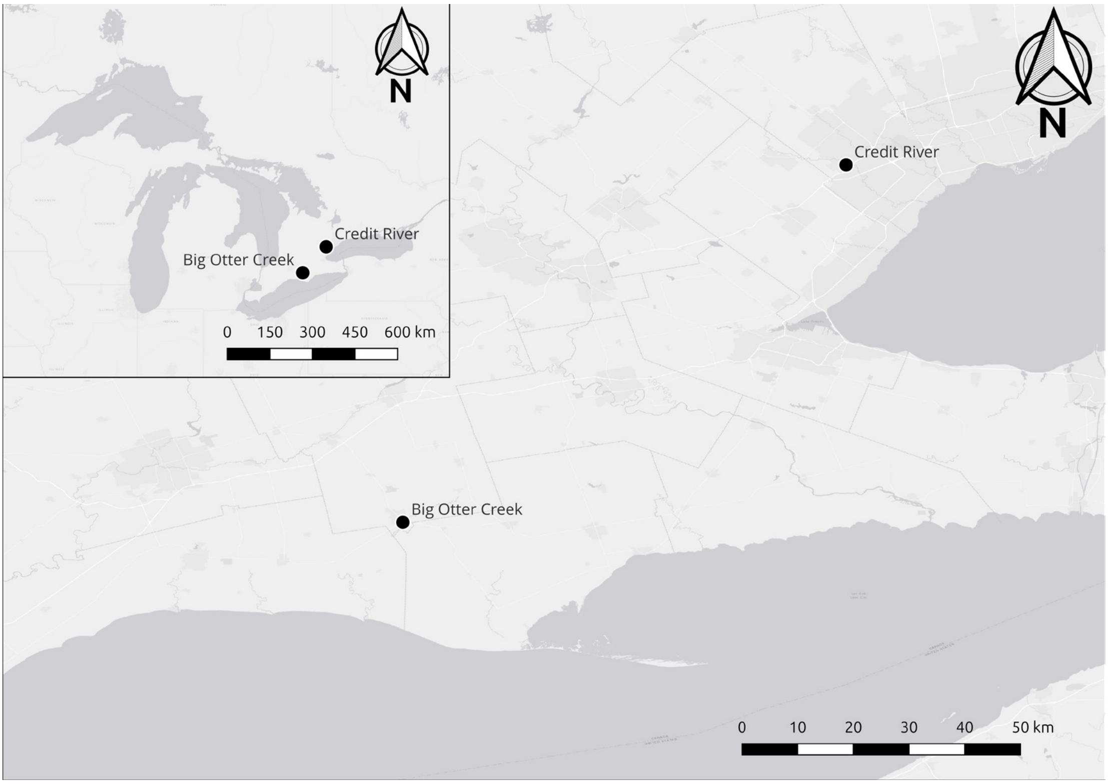

FIGURE 1. Locations of Big Otter Creek and Credit River, Ontario, where eDNA sampling was conducted for the filter comparison experiment (Experiment 1). At each sampling location, four biological replicates and one field negative control were collected for each of the four filter types. Map was created using QGIS Desktop (available at https://qgis.org/).

Filter Selection

The four filter types selected for the experiment were 1.5-µm GF, and 0.45-µm, 1.2-µm, and 5-µm CN filters. The 1.5-µm GF filters were included based on their previous use in eDNA monitoring of Sea Lamprey (Gingera et al. 2016; Schloesser et al. 2018). Additionally, CN filters were tested, as previous research suggested a positive interaction between CN material and the DNeasy® Blood & Tissue Kit (Qiagen, Hinlo et al. 2017) used for DNA extractions. The three pore sizes for CN filters were chosen to assess the potential influence of filter pore size on

DNA yield. Multiple pore sizes for GF filters were not included due to limited availability from manufacturers.

Extraction and qPCR Analysis

DNA extraction from the collected filters was conducted within four months of the sample collection. DNA extraction followed a modified manufacturer's protocol (see Supplementary Information S2: DNA Extraction Protocol One in the online Supplement of this publication) using a DNeasy® Blood & Tissue Kit (Qiagen) and a QIAshredder (Qiagen). Each batch of extractions included a new 1.5-μm GF filter as an extraction negative control to test for contamination during this step. The DNA extracts were stored at -20°C until needed for qPCR analysis. To quantify Sea Lamprey DNA in each sample, a TaqMan™ Assay (Thermo Fisher Scientific, Waltham, Massachusetts) was used (Table 1). The assay targeted a 154-base pair segment of the Cytochrome B (CytB) gene, as described by Schloesser et al. (2018), which was duplexed with an internal positive control (IPC, TaqMan™). The IPC consisted of synthetic DNA, along with primers and a probe, which were included in the qPCR reaction. This control served to differentiate genuine negative detections from false negatives caused by PCR inhibitors (Gasparini et al. 2020). The qPCR reactions also included a PCR inhibitor-resistant Environmental Master Mix (EMM 2.0, TaqMan™) to further mitigate the impact of inhibitors. The cycling conditions of the duplexed qPCR assay were a 10-minute hold at 95°C, followed by 45 cycles of 15 seconds at 95°C, and 60 seconds at 60°C.

TABLE 1. Sea Lamprey qPCR assay details. Forward primer, reverse primer, and probe (with a (Fluorescein (FAM) dye label) for the TaqMan™ qPCR assay targeting the Cytochrome B (CytB) gene of the Sea Lamprey and the sequence of the resulting 154-base pair amplicon. In the probe sequence, ZEN™ is the internal quencher (Integrated DNA Technologies, Coralville, Iowa). From Schloesser et al. (2018).

Component	Sequence
Forward Primer	5'-GGTTTTGTTATTCTACTGGGCAT-3'
Reverse Primer	5'-GTAGAATGGCATAGGCAAATAGA-3'
Probe	5'-TTCCCTTTT/ZEN/AGCCCCTAATGCACT-3'
154 bp Gene Fragment of CytB	5'-GGTTTTGTTATTCTACTGGGCATTCTTTTCATAATTTCCCTTTTAGCCCCTAATGCACTAGGTGAACCAGACAACTTTATTTATGCTAATCCTCTTAGTACCCCTCCCCATATTAAACCAGAATGATACTTTCTATTTGCCTATGCCATTCTAC-3'

The samples collected from the Big Otter Creek sampling station were analyzed on the QuantStudio™ 7 real-time PCR system (Thermo Fisher Scientific), with four qPCR technical replicates per sample. The samples collected from the Credit River sampling station were analyzed on the Mic real time PCR system (Bio Molecular Systems, Dural, New South Wales, Australia), with eight qPCR technical replicates per sample. This doubling of the qPCR replicates for the second set of samples aimed to investigate if heightened sensitivity, achieved through more qPCR replicates (Klymus et al. 2020), would significantly alter the experimental results. Additionally, using eight technical replicates aligns closely with the intended field application methods. The change of qPCR platform from the QuantStudio™ 7 for samples from Big Otter Creek to the Mic for samples from the Credit River location was made for practical reasons, as the QuantStudio™ 7 was experiencing technical issues at the time. However, the same assay, reagents, and cycling conditions were used for both locations and, most importantly, the data were not statistically compared between the two platforms. Although rigorous comparisons between platforms would be needed before both were used interchangeably during routine eDNA monitoring. The change in platforms here should not impact the interpretation of the results. During each analysis, qPCR technical replicates for each sample were evenly distributed across four qPCR runs. This distribution captured the inter-run variation within individual sample variances. Additionally, each qPCR run included three no-template controls, which served as PCR negative controls to test for contamination at this step. For samples from the first location (Big Otter Creek), a no-amplification control (TaqMan™) was incorporated into each qPCR run. The no-amplification control inhibited the enzyme activity required for PCR reactions and controlled for any non-PCR fluorescence. Additional details about the qPCR assay can be found in Supplementary Information S3: qPCR Assay Details in the online Supplement of this publication.

Data Analysis

The average estimated DNA copies/µL of the DNA template for each qPCR reaction was calculated following the method outlined in Klymus et al. (2020). The dilution series used to estimate DNA copies/µL, and establish the limit of quantification (LOQ) and limit of detection (LOD) consisted of a six-fold 1:5 dilution series of synthetic Sea Lamprey DNA gBlock™ from Schloesser et al. (2018), with 15 technical replicates per dilution, which was analyzed on the QuantStudio™ 7. The LOQ was determined to be 24 DNA copies/µL, and the LOD for four and eight technical replicates per sample was 1.858 DNA copies/µL and 1.068 DNA copies/µL, respectively (see Supplementary Information S3: qPCR Assay Details available in the online Supplement of this publication). The standard curve used to assess the efficiency of the assay consisted of a five-fold 1:10 dilution series of synthetic Sea Lamprey DNA gBlock™ from Schloesser et al. (2018), run on the QuantStudio™ 7 with eight technical replicates per dilution, with an efficiency of 96.58% and an R^2 of 0.9842 (see Supplementary Information S3: qPCR Assay Details available in the online Supplement of this publication). The synthetic Sea Lamprey DNA gBlock™ sequence allows for a known quantity of DNA to be aliquoted into a qPCR reaction to serve as a positive control to determine qPCR run quality and to also establish performance metrics such as sensitivity, and the conversion of an unknown sample to an estimated average DNA copy number using a standard curve.

In our study, a number of qPCR replicates amplified below the established LOQ, necessitating a method to handle these values statistically. Klymus et al. (2020) note that, in analytical chemistry, it is common to assign qualitative or semi-quantitative values to data falling between the LOD and LOQ. However, in the field of eDNA, there is limited precedent for managing qPCR data points in this range. A frequent approach in various analytical chemistry methods involves using half-LOQ or midpoint values for all amplifications below the LOQ when conducting statistical analyses (Warth et al. 2012; Abia et al. 2013; Li et al. 2018; EMA 2022). Other common methods include excluding the data points, using a likelihood estimation to impute values, and including the values as is (Keizer et al. 2016). Of these methods, the half-LOQ method is suitable when only a small portion of the data falls below the LOQ. However, when a larger portion of the data set includes values below the LOQ, using those values without adjustment introduces the least bias into the analysis (Keizer et al. 2016). As such, the copy number values below the LOQ were included as estimated in the subsequent statistical analysis and reactions without a machine-registered quantification cycle (Cq) value were assigned a DNA copy number of 0 DNA copies/μL. While this decision to include all values as is may introduce bias into the statistical analysis, it is likely preferable to the other approaches.

