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Genetic origins of a resurging lake whitefish, *Coregonus clupeaformis*, population in the Detroit River, Laurentian Great Lakes

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Abstract – The Detroit River connects Lake Huron and Lake Erie of the Laurentian Great Lakes. The river once supported a substantial lake whitefish (*Coregonus clupeaformis*) fishery until the early 1900s, when habitat loss, pollution, and overfishing contributed to the collapse of the fishery and loss of spawning populations in the river. In the early 1970s, efforts were initiated to improve water and habitat quality, and in December 2005 a spawning male lake whitefish and viable eggs were collected; the first documented evidence of spawning since 1916. Researchers have tracked the spawning magnitude of the lake whitefish population in the Detroit River since 2005 by assessing the number of eggs deposited on egg mats. Genetic analysis of larval fish hatched from eggs collected in the field between 2005 and 2018 was used to determine the relative contributions of Lake Erie and Lake Huron to the resurging population. Over 80% of the hatched larvae had parents originating from Lake Erie in all the years sampled. The estimated number of full-sibling families sampled at Belle Isle was the same in 2010 and 2014 and varied between 2009 and 2016 at Fighting Island. The estimated number of lake whitefish parents at Fighting Island decreased in the most recent collections possibly due to loss of habitat on spawning reefs due to sedimentation. Our results provide additional evidence that restored spawning habitat in the Detroit River is again being used by lake whitefish and continued reproduction at these sites may improve the Great Lakes portfolio of ecological and genetic diversity.

Keywords: Spawning habitat / restoration / individual assignment / effective number of breeders / Great Lakes

1 Introduction

Rocky reefs and coarse substrates in freshwater lakes and streams provide habitat for aquatic species at different life history stages. For example, they are used by lithophilic fishes for spawning. The location and composition of reef habitat in rivers can make them prone to degradation caused by sedimentation related to pollution or water control, or reefs may be removed to clear passage for shipping lanes or for dam construction (Gunderson *et al.*, 2008; Bennion and Manny, 2011). The St. Clair and Detroit rivers in the Laurentian Great Lakes are examples of rivers where fish spawning habitat was removed and degraded with a corresponding loss in fish abundance and species diversity (Fig. 1; Manny 2003), but more recently there have been environmental improvements resulting from regulation changes and targeted habitat restoration projects (Hartig *et al.*, 2018, 2020).

The Detroit River supported a large lake whitefish (*Coregonus clupeaformis*) fishery until the early 1900s (Baldwin *et al.*, 2009). Most of the fishing effort was located between the city of Detroit, MI, USA (42.3314 N, –83.0457 W), and the entry of the Detroit River into Lake Erie (Milner, 1874; Baldwin *et al.*, 2009) when fishing occurred during the fall during spawning runs of lake whitefish and cisco (*C. artedii*). While the fishery had started to decline in the late 1800s due to overfishing and pollution (Milner, 1874), the construction of shipping channels also affected lake whitefish and other fish using the river for spawning (Smith, 1915). During the construction of shipping channels in the early 1900s, cobble and bedrock used as spawning habitat at Grassy Island (42.2236 N, –83.1342 W; Detroit River) were removed and water flows through the river were restricted or halted (Smith, 1915). The total area of habitat lost is unknown (Bennion and Manny, 2011), but the loss is one of the main reasons for the decline and limited recovery of several fish species in lakes Erie and Huron (Roseman *et al.*, 2007; Manny *et al.*, 2015). In the early 1970s, ecological restoration

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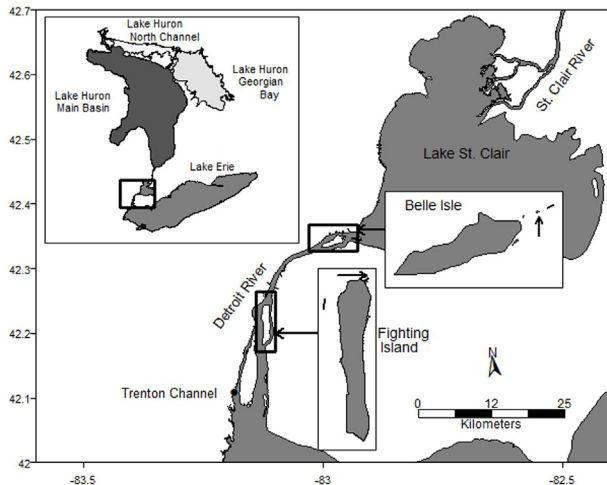


Fig. 1. Locations of sample collection sites in the Detroit River at Belle Isle and Fighting Island. Insets show the location of the reference populations in Lake Erie and Lake Huron, Lake Erie, Lake Huron-Main Basin, Lake Huron-Georgian Bay, and Lake Huron-North Channel (top right) used for the analysis of the samples. Arrows in insets show location of sampled spawning reefs.

programs were initiated to improve water and habitat quality. In December 2005, a spawning male lake whitefish and viable eggs were collected; the first documented evidence of spawning since 1916 (Roseman *et al.*, 2007). From 2004 to 2018, seven artificial spawning reefs were constructed near historical spawning areas for lake sturgeon (*Acipenser fulvescens*), walleye (*Sander vitreus*), and lake whitefish near Belle Isle, Fort Wayne (42.2974 N, -83.0933 W), Fighting Island, and Grassy Island in the Detroit River (Fig. 1; Manny *et al.*, 2015). While there was little formal assessment of spawner abundance in the river prior to reef construction, annual indicators of egg deposition on reefs and the surrounding areas have been conducted since 2005 to document the continued use of the spawning habitat, suggesting that improvements in water quality have played a role in restoration of the river habitat as well as the addition of spawning habitat (Prichard *et al.*, 2017; Fischer *et al.*, 2018).