Normal quantile-quantile plots were used to visually assess the distribution of estimated DNA copies/μL values grouped by filter type, followed by a Shapiro-Wilk test of whether the assumption of normality had been violated within each group. For samples from both locations, the assumption of normality was violated as evidenced by the skew in the quantile-quantile plots, particularly at the Credit River location (Figure 2). In addition, only the average eDNA counts for the CN5 filters from Big Otter Creek were not statistically significant and did not violate normality (Table 2). Given the non-normal distribution of the data, the non-parametric Kruskal-Wallis test was used to evaluate if there were significant differences in the median DNA copy number among the four filter types. If the Kruskal-Wallis test results were significant ($P < 0.05$), a Dunn's multiple comparison test was used as a post hoc test. This test was used to identify the filter type with the highest median DNA copies/μL, indicating its suitability for future applications. Samples from each location were analyzed separately. Additionally, values for the 1.5-μm GF filter from the Big Otter Creek samples were excluded from the analysis because the field negative control for those samples was contaminated, rendering any subsequent results from those samples untrustworthy.

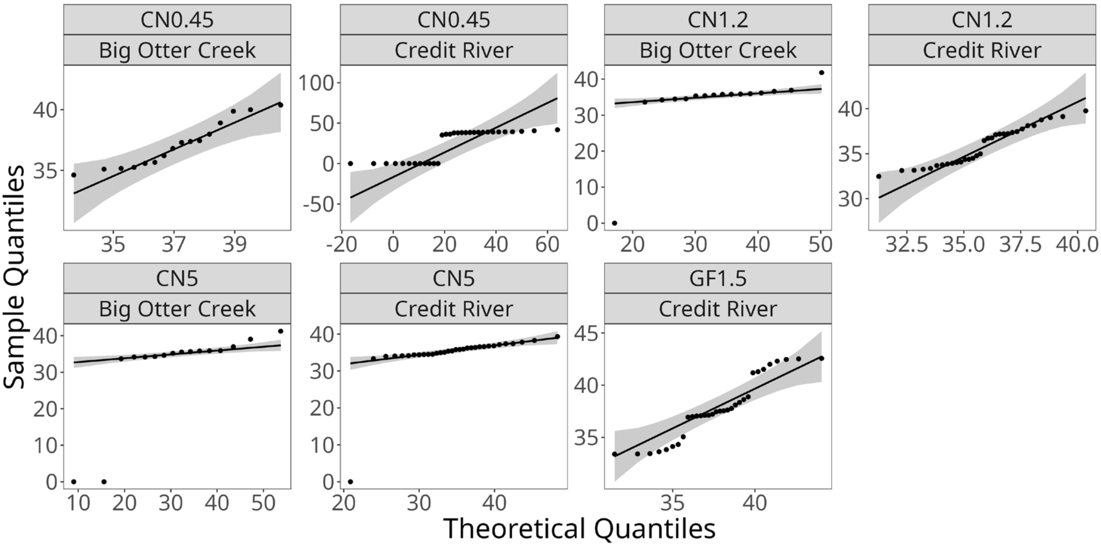

FIGURE 2. Normal quantile-quantile plots for the estimated average DNA copy number for each qPCR replicate from biological samples collected from the Big Otter Creek and Credit River sampling stations in Experiment 1. The plots are a visual check to see if the data are normally distributed; the more closely the data fit the central line, the more closely they approximate a normal distribution. For the filter-type abbreviations, the first two characters are the filter material, and the following number is the filter pore size in μm.

TABLE 2. dProbability values estimated with the Shapiro-Wilk test to assess normality of DNA copy number for each filter type for Experiment 1 on the Big Otter Creek and Credit River, Ontario, locations. If the *P*-value was significant at the 5% level, the normality assumption was considered violated and is indicated with an asterisk. For the filter-type abbreviations, the first two characters represent the filter material, and the numbers represent the filter pore size in µm.

Filter type	Big Otter Creek	Credit River
CN0.45	$4.10 \times 10^{-2*}$	$1.05 \times 10^{-8*}$
CN1.2	$2.89 \times 10^{-2*}$	$1.75 \times 10^{-4*}$
GF1.5	N/A	$1.76 \times 10^{-7*}$
CN5	8.19×10^{-2}	$3.64 \times 10^{-3*}$

Experiment 2: Comparing Two Filtration Devices for eDNA Sample Collection

Study Design and Location

Experiment 2 aimed to compare two water filtration devices for eDNA sampling. The experiment involved paired eDNA sampling for larval Sea Lamprey at 28 sampling locations. The comparison was based on three key criteria: (1) the frequency of contaminated field negative controls, (2) the frequency of PCR inhibition in field samples, and (3) the frequency of samples containing Sea Lamprey DNA.

In collaboration with the U.S. Fish and Wildlife Service (USFWS) and DFO Sea Lamprey larval assessment teams, paired peristaltic pump and OSMOS sampling occurred at 28 stations across nine tributaries in the USA and Canada during the fall of 2021 (Figure 3). Though other autosamplers exist, the OSMOS was selected due to the flexibility and cost effectiveness of allowing us to use our own filters. At each station, four samples were collected using the OSMOS aquatic eDNA sampler and the peristaltic pump, consisting of one negative control collected from a bucket of tap water, as described in the previous section, followed by three biological replicates. For each sample, 2 L of water was filtered through a 1.5-µm GF filter; however, approximately 10% of the filters clogged before reaching the 2 L mark. The 1.5-µm GF fiber filters were chosen due to their prior use in Great Lakes Sea Lamprey eDNA sampling (Gingera et al. 2016; Schloesser et al. 2018) and because the results of the filter optimization experiment were not yet available. After water filtration, each filter was placed into a uniquely labeled coin envelope and preserved with 100 g of desiccated silica. The coin envelope with the field negative control was stored in its own resealable plastic bag, separate from the three coin envelopes containing filters from the biological replicates (see Appendix). In total, 232 water samples were collected, 116 with the peristaltic pump and 116 with the OSMOS aquatic eDNA sampler.

As in Experiment 1, the field data sheet (see Supplementary Information S1: Environmental DNA Sampling Field Data Sheet available in the online Supplement of this publication) used to record sampling metadata was a modified version of the GEN-FISH Lotic eDNA Collection Data Sheet (GEN-FISH 2022a). For the coordinates of the 28 sampling stations, see Supplementary Information S4 (Sampling Station Coordinates for the Filtration Device Comparison available in the online Supplement of this publication).

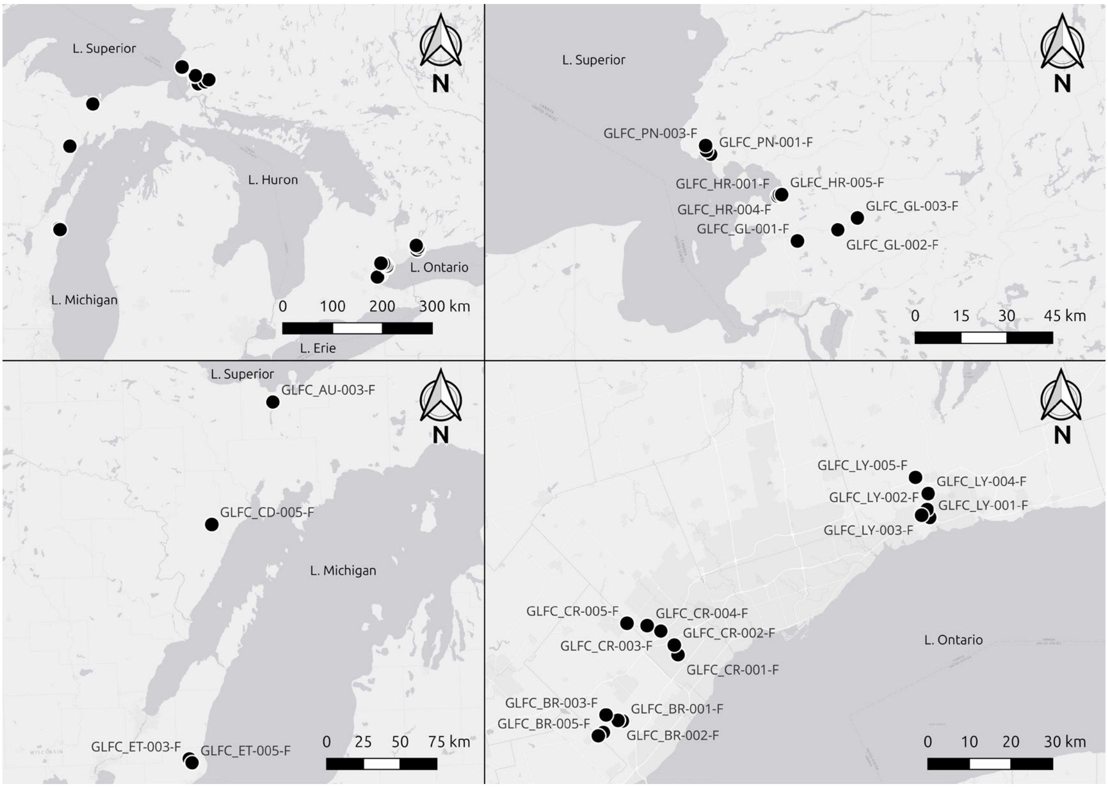

FIGURE 3. Map of the 28 stations sampled for Experiment 2. At each sampling station, three biological replicates and one field negative control were collected with an automated OSMOS aquatic eDNA sampler (Halltech OSMOS) and a do-it-yourself peristaltic pump, described in Gingera et al. (2016). Samples were then analyzed to compare each filtration device with respect to the frequency of detection, contamination of the field negative control, and prevalence of PCR inhibition. Map was created using QGIS Desktop (available at https://qgis.org/).

DNA Extraction and qPCR Analysis

The eDNA sample extraction and analysis followed the same methods as Experiment 1, with the following modifications: in the winter of 2022, the analysis was conducted using 96-well qPCR plates on the QuantStudio™ 7, with four qPCR technical replicates per sample. Additionally, each 96-well qPCR plate included a dilution series of Sea Lamprey DNA gBlock™ (with concentrations of 724, 7,240, and 72,400 DNA copies/µL) as a positive control, four no-template controls, and one no-amplification control.

Data Analysis

The fractional number of cycles in a PCR reaction required for fluorescence to reach a quantification threshold is called the quantification cycle (Cq). The reaction conditions for the qPCR analysis used 45 cycles (see Supplementary Information S3: qPCR Assay Details available in the online Supplement of this publication), so the potential range of Cq values was from 0 to 45. Each sample was classified as inhibited or uninhibited by averaging the Cq value for the IPC across the four qPCR technical replicates. A threshold for inhibition was established by averaging the Cq values from the no-template controls (27 Cq) and extending above this value by 3 Cq (Hartman et al. 2005), corresponding to approximately a one order of magnitude lower concentration than expected. This reduction in concentration is well beyond the expected qPCR error variance and signifies a significant decrease in assay sensitivity due to inhibition (Hartman et al. 2005). Biological samples with an average IPC Cq value below 30 were classified as uninhibited (0), and values above this threshold were classified as inhibited (1). The inhibition outcome (0,1) for the eDNA samples was compared with the filtration device as the treatment level.

To test the effect of the two filtration devices on each of the three response variables, a generalized linear mixed model (GLMM) fitted by maximum likelihood (Laplace approximation) with a binomial error distribution was implemented in R Statistical Software (v4.3.1; R Core Team 2023) using the lme4 package (version 1.1-35.5; Bates et al. 2015). This model allowed exploration of the filtration device effect on the likelihood of sample inhibition. The GLMM was structured with the response variable representing the proportion of inhibited samples. The instrument used was introduced as the fixed effect, while random effects were incorporated to address variations at the river level.

Similarly, an analysis implementing a GLMM, as described above, was used to investigate potential differences in the proportion of contaminated field negative controls between the two filtration devices. The GLMM was structured with the response variable representing the proportion of contaminated field negative controls collected by each filtration device. Each negative control sample collected was classified as contaminated (1) or non-contaminated (0), with any amplification of the CytB assay in the four technical replicates classified as contamination. Following this, stations where inhibited or contaminated eDNA samples were collected by either filtration device were removed from the data set.

The last analysis focused on evaluating potential differences in the proportion of detected samples between the two filtration devices. The GLMM was structured with the response variable representing the proportion of detected samples, with the filtration device as the fixed effect. Detection was classified as positive if any of the four qPCR replicates from the field samples amplified.

Experiment 3: Determining the Attenuation of Adult Sea Lamprey eDNA Signal in a Great Lakes Tributary

Study Location

River selection was crucial for experiment accuracy. The chosen river needed a reliable history of large spawning runs and minimal to no larval Sea Lamprey presence. This choice was driven by the need to accurately determine when the eDNA from spawning Sea Lamprey had dissipated from the system, without ambiguity caused by the presence of eDNA from larval Sea Lamprey. The Humber River (Ontario) has a large spawning run of Sea Lamprey in the spring up to a series of six Sea Lamprey barriers in the 1-km reach upstream of our sampling site, with the first barrier being approximately 150-m upstream of our sampling location (Sea Lamprey Control Map; http://data.glfc.org). Furthermore, despite evidence of egg survival to the pro-larval stage, no larval Sea Lamprey have been detected in recent years by electrofishing or Bayluscide surveys (R. Booth, DFO, personal correspondence). Bayluscide (Bayer 73) is a toxin sprayed on top of the water that causes larval Sea Lampreys to die and rise to the water's surface; it is used both for population control and abundance estimations (Howell et al. 1964). Thus, since larvae are not present in the Humber River, the eDNA signal was expected to drop to zero when spawner eDNA had left the system.

eDNA Sample Collection

The eDNA sampling took place at five time points on the Humber River (43.652 276 6° N, -79.492 065 7° W) beginning on July 2, 2022, with each sampling activity spaced two weeks apart. During each eDNA sampling activity, a field negative control was filtered from a sterile bucket of tap water, followed by the collection of three biological replicates from the river, using the OSMOS aquatic eDNA sampler in conjunction with 5.0-µm CN filters (Cytivia), a choice informed by the results of Experiment 1. Filtration proceeded until either 2 L of water had been filtered or the filter became clogged. Out of the 15 field samples collected, one filter became clogged, filtering only 0.5 L of water instead of the intended 2 L. Following sample collection, each filter was placed into a uniquely labeled coin envelope and then preserved in 100 g of desiccated silica in resealable plastic bags. After returning from the field, the filters, still preserved within the coin envelopes and desiccated silica, were stored at -20°C to await DNA extraction. The same field data sheet used to record sampling metadata in Experiments 1 and 2 was used for this survey (see Supplementary Information S1: Environmental DNA Sampling Field Data Sheet available in the online Supplement of this publication; GEN-FISH 2022a). See Appendix for the procedure for eDNA sample collection.

DNA Extraction and qPCR Analysis

DNA extraction from the filters occurred 2–4 months after collection. DNA extraction from the filters was performed using a Qiagen DNeasy® Blood & Tissue Kit and QIAshredder, following a modified version of the manufacturer's protocol (see Supplementary Information S2: DNA Extraction Protocol Two available in the online Supplement of this publication). Each extraction batch included an unused 5.0-µm CN filter as an extraction negative control to check for lab-based contamination. Extracts were stored at -20°C until qPCR analysis. All samples underwent qPCR analysis with eight technical replicates using the duplexed assay described in Experiment 1. Each qPCR run also included a positive control (7240 DNA copies/µL), six no-template controls, and a no-amplification control.

Data Archiving

For all three experiments, field data sheets, photographs, lab data, and qPCR RDML files were archived in the Hanner Lab information system (Borisenko et al. 2024).

RESULTS

Experiment 1: Assessing Filter Material and Pore Size

The Kruskal-Wallis test indicated significant differences in median DNA copy numbers among filter types for the eDNA samples from the Credit River ($P = 7.631 \times 10^{-10}$), but not for samples from Big Otter Creek ($P = 3.449 \times 10^{-1}$), at $a = 0.05$. As the Kruskal-Wallis test was not significant for the samples from the Big Otter Creek location (Figure 4), a Dunn's multiple comparison test was not applied. At the Credit River sampling station, the median DNA copies/µL was significantly higher for the 1.5-µm GF filter and the 1.2-µm and 5.0-µm CN filters compared to the 0.45-µm CN filters (Table 3, Figure 5). Notably, no significant difference in median DNA copies/µL was observed between the 1.2-µm CN and 5.0-µm CN filters in either location (Table 3; Figures 4 and 5). In the analysis of samples from Big Otter Creek, no fluorescence was observed in the no-amplification controls. This observation led to the decision to not include no-amplification controls for the qPCR runs for samples from the Credit River.

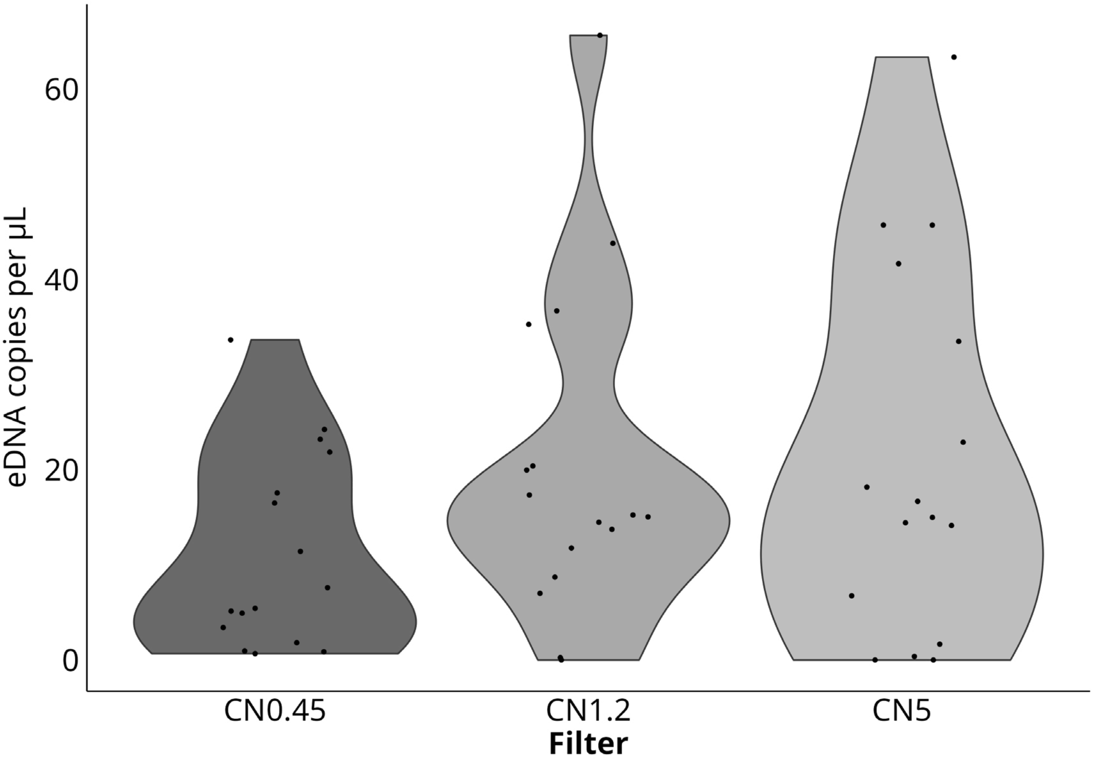

FIGURE 4. Number of eDNA copies per µL by filter type for water samples collected during Experiment 1, Big Otter Creek, Ontario, September 2021. For the violin plots, areas of greater width correspond to value ranges where data points are more concentrated, whereas narrower sections represent ranges where observations are sparser. For the filter-type abbreviations, the first two characters are the filter material, and the following number is the filter pore size in µm.