Little is known about what the genetic population structure of lake whitefish was historically in the Great Lakes. Multiple spawning grounds were documented at Belle Isle, Grassy Island, and the Livingstone Channel (42.0922 N, -83.1294 W) on the Detroit River (Goodyear *et al.*, 1982a) and earlier reports suggest that lake whitefish once spawned in the St. Clair River (Milner, 1874). These spawning areas appear to have been used predominantly by lake whitefish migrating from the eastern basin of Lake Erie (Milner, 1874; Wright, 1955). Therefore, Detroit River lake whitefish may have been genetically distinct from Lake Huron, and the Lake Erie lake whitefish that used the river for spawning may have been part of a meta-population. Allozyme and microsatellite DNA data have been used to describe population structure in Lake Huron (Casselman *et al.*, 1981; Stott *et al.*, 2010, 2012). Single nucleotide polymorphisms (Euclide *et al.*, 2022) and microsatellite DNA (Stott *et al.*, 2013) data have been used to describe lake whitefish populations in Lake Erie. Several genetic populations were identified in Lake Huron; including

the northern portion of the main basin, the North Channel, and Georgian Bay (Fig. 1; Casselman *et al.*, 1981; Stott *et al.*, 2010; Stott *et al.*, 2012). Microsatellite DNA data identified little structure in contemporary or historical samples from Lake Erie but found that Lake Erie and Lake Huron were genetically distinct (Stott *et al.*, 2013), while Euclide *et al.* (2022) found modest differentiation between samples from the western and eastern areas of Lake Erie.

While reef restoration and programs to improve water quality aim to create a self-sustaining and resilient system, biological factors are also important for successful restoration of native species. For example, genetic variation is a prerequisite for an evolutionary response to environmental changes encountered as a species enters a novel habitat (Levitan, 2004). The number of migrants moving into a restored habitat and the genetics of their source populations can have an impact on the probability that a restored habitat will be able to support a self-sustaining population. If the increased amount of spawning habitat is used by a small number of individuals, their fertilization rates may be impacted by reduced spawner density which in turn may have an impact on effective population size (Levitan, 2004). Therefore, monitoring resurging and restored populations by determining relative contributions of source populations and measuring spawning success of adults is important. In this study, we quantified the use restored reef habitat in the Detroit River by estimating the number of adults producing offspring at the reefs and determining the relative contributions of Lake Erie and Lake Huron populations using microsatellite DNA data. We hypothesized that most of the lake whitefish using the spawning grounds were from Lake Erie because it is consistent with previous observations of lake whitefish movement in the St. Clair and Detroit rivers (Milner, 1874; Wright, 1955).

2 Materials and methods

2.1 Study area

The Detroit and St. Clair rivers and Lake St. Clair connect Lake Huron to Lake Erie. The St. Clair River starts at the outlet of Lake Huron and splits into several channels as it flows into Lake St. Clair, forming a delta. The Detroit River starts at the outlet of Lake St. Clair as a single channel and water flows around several islands before entering Lake Erie. Egg samples for this study were collected at the locations of the constructed spawning reefs at Fighting Island and Belle Isle (Fig. 1) and genetic samples from adults were collected from the Detroit River.

2.2 Sample collection

Fish eggs and adult fish were collected as part of an ongoing study to document the use of the constructed spawning reefs by several species (e.g., Roseman *et al.*, 2011; Prichard *et al.*, 2017; Fischer *et al.*, 2018). The methods have been described elsewhere (e.g., Roseman *et al.*, 2011; Fischer *et al.*, 2018), but in brief, eggs were collected from gangs of egg mats placed on the river bottom at multiple sites. Sampling started in the fall when water temperatures dropped below 10 °C and ended when ice formed or poor weather made

Table 1. Summary of lake whitefish (*Coregonus chupeaformis*) samples collected from sample sites in the Detroit River each year. Sample counts include adults and larvae hatched from eggs.

Sample site	Year											Site total
	2005	2006	2007	2009	2010	2013	2014	2015	2016	2017	2018	
Belle isle					21	1	26	12	6		4	70
Detroit river	2	9	11								106	128
Fighting island			2	55	183	139	115	56	63	17	10	640
Trenton channel		1										1
Year total	2	10	13	55	204	140	141	68	69	17	120	

working on the river impossible. Sampling usually occurred between October and December each year. Each week, egg mats were retrieved, eggs were removed, counted, and transported to the US Geological Survey Great Lakes Science Center (Ann Arbor, MI USA) where they were incubated until they hatched. The species was determined using keys provided by Auer (1982) and then larvae were sacrificed and stored in 95% ethanol for genetic analysis (Tab. 1). Adult lake whitefish were collected during gillnet surveys conducted in the fall on the Detroit River.