TABLE 3. Adjusted probability values resulting from the post-hoc Dunn's multiple comparison test for Experiment 1, Credit River, Ontario, to assess differences in DNA copies/µL among independent groups of biological samples collected from each location. The test followed a significant Kruskal-Wallis analysis, aiming to identify pairwise differences in median DNA copies/µL among filter types. For the filter-type abbreviations, the first two characters are the filter material, and the following number is the filter pore size in µm. Adjusted probability values significant at the 5% level are denoted by an asterisk.

Filter comparison	Credit River
CN0.45 - CN1.2	$1.26 \times 10^{-8*}$
CN0.45 - CN5	$2.26 \times 10^{-8*}$
CN1.2 - CN5	1.00×10^{0}
CN0.45 - GF1.5	$1.66 \times 10^{-3*}$
CN1.2 - GF1.5	$4.37 \times 10^{-2*}$
CN5 - GF1.5	5.71×10^{-2}

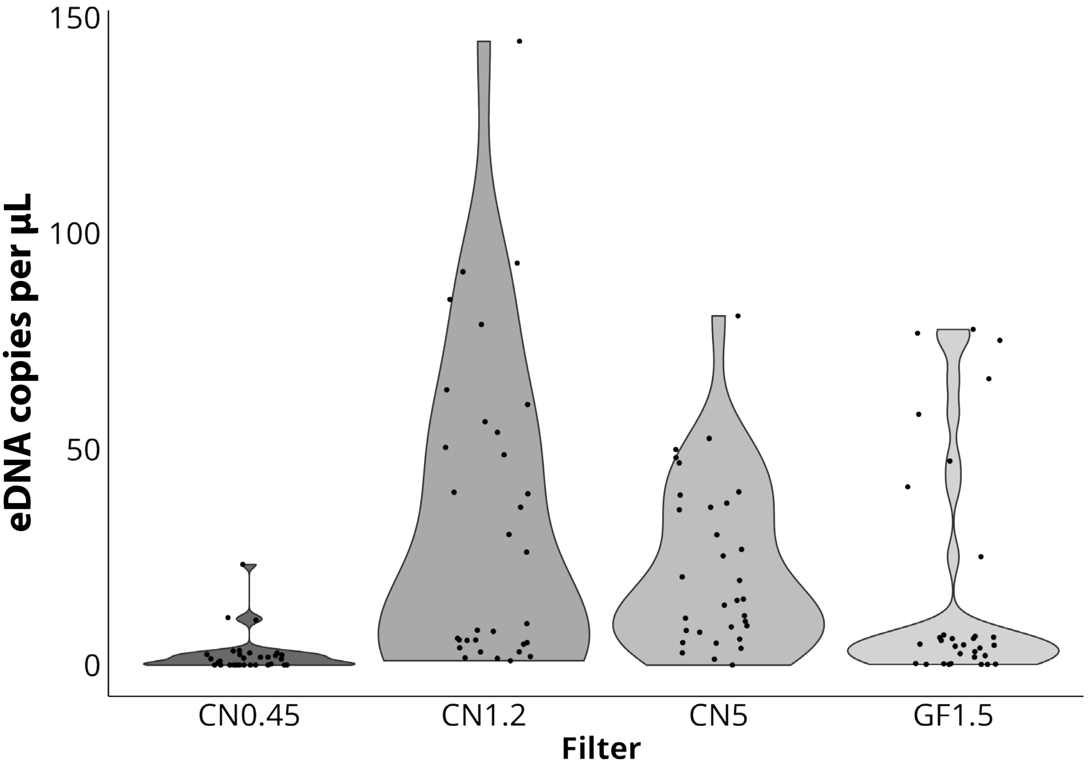

FIGURE 5. Number of eDNA copies/µL by filter type for water samples collected during Experiment 1, Credit River, Ontario, June 2022. For the violin plots, areas of greater width correspond to value ranges where data points are more concentrated, whereas narrower sections represent ranges where observations are sparser. For the filter-type abbreviations, the first two characters are the filter material, and the following number is the filter pore size in µm.

Experiment 2: Comparing Two Filtration Devices For eDNA Sample Collection

The difference in frequency of inhibited samples between the two filtration devices was not statistically significant (GLMM, $P = 0.16134$) and was influenced by the river of collection. For the OSMOS aquatic eDNA sampler, 17.86% of the 84 non-negative-control samples were inhibited compared to 23.81% of the 84 non-negative-control samples collected with the peristaltic pump (Figure 6). The standard deviation of the GLMM was 15.06 with river as a fixed effect, which suggests that the log odds of inhibition vary greatly among river systems.

For contamination, 2 of the 28 field negative controls collected with the OSMOS aquatic eDNA sampler were contaminated (7.14%), and five of the 28 field negative controls collected with the peristaltic pump were contaminated (17.86%) (Figure 6). However the GLMM analysis suggests the difference was not statistically significant ($P = 0.2099$).

There were no statistically significant differences in the frequency of positive non-negative-control samples between the OSMOS aquatic eDNA sampler and the peristaltic pump after samples from stations with contaminated field negative control and inhibited field were excluded from the GLMM analysis ($P = 0.831$). The frequency of positive non-negative-control samples for the OSMOS aquatic eDNA sampler was 57.79% of 45 samples compared to 55.56% of 45 samples for the peristaltic pump (Figure 6).

The three-fold dilution series included with each qPCR run amplified with varying quality: some amplified as expected, while others showed variations in Cq value. While not ideal,

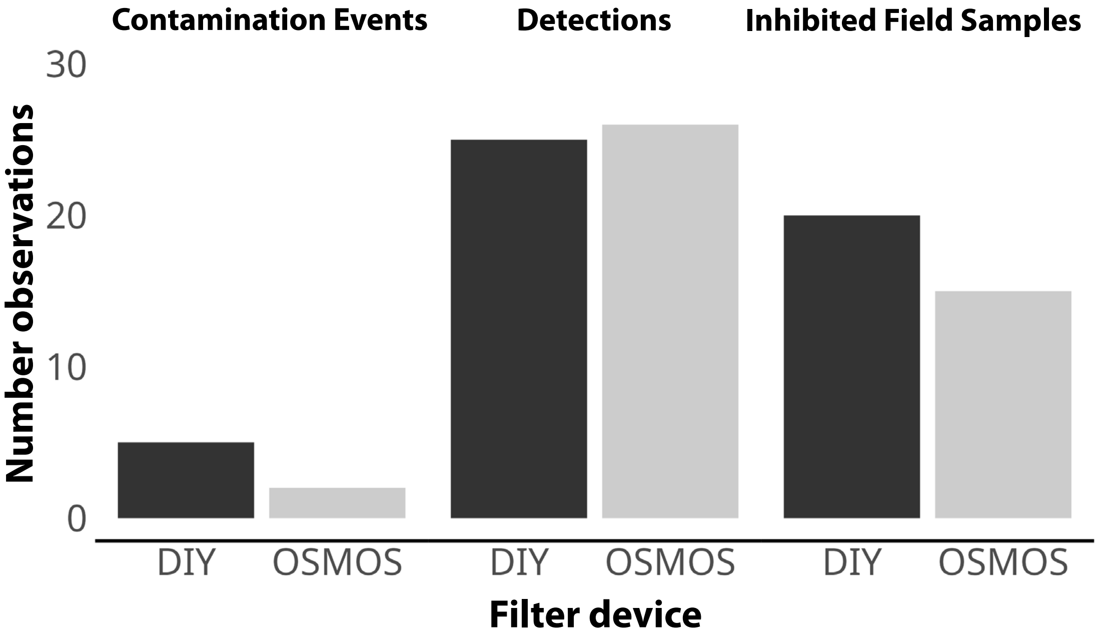

FIGURE 6. Number of eDNA contaminated field negative controls, detections, and inhibited samples for the OSMOS aquatic eDNA sampler and peristaltic do-it-yourself pump filtration devices that occurred at 28 stations across 9 tributaries in the U.S. and Canada during the fall of 2021.

the conclusions drawn from the analysis are based on qualitative presence-absence and do not depend on the data from the dilution series. Additionally, there is no evidence that the sensitivity of the analysis changed among runs, as the mean and variance for each run do not differ significantly. For the analysis of qPCR plate variance, see Supplementary Information S5: Analysis of Cq Mean and Variance by Run for the Filtration Device Comparison Experiment available in the online Supplement of this publication.

Experiment 3: Determining the Attenuation of Adult Sea Lamprey eDNA Signal in a Great Lakes Tributary

Sea Lamprey eDNA detections were observed in field samples during the first (July 4) and second (July 18) sampling activities but not during any of the three sampling dates in August (Figure 7). Importantly, there was no contamination detected in any of the field negative controls, lab negative controls, or qPCR no-template controls. Furthermore, the consistent amplification of positive controls across all qPCR runs provides strong evidence that the absence of Sea Lamprey eDNA in the latter samples is likely due to the actual absence of eDNA rather than issues with the qPCR reagents or conditions. This pattern suggests that the spawning Sea Lamprey eDNA signal attenuated between the second and third sampling activity.

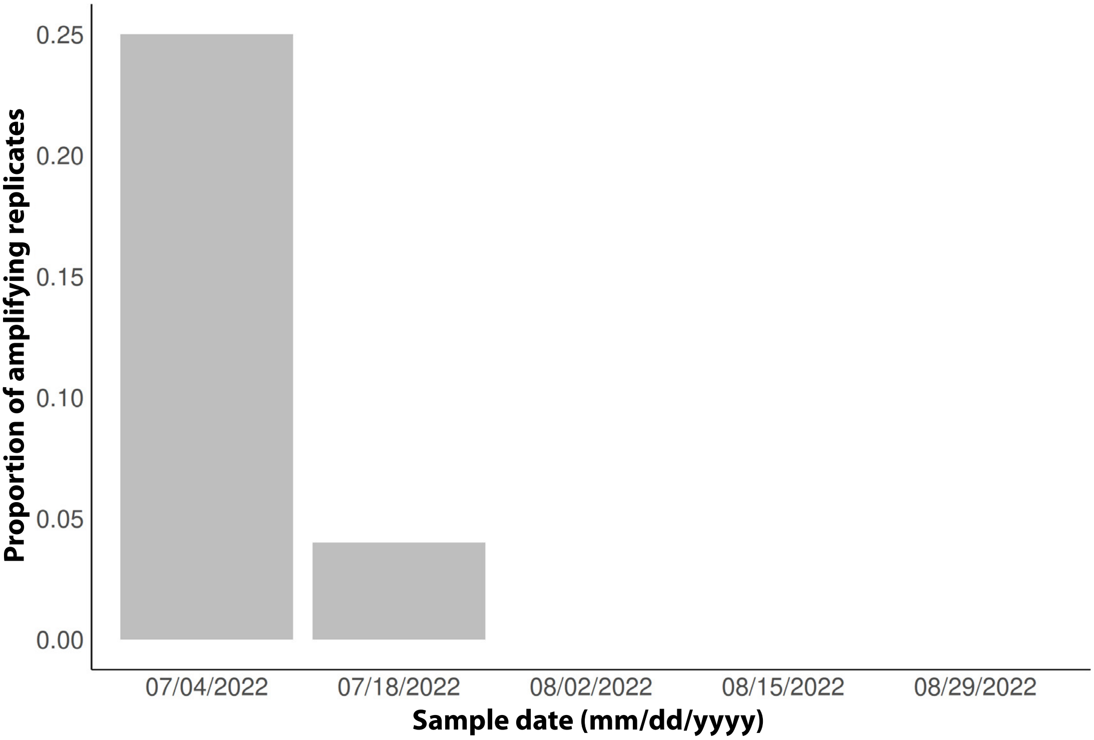

FIGURE 7. The proportion of qPCR replicates that registered a Cq value for eDNA samples of adult Sea Lamprey collected in Experiment 3 from the Humber River, Ontario, on five dates in 2022. Proportion of qPCR replicates amplifying is shown for each date the samples were collected; the last adult spawning Sea Lamprey was trapped on June 17, 2022.

DISCUSSION

By synthesizing the results from the three experiments into a comprehensive eDNA sampling protocol, we hope to provide a practical solution for ecosystem managers seeking to survey larval Sea Lamprey with eDNA methods. Our thoroughly tested protocol aims to promote wider adoption of eDNA collection methods for management applications. Postponing sampling for a minimum of six weeks after the spawning run ends, using a 5.0-µm CN filter in combination with the OSMOS aquatic eDNA sampler (or the backup peristaltic pump), and following rigorous negative control and sterile-technique protocols represent substantial advancements in determining the most effective eDNA methods for monitoring larval Sea Lamprey.

The 5.0-µm CN filter was selected for routine use, despite the absence of a statistically significant difference in median DNA yield between the two consistently highest-performing filters (1.2-µm and 5.0-µm CN) from either sampling location. This decision was based on additional considerations, particularly the expected reduction in clogging risk due to its larger pore size. Along this line, using a larger pore-size filter may improve eDNA yield. Research by Thomas et al. (2018) suggests that high filtration pressures can lead to reduced eDNA retention on filters. Therefore, using a larger pore-size filter may decrease the likelihood of high filtration pressures, potentially enhancing eDNA retention. The 5.0-µm CN filter was also determined to be the best choice for the OSMOS aquatic eDNA sampler's pump performance (M. Hall, Halltech, personal correspondence), which may help lower filtration pressure, enhancing DNA retention and reducing stress on the filtration device.

Our study supports the findings of Toshiaki et al. (2020), which concluded that reduced eDNA yields were not caused by larger filter pore sizes, despite the concern that large pore sizes may allow smaller DNA fragments to pass through the filter (Turner et al. 2014). Research has indicated that factors beyond pore size significantly influence DNA yield. For example, the choice of extraction kit and its interaction with filter material may play a significant role. Our use of the DNeasy® Blood & Tissue Kit, as shown in the study by Hinlo et al. (2017), may result in higher DNA yields when combined with CN filters instead of GF filters. This finding may explain why the 1.2-µm CN filter performed notably better than the 1.5-µm GF filter, despite their similar pore sizes. In our study, CN filters with a pore size of 1.2 µm or greater consistently captured the highest amount of eDNA and were chosen for subsequent use in Sea Lamprey eDNA monitoring. However, it is important to note that this choice, despite evidence of reduced clogging risk and efficient pump performance, may not be applicable for all species. Contexts may exist where smaller pore sizes, ranging from 0.2 µm to 0.6 µm as suggested by Eichmiller et al. (2016), could be more appropriate, and the specifics of the target organism and sampling environment should be taken into consideration when selecting a filter.

No significant performance differences were observed between the OSMOS aquatic eDNA sampler and the do-it-yourself peristaltic pump, indicating that both devices can be reliably used for Sea Lamprey eDNA sampling. For subsequent experiments, the OSMOS sampler was chosen as the primary device for field sample collection, while the peristaltic pump was included as a backup in the equipment list. In the field, equipment can be susceptible to damage, leading to temporary malfunctions in both filtration devices. Consequently, a backup filtration device became essential. The peristaltic pump is the more budget-friendly option, priced at approximately Can$800 for the pump alone and Can$1,200–$1,800 for the complete package including the power drill, replacement battery, and tubing. The peristaltic pump is also lighter and more compact, which might be desirable for remote sampling locations. However, feedback from field crews indicated that they generally favored the OSMOS sampler, which costs approximately Can$8,500–$10,000; they found it easier to use and better suited for routine monitoring. The OSMOS is also faster when it comes to the filtration time of individual samples, although this time savings was relatively minor in the course of the entire field sampling workflow. A multitude of eDNA sample filtration devices, including other autosamplers, are available on the market; users should select and test filtration devices based on project needs and budget constraints. Another crucial point to address is the handling of inhibition in eDNA samples. As shown in the results, samples collected using both filtration devices were affected by inhibition. Commercial products, such as the OneStep PCR Inhibitor Removal Kit (Zymo Research), are often used to remove PCR inhibitors from eDNA samples, although their efficacy can be inconsistent (Loeza-Quintana et al. 2021). Noncommercial methods of PCR inhibitor removal have also been employed in eDNA research (Milián-García et al. 2021).

Trapping data from the Humber River indicated that the highest number of adult spawners entered the system between May 8 and May 30, 2022, and the last adult spawning Sea Lamprey was trapped on June 17 (R. Booth, unpublished data). Sea Lamprey eDNA could still be detected (at lower rates) by July 18, but the lack of detections by August 2 indicates that the spawner eDNA signal had left the system within 4–6 weeks after the last adult Sea Lamprey capture. This pattern aligns with findings of Gingera et al. (2016), although without the confounding effects from a larval signal. Most Sea Lamprey spawning occurs in June–July, but the timing varies considerably across the Great Lakes. Adults usually begin entering tributaries when the water temperature reaches 15°C, and spawning can occur as early as May or as late as September (Manion and Hanson 1980). Therefore, the absolute timing (i.e., calendar date) of eDNA sampling for larval assessment will differ depending on regional spawning time but should be at least six weeks after spawning ends. Beyond the differences in the timing and duration of Sea Lamprey spawning, various environmental conditions can influence eDNA degradation and the duration that spawner eDNA remains viable for analysis. For instance, eDNA degradation may be affected by factors such as water temperature, ultraviolet B (UV-B) levels, and pH (Strickler et al. 2015), and dispersion will be impacted by river flow and hydrology (Harrison et al. 2019). Additionally, environmental variables, particularly temperature, are likely to affect the rate of eDNA production by spawning Sea Lamprey (Lacoursière-Roussel et al. 2016; Stewart 2019). To improve our estimates, future research endeavors should investigate how biotic and abiotic environmental factors, including water temperature, UV-B levels, and pH interact with eDNA and impact spawning Sea Lamprey eDNA production and attenuation.

While it is anticipated that Sea Lamprey eDNA from adult spawners will typically dissipate from a tributary about 4–6 weeks after spawning concludes, it is important to consider the potential impacts of climate change on this time frame. Lennox et al. (2020) have projected that climate change could alter the life history of invasive Sea Lamprey and, as surface water temperatures in the Great Lakes continue to rise (Trumpickas et al. 2009), the timing and duration of Sea Lamprey spawning may change. In addition to these factors, climatic changes are also expected to modify the peak daily flow of Great Lakes tributaries (Byun et al. 2019). However, the precise effects of these changes on the attenuation of the eDNA signal after the spawning run remain uncertain. Understanding these complex interactions between environmental variables and eDNA dynamics is crucial for accurate monitoring and management of Sea Lamprey.

Contamination is a common and ongoing concern in eDNA studies (Sepulveda et al. 2020) and, as reflected in our results, this study was also impacted by contamination. Although we adhered to rigorous protocols for collecting negative controls and decontaminating equipment, it is important to further improve these methods to reduce the incidence of contamination in future research. Several guidelines for eDNA sample collection in aquatic systems have been developed (Carim et al. 2016; Welsh et al. 2019; Amberg and Hunter 2022; Vazquez et al. 2023), providing valuable resources for strategies to mitigate contamination. Based on the results of our study, contamination was observed only in the field blanks and was absent from the DNA extraction and qPCR negative controls. Therefore, our efforts should focus on maintaining sterile techniques during field collection. Potential ways to reduce contamination include increased use of sterile single-use materials, adopting a tiered approach to decontamination (Vazquez et al. 2023), and providing additional training and practice for field crews.

In summary, we recommend initiating eDNA sampling for larval Sea Lamprey at least six weeks after the estimated regional spawning period, using a 5.0-µm CN filter in combination with the OSMOS aquatic eDNA sampler (or the peristaltic pump as a backup), and adhering rigorously to negative control and sterile-technique protocols. Other eDNA samplers are available and could be used for eDNA collection; however, significant differences can exist, and their performance would need to be tested (e.g., Nolan et al. 2023) before using. The protocol for eDNA sample collection is presented in the Appendix.

Our proposed approach considered not only the ecology of the target species, but also the need for rigorous contamination prevention and the nuances of collection and filtration methods, thus representing a significant advancement in optimizing eDNA methods for larval Sea Lamprey monitoring. This well-validated approach improves the efficacy and reliability of eDNA-based monitoring efforts, providing a valuable tool for the sustainable management of aquatic ecosystems and addressing critical gaps in the field, thereby supporting long-term sustainability.

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APPENDIX

Standard Operating Procedure: Environmental DNA Field Sample Collection for Invasive Sea Lamprey Monitoring

Document Control

Version	Date	Description of changes	Authors
1.0	January 1, 2024	Initial creation	Cameron D. Brown, Robert H. Hanner, Margaret F. Docker

1 Purpose and Scope

The primary goal of this Standard Operating Procedure (SOP) is to standardize the collection of environmental DNA (eDNA) samples for monitoring the invasive Sea Lamprey *Petromyzon marinus* in Great Lakes tributaries. This SOP provides a detailed, step-by-step methodology for eDNA sample collection, ensuring accurate and reliable eDNA data collection that is essential for decision making.

This SOP specifically addresses the collection of eDNA samples targeting the larval stage of the Sea Lamprey. It is applicable to the environmental conditions present in Great Lakes tributaries. For adaptations of this protocol to different environmental conditions, life stages, or target species, consultation with an expert is advised, as variations to the sampling parameters (e.g., life stage, sampling environment, target species) may necessitate modifications to the procedure.

2 Responsibilities

While many individuals may participate in the eDNA sample collection process, a minimum of two people is required for samples to be collected effectively. The roles are data recorder and sample handler.

2.1 Data Recorder

The data recorder is responsible for accurately capturing all necessary data during eDNA field sample collection. This includes recording essential details on the field data sheet, such as site name, station name, date, sample ID, time of collection, and volume of water filtered. The data recorder must also ensure that photographs of the physical sample and sampling station, if required, are taken and appropriately documented. Although others may assist, one individual must be assigned this responsibility to ensure the accuracy and completeness of all recorded data.

2.2 Sample Handler

The primary responsibility of the sample handler is to manage the filter used for collecting eDNA from the water. This includes placing the unused filter into the filtration device before collection and transferring the filter into the appropriately labeled coin envelope preserved in desiccated silica after sample collection. Proper handling is crucial to avoid contamination or damage to the filter, which could significantly reduce the likelihood of obtaining usable results.

2.3 Additional Duties

Additional tasks in eDNA sample collection include setting up and dismantling equipment, collecting supplementary data (e.g., water-quality measurements), and managing plastic waste. After the primary responsibilities of data recorder and sample handler are assigned, field teams are encouraged to distribute additional duties among themselves as they see fit. This distribution should occur in a manner that ensures efficiency and adherence to the SOP.

3 Materials and Equipment Sampling Checklist

3.1 Common Items for OSMOS and DIY Sampling (GEN-FISH 2022b, 2022d)

- Sterilized buckets: individually wrapped in plastic bags
- Tap water: stored in a sterilized container for the field negative control
- Backpack or field bin: for transporting supplies to the field location
- Sterile nitrile gloves: ensure sizes fit all team members
- Kimwipes™ or paper towel: for on-site sterilization or equipment maintenance
- ELIMINase™: for cleaning and decontaminating equipment
- First aid kit: fully equipped for emergency scenarios
- Garbage bags: for waste disposal
- Clipboard: to hold and manage field data sheets
- Field data sheets: for recording sampling data
- GPS: for accurate location tracking.
- Water chemistry probe (optional): for collecting water chemistry metadata
- Forceps and filters: sufficient for planned sampling, pack five extra as a buffer
- Coin envelopes: sufficient for planned sampling, plus additional spare envelopes
- Plastic bags: small and large, resealable, for sterile material storage and sample preservation
- Silica beads: 30 g per negative-control bag, 90-100 g per bag for biological replicates
- Labels for each coin envelope and station bag, plus blank extras
- Filters, forceps, coin envelopes, and silica beads packed according to the infographic below (Figure A.1).

For a visual summary of materials needed across sampling stations, refer to Figure A.2.

FIGURE A.1. Infographic showing the consumable materials required for OSMOS sampling: the set includes sterile forceps with a preloaded filter canister, three filters for biological replicates, a sterile bucket sealed in a garbage bag for collection of the negative control, three coin envelopes filled with silica for the biological replicates, and one coin envelope with silica for the negative control. It is essential to fill coin envelope bags with the specified amounts of silica beads—30 g for the negative-control bag and 90–100 g for each biological-replicate bag—to ensure proper preservation of eDNA samples (GEN-FISH 2022d).

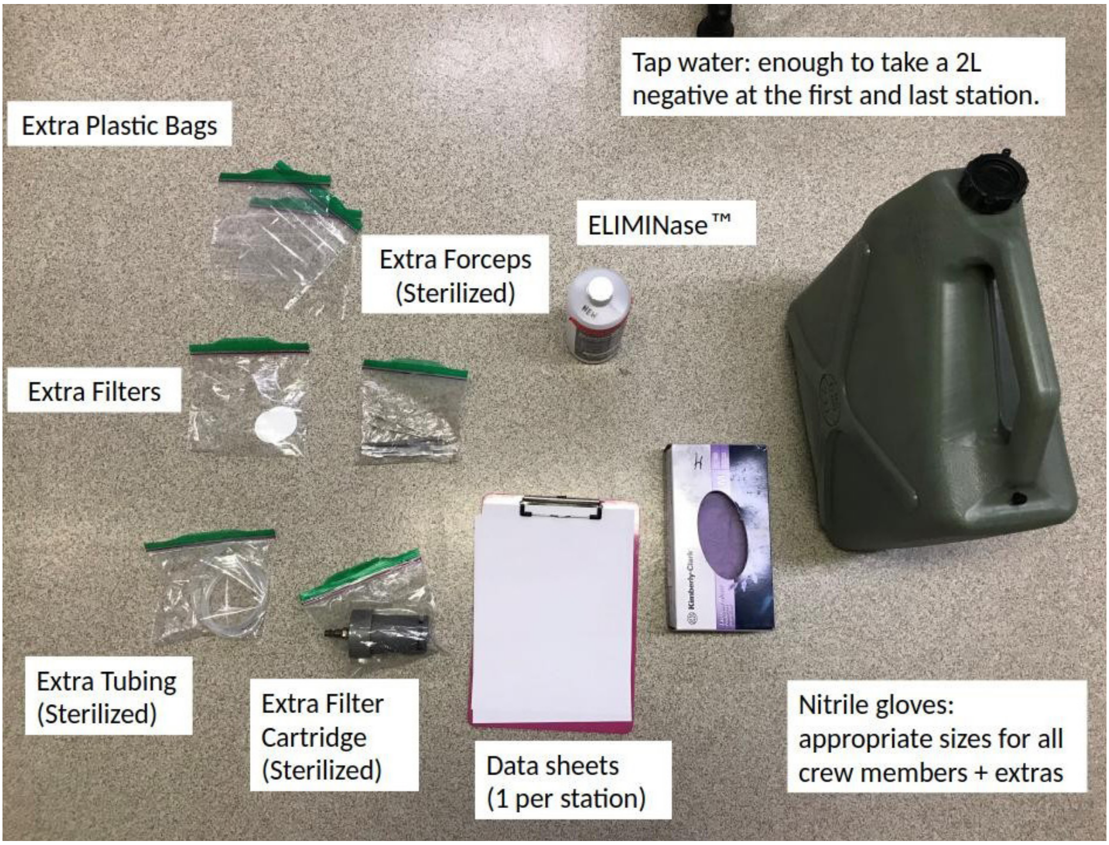

FIGURE A.2. Infographic showing the materials needed cross-station: sterilized container carrying enough tap water to collect a 2L negative at the first and last sampling station, nitrile gloves in appropriate sizes for all team members plus extras, five UV-irradiated plastic bags containing extra filters, five extra sterilized forceps, one extra filter cartridge (sterilized) for the OSMOS, extra tubing for peristaltic pump (sterilized), additional resealable plastic bags (both large and small sizes). Also bring field data sheets (1 per station) and ELIMINase™ (GEN-FISH 2022b, 2022d).

3.1.1 Specific Items for OSMOS Sampling (GEN-FISH 2022d)

- OSMOS
- Telescoping pole for OSMOS
- Inflow tube for OSMOS
- Outflow tube for OSMOS

3.1.2 Specific Items for DIY Peristaltic Pump Sampling (GEN-FISH 2022b)

- Reaching pole: or suitable alternative, such as a dip net, for sample collection
- Flat-head screwdriver
- Wrench
- Filter holder: part of the peristaltic pump kit
- Cordless drill Nalgene® bottles: ensure an adequate number are available for planned sampling
- Drill battery: charge fully before leaving for sampling site
- Duct tape: for securing components or making quick repairs in the field

See Figure A.3 for a visual guide of the consumable materials needed for aquatic eDNA sample collection with the DIY peristaltic pump.

FIGURE A.3. Infographic showing the consumable materials required for the DIY peristaltic pump sampling. The set includes one coin envelope in desiccated silica for the negative control, and three coin envelopes in desiccated silica for the biological replicates. Additionally, there are four sterile forceps, four filters for collection of the biological replicates and the negative control (NC), sterile tubing, and a Nalgene® bottle. For adequate preservation, the sealable plastic bag containing the coin envelopes should be filled with silica beads—30 g for the negative-control bag and 90–100 g for the bag containing the biological replicates (GEN-FISH 2022b).

3.1.3 Important Note

Collecting eDNA samples often incurs significant costs and time commitments, primarily due to the transportation of personnel to and from sampling stations. To ensure efficient sample collection and to mitigate the risk of equipment failure, it is advised that sampling teams prepare and bring both types of filtration devices on the sampling venture. This strategy helps ensure successful sampling, even if one of the devices encounters mechanical issues.

3.2 OSMOS Material Preparation

Prior to eDNA sampling using the OSMOS sampler, a rigorous sterilization process is required to ensure the integrity of collected samples. Prior to any sampling event, perform the following steps.

3.2.1 Clean OSMOS Canisters and Forceps

OSMOS filter canisters and forceps are to be cleaned using a standardized protocol. A bucket capable of holding a minimum of 3 L of water (for the field negative control) is sterilized by either soaking it in a 10% bleach solution for 30 min or wiping it down with ELIMINase™. The sterilized bucket is then sealed inside a clean garbage bag.

3.2.2 Sterilize Jerrycan

A jerrycan (or similar water container) is sterilized by rinsing the inside with 10% bleach solution and then filling the container with tap water to transport the water used for the field negative control to the sampling station.

FIGURE A.4. Infographic showing the recommended organization of consumables for eDNA field sample collection with the OSMOS and the DIY peristaltic pump. Each station's consumables are placed into a single large plastic bag, clearly labeled with the station ID for easy identification and organization: A = OSMOS sampling bag containing a loaded filter cartridge, forceps, three filters for the biological replicates, and three coin envelopes in desiccated silica for the biological replicates; B = DIY peristaltic pump sampling bag containing sterile forceps, tubing, four filters, three coin envelopes for desiccated silica for the biological replicates, and one coin envelope in desiccated silica for the negative control (GEN-FISH 2022b, 2022d). Such organization is critical for efficient and accurate eDNA sample collection.

3.2.3 Prepare Consumable Materials

Forceps, filters, and gloves are placed into separate resealable plastic bags. The plastic bags containing the forceps and gloves are then subjected to 15 min of ultraviolet (UV) irradiation (GEN-FISH 2022b, 2022d). Filters should not be exposed to UV irradiation as this can damage the filters. See Figure A.4 for the recommended organization of consumables for eDNA sample collection with both the OSMOS Aquatic eDNA Sampler, and the DIY peristaltic pump.

3.2.4 Store Silica Beads

Resealable plastic bags are filled with desiccated silica beads, with each bag containing 30 g of silica per coin envelope (GEN-FISH 2022b, 2022d). One coin envelope per plastic bag is used for field-negative controls, while three envelopes per plastic bag are used for each of the biological replicates.

3.2.5 Label Coin Envelopes

Coin envelopes are labeled with a unique sample code and sample bags are labeled with unique station codes.

3.2.6 Treat with UV Irradiation

All materials, including silica beads, coin envelopes, and labels, are subjected to 15 min of UV irradiation within a sterilized UV cabinet before use in the field (GEN-FISH 2022b, 2022d).

3.2.7 Decontamination Protocol for OSMOS

3.2.7.1 OSMOS Unit

- Set volume and filter 10 L of tap water through the unit.
- Wipe down OSMOS unit, telescoping pole, tripod, and inflow tubing with ELIMINase™ and paper towel or bleach wipes. Rinse with tap water or wipe down with wet paper towel. Look for scrapes on the OSMOS unit, fraying and ripping of backstraps, and permanent marks on the OSMOS control panel glass.
- Soak outflow tubing in 1% bleach for 30 min.
- Thoroughly rinse the tubing and dry with a paper towel, then hang to air-dry.

3.2.7.2 Filter Cartridge

- Separate the filter housing into its three components.
- Soak rubber gaskets in ELIMINase™ for 5 min.
- Submerge inlet- and final-stage components in 10% bleach for 30 min.
- Rinse each component three times in distilled water; allow draining between each rinse.
- Allow to air-dry.
- Repeat rinsing if bleach residue is present.
- Rinse rubber gaskets with distilled water.

3.2.7.3 Forceps

Soak in 10% bleach for 30 min, rinse three times with distilled or tap water, then air-dry. This can be done with cartridges.

4 Station Workflow for eDNA Sample Collection

4.1 Workflow for eDNA Sample Collection at Each Station

4.1.1 Set up the OSMOS or DIY peristaltic pump system at the station.

4.1.2 Filter the negative-control sample (this step is performed only at the first and last stations).

4.1.3 Filter biological-replicate samples.

4.1.4 Take a photograph of the OSMOS/DIY setup to document the setup conditions.

4.1.5 Collect water-quality data or other additional environmental metadata.

4.1.6 Take photographs of the site to visually document the sampling environment.

4.1.7 Enter all necessary information into the field data sheet.

Note: Refer to the Halltech OSMOS manual and GEN-FISH guidance documents (GEN-FISH 2022b, 2022d) for how to properly assemble and disassemble each filtration device.

4.2 Workflow for Collection of Individual Sample

Maintain sterility during and after sampling to prevent contamination.

4.2.1 Handle the filter cartridge/pump head, filter, coin envelope, and forceps only with sterile gloves.

4.2.2 Be aware of contamination sources, including the outside of plastic bags, the OSMOS unit, telescoping pole (including brass fitting), drill, reaching pole, clothing, and the ground.

4.2.3 If your gloves, forceps, or filter come into contact with any contamination source, they must be considered not sterile and replaced immediately.

4.2.4 Sterile Sample Handling

The filter should always remain at least two sterile layers away from any potential source of contamination, such as filter, forceps, gloves, hand (source of contamination), or filter, coin envelope, plastic bag, larger sample bag for transport (source of contamination).

4.2.5 Material Separation

Materials that come into direct contact with the filter (forceps, gloves, coin envelopes, filtration canisters) are never shared across sampling stations and are always kept separate from materials used at other stations.

5 Sample Collection Procedure Overview

5.1 Individual eDNA Sample Collection Procedure

5.1.1 Prepare

Review the sample collection steps and set up the DIY peristaltic pump or OSMOS according to the manual upon arrival at the sampling station.

5.1.2 Prepare the Negative Control

Collect 1–2 L of tap water in a clean Nalgene® bottle or sterilized bucket to serve as a negative control sample.

5.1.3 Install Filter

Install a new filter into the pump head or filter housing using sterilized forceps while wearing gloves to maintain sterility.

5.1.4 Collect Sample

Use the OSMOS or DIY peristaltic pump to filter the negative control sample through the installed filter.

5.1.5 Store Filter

After filtration, carefully remove the filter, photograph it for documentation, fold it appropriately, and place it into the designated coin envelope to avoid contamination.

5.1.6 Collect and Filter Biological Samples

Repeat the filtering process for the biological water samples, following the same steps as above, ensuring sterility and proper handling for each sample.

This overview provides a quick reference of the sample collection process. Following is a detailed, step-by-step procedure.

6 DIY Peristaltic Pump Procedure (GEN-FISH 2022b)

6.1 Preparation

6.1.1 Prepare

- Start by reviewing the basic steps for sample collection using the DIY peristaltic pump.
- Set up everything according to the manual upon arrival at the sampling station.
- Ensure the DIY peristaltic pump is correctly and appropriately assembled.

6.1.2 Prepare the Negative Control

- Remove the clean Nalgene® bottle from the plastic transport bag.
- Open the Nalgene® bottle carefully while wearing gloves.
- Have a second person use a sterilized container to fill the Nalgene® bottle with tap water.
- Fill the bottle up to 1 L.

6.1.3 Install Filter

- Unscrew the pump head (filter handler wearing gloves).
- Hold the pump head during the process with the second person's assistance.
- Change gloves for sterility (filter handler).
- Carefully take a clean filter from the filter bag using fresh gloves.
- Grab a filter from the filter bag using sterilized forceps.
- Carefully place the filter into the pump head using the forceps.
- Place the orange O-ring over the top of the filter and the pump head.
- Securely screw the pump head shut. See Figure A.5 for an important note about differentiating the filter from the paper filter divider.

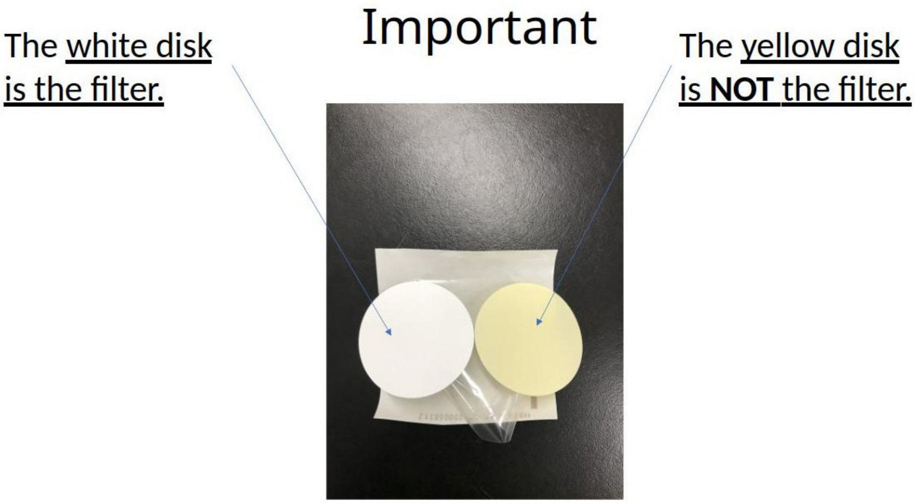

FIGURE A.5. The white disk is the filter. The yellow disk is NOT the filter. The color of the filter and the paper filter divider may differ depending on the brand and type of filter used. Always double check the product details.

6.1.4 Collect Sample

- Be conscious of the surrounding space during the process. To avoid contamination, ensure the pump head does not swing into and hit any objects like waders or the ground to avoid contamination.
- Have one person wearing gloves hold the Nalgene® bottle filled with tap water.
- Have a second person hold and operate the filtration system with the drill.
- Coordinate roles and actions to manage the equipment effectively during sample collection.
- Run the drill at a slow, steady pace to avoid tearing the filter.
- Drain and filter the entire 1 L of tap water from the Nalgene® bottle.
- Stop the process after the first liter is filtered.
- Refill the Nalgene® bottle with tap water from the sterilized water container.
- Filter the entire second liter of tap water, ensuring a total of 2 L has been pumped through the filter.

6.1.5 Store Filter

- Have the non-filter handler hold the filtration apparatus after sample filtration.
- Have the filter handler carefully unscrew the filter head.
- Remove the orange O-ring from the pump head using sterilized forceps.
- Have the secondary person take a photo of the filter in its current position for documentation.
- Have the filter handler then open the appropriate negative-control sample bag and open the coin envelope.
- Prevent contamination by only touching the coin envelope with the forceps.
- Place the filter inside the coin envelope to ensure minimal contact and contamination risk.

6.1.6 Collect and Filter Biological Samples

- Reuse the Nalgene® bottle for biological samples if it is clean, following the same procedures as for the negative control.
- Secure the Nalgene® bottle to the reaching pole or other suitable devices as per the instructions.
- Position the opening of the Nalgene® bottle facing upstream while collecting samples.
- Submerge the bottle about 15 cm below the waterline, stretching out as far as possible into the water.
- Focus on collecting samples from flowing water rather than still water or eddies.
- Filter each biological sample using the same steps as in the Sample Collection procedure.
- Follow the Store Filter procedure after filtering each biological sample. However, instead of placing the filter in the negative-control bag, use the designated coin envelopes and sample bag for each biological sample.
- Repeat the process for each biological sample, ensuring fresh gloves and sterilized forceps are used each time.
- Continue the process until all required biological samples have been collected and filtered.

6.1.7 Additional Notes

- When securing parts in place during the process, ensure they are firm but avoid overtightening to prevent breakage.
- The non-filter handler is responsible for managing the Nalgene® bottle used for the negative control, which can be reused for subsequent biological samples at that sampling station.
- Remember to record the estimated amount of water filtered and the time the filter photo was taken for each sample.

7 OSMOS Procedure (GEN-FISH 2022d)

7.1 Prepare

- Begin by setting up the OSMOS by the riverside, following the setup protocols provided in the Halltech OSMOS manual.
- Ensure the data recorder has noted station ID, latitude, longitude, additional metadata, and time of arrival.
- Perform any required pre-priming as dictated by the OSMOS model being used (GEN-FISH 2022c).

7.2 Prepare the Negative Control

- Take the container (such as a plastic bucket) out of its clean storage bag.
- Use a jerrycan or other sterilized water container to fill the bucket with about 3 L of tap water.

7.3 Set Up the OSMOS

- Take a sterilized filter housing from its sealed plastic bag.
- Firmly connect the filter housing to the OSMOS and its telescoping pole.
- Always handle the OSMOS canister with clean plastic gloves to minimize the risk of contamination.

7.4 Install the Filter

- Open the OSMOS canister.
- Remove the bottom part of the canister to access the filter placement component.
- Remove the O-ring from the inside of the canister using sterile forceps.
- Carefully place the filter flat on the metal mesh inside the canister.
- Replace the O-ring on top of the filter and reassemble the canister.
- Ensure all components are secure, including the connection of the canister to the telescoping pole.

See Figure A.5 for an important note about differentiating the filter from the paper filter divider.

7.5 Filter the Negative Control

- Lower the canister into the bucket containing tap water using the telescoping pole.
- Be careful to avoid having any part of the telescoping pole, including the brass knuckle, touch the waterline as this poses a contamination risk; see Figure A.6 for proper positioning of the filter cartridge to avoid contamination during negative control sampling.
- Follow the instructions in the Halltech OSMOS manual to filter 2 L of water from the negative-control bucket.

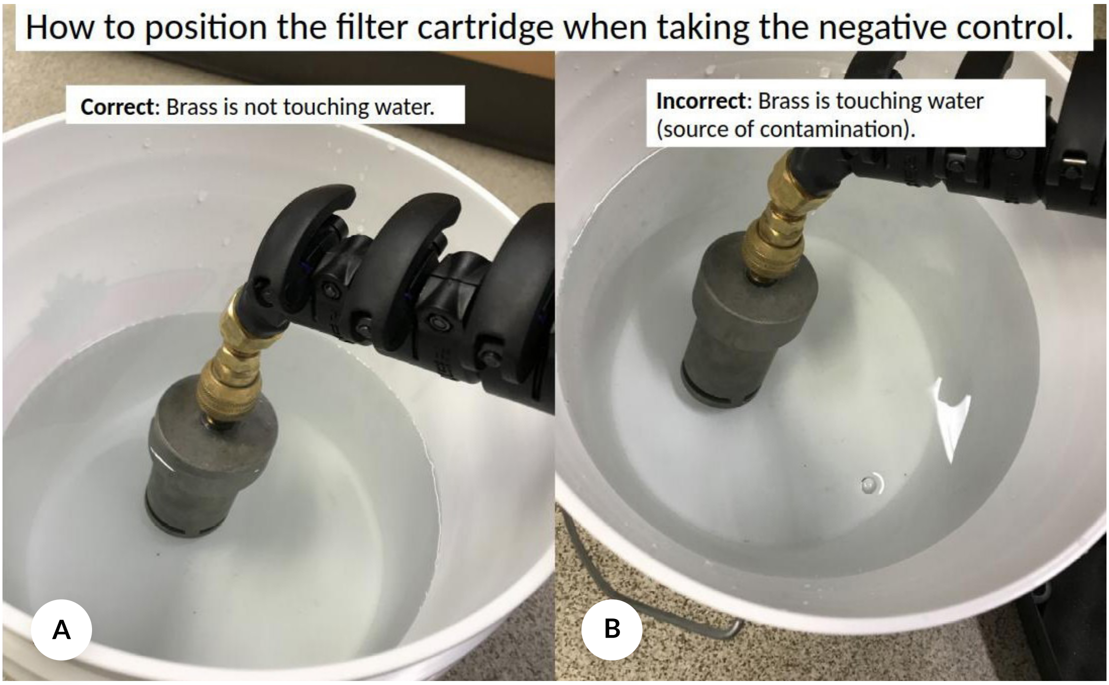

FIGURE A.6. How to position the filter cartridge correctly when taking the negative control: A = correct position—the brass on the telescoping pole is not touching the water. B = incorrect position—the brass on the telescoping pole (source of contamination) is touching the water.

7.6 Store the Filter

- Invert the OSMOS telescoping pole to drain the water after filtration.
- Carefully remove the bottom portion of the OSMOS canister to access the filter.
- Using sterile forceps, remove the O-ring so the filter is fully visible.
- Have a secondary person take a photo of the filter for documentation.
- Fold the filter in half with the dirty side facing in, and place it in the appropriately labeled coin envelope.
- Seal the coin envelope and the plastic bag.

7.7 Collecting and Filtering Biological Samples

- Repeat the filter installation steps for each biological sample.
- Extend the telescoping pole as far into the river as possible to collect the sample from flowing water.
- Lower the OSMOS canister so that the bottom of the canister is about 15 cm below the waterline.
- Notice that, for biological samples, it is acceptable for the telescoping pole to enter the water.
- Use the default OSMOS settings to filter 2 L of water from the river.
- Follow the same Store the Filter procedure as for the negative control.
- Record sample summary information including average sampling time, flow rate, total volume collected, and filter photo time.
- Repeat this process for each biological sample, ensuring sterilized gloves and forceps are used for each sample.

7.8 Key Parameters for Sampling

For consistent and accurate biological-replicate sampling, adhere to the following key parameters:

- Sample depth: The sample depth for biological replicates should be approximately 15 cm. This depth corresponds to the waterline being just above the first brass knuckle on the telescoping pole.
- Sample volume: The sample volume should be 2 L. Always use the default settings on the OSMOS system for this purpose.
- Pole extension: Extend the telescoping/reaching pole to its maximum length or to the center of the river, whichever is closer to your current position.
- Sampling location: Avoid sampling in eddies or still water. Always aim to sample from the flowing part of the river.

Collecting eDNA samples in accordance with these parameters is crucial for consistent and accurate data collection.

8 After-Sampling Procedures

8.1 Sample Storage and Disposal

- Place all sample storage bags (which include used filters, coin envelopes, and silica) into a large resealable plastic bag. Make sure this bag is labeled with the site ID.
- Used filter cartridges, forceps, gloves, and other contaminated materials should be disposed of in the garbage bag that was originally used to store the bucket.

8.2 Field Data Sheets

Field data sheets should be completed for each sampling-station visit. Record site information, crew details, date, weather conditions, water-quality parameters, volume filtered, and time of filter photographs.

8.3 Photographic Documentation

It is recommended that a series of photos be taken during each sampling event, capturing various angles of the sampling station, including the OSMOS aquatic eDNA sampler or DIY peristaltic pump during sampling. For guidance on photographing the filter, refer to Figure A.7. Ensure the rubber gasket is removed to fully display the filter, as shown in the left panel.

FIGURE A.7 A = this is the correct image; the rubber gasket has been removed, and the filter is completely visible. B = this is the incorrect image; the rubber gasket is still on the filter.

8.4 Sample Storage

After returning from the field, the filters are kept within the coin envelopes, and they (along with the desiccated silica in the labeled sample bag) should be stored at -20°C.

9 Troubleshooting and Contingencies

9.1 When to Retake a Sample

There are specific instances during the sampling process when a sample must be retaken to ensure the integrity of the results:

- The filter gets dropped or is otherwise contaminated.
- The filter cartridge or pump head gets dropped during filtration.
- A large tear in the filter occurs during sampling (it’s ok if the filter tears while folding it).

In the event that any one or more of these three circumstances occur, do not attempt to save the filter. Instead, discard it and start again with a clean spare.

9.2 Troubleshooting Filter Clogs

During the sampling process, it is possible to encounter filter clogging. This section outlines the steps to identify and document this issue for both the OSMOS and DIY peristaltic pumps.

- OSMOS: The filter is considered clogged when the average flow rate reduces to less than 0.05 L/min for a duration of 1 min.
- DIY: The filter is considered clogged if the pump is running but water has stopped exiting the pump head.

After a filter is determined to be clogged, proceed with the following steps:

- Place the clogged filter in its designated coin envelope.
- Make a note on the data sheet indicating that the filter has clogged (include the filter code on the coin envelope in the note).

9.3 Troubleshooting Common OSMOS Error Codes

When operating the OSMOS, certain errors may be encountered. Below are the recommended actions for the most common errors:

- Error #1 (Pressure exceeded warning): Press the "Ent" button and continue to collect the sample until completion.
- Error #2 (No filtration): (1) Be patient—the OSMOS may start filtering after a couple of minutes; (2) if filtration does not commence, stop the run, adjust the pressure to 80-90 kPa, and attempt to filter again.

10 Training and Competency

While reference manuals and SOPs, such as this document, can be helpful in facilitating the consistent and accurate collection of environmental DNA samples, it is critical to remember that the environmental DNA sample collection is a highly technical physical process that requires many hours of in-person training and repeated practice—things that an SOP alone cannot substitute for.

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Optimization of Methods for the Collection of Larval Sea Lamprey Environmental DNA (eDNA) from Great Lakes Tributaries

Cameron D. Brown, Robert H. Hanner, and Margaret F. Docker