2.3 Genetic methods

Determining the origins of adult and eggs collected from the Detroit River required reference genotype data for lake whitefish that represented potential lake whitefish source populations for assignment tests. Lake whitefish representing potential source populations from lakes Erie and Huron were collected during or close to the spawning season from sites across both lakes. The details of the sources, collection methods, and observed population structure for these reference populations have been described elsewhere (Ebener *et al.*, 2010; Stott *et al.*, 2010, 2012, 2013).

We extracted DNA from the hatched lake whitefish larvae using the DNeasy protocol and reagents (Qiagen Inc., Hilden Germany) and determined the concentration of DNA using a DyNA Quant 200 (Hoefer, Inc., Holliston, MA, USA) fluorometer or an UV-Vis Cary spectrophotometer (Agilent Technologies, Inc., Santa Clara, CA, USA). Sample concentration was standardized to 100 ng μL^{-1} for amplification of microsatellite DNA loci via the polymerase chain reaction (PCR). Eight microsatellite DNA primers designed to amplify loci in lake whitefish (Bernatchez, 1996; Turgeon *et al.*, 1999; Rogers *et al.*, 2004), two primers designed for broad whitefish (*C. nasus*; Patton *et al.*, 1997), and two primers designed for brook trout (*Salvelinus fontinalis*; Angers *et al.*, 1995) were used. One of the two primers for each locus was labelled with a fluorescent dye. Each PCR reaction was carried out in a volume of 15 μL containing 0.25 to 0.75 U *Taq* polymerase, the manufacturer's (Promega Co., Madison, WI, USA) buffer at 1 $\times$ concentration, 0.2 mM of each dNTP, 0.3–0.5 μM of each primer and 80 to 100 ng of template DNA. The PCR thermal profile was similar for all loci; only the annealing temperature changed depending on the primer set being amplified (Tab. 2). An initial denaturation step of 2 min at 94 °C was followed by 35 cycles of 1 min at 94 °C, 1 min at the annealing temperature, and 1 min extension at 72 °C, followed by one final extension

for 5 min at 72 °C. Several loci were amplified in multiplex reactions to increase sample throughput efficiency (Tab. 2).

PCR products were prepared according to manufacturer's guidelines (Life Technologies, Carlsbad, CA, USA) for capillary electrophoresis. The PCR product was diluted with 9 μL of water. Formamide (10 μL) and 0.5 μL of a 400 base pair size standard (Applied Biosystems GeneScan 400HD ROX™ Size Standard, Life Technologies, Carlsbad, CA, USA) was added to 1 μL of the diluted PCR product. Samples were denatured for 4 min and then chilled for 3 min before they were loaded on the ABI 3130-AVANT Genetic Analyzer. Fragment size data were collected using the manufacturer's (Life Technologies, Carlsbad, CA, USA) software. Allele calls were assigned using the GeneMapper software (Life Technologies, Carlsbad, CA, USA) and then reviewed at least twice. Alleles for each fish were summarized into a genotype used for subsequent analyses. Any sample missing data at more than four loci was removed from the data set.

2.4 Data analysis

The morphological assignment of the hatched larval samples as lake whitefish was confirmed using genetics following the approach described by Stott *et al.* (2021) because cisco and bloater (*C. hoyi*) have also been captured in the Detroit River (Stott *et al.*, 2021). We then used two approaches to determine the contributions of Lake Erie and Lake Huron lake whitefish populations to the samples collected from the Detroit River. Individual assignment tests were performed using GENECLASS (Piry *et al.*, 2004) and Structure 2.3.4 (Pritchard *et al.*, 2000) software packages. Both software packages use a Bayesian approach but use different algorithms and present the results in a different format. GENECLASS estimates the probability that an individual belongs to each reference population and assigns it to the most probable source while Structure estimates the proportional contribution of each reference population to an individual's genotype after grouping individuals into a user-defined number (K) of genetic clusters. Using two approaches will provide additional validation of results and allow us to analyze trends in different ways. Based on past analyses of the population structure of lake whitefish in lakes Erie and Huron (Stott *et al.*, 2010, 2012, 2013), we divided the reference data set into four groups that represent the main sources of genetic variation: North Channel-Lake Huron, Main Basin-Lake Huron, Georgian Bay-Lake Huron, and Lake Erie (Fig. 1). These groups were used as reference populations for the analysis of larval samples using

Table 2. Loci used in analysis of lake whitefish (*Coregonus clupeaformis*), their PCR conditions, fluorescent labels used, and reference for each locus.

Multiplex/ PCR Cycle	Locus	MgCl ₂ (mM)	Concentration primer (μM)	Label	Size range (base pairs)	References
1a	<i>Bwf1</i>	1.25	0.1	56FAM	190–268	Patton <i>et al.</i> , 1997
2b	<i>Bwf2</i>	1.5	0.15	56FAM	139–167	Patton <i>et al.</i> , 1997
1a	<i>C2-157</i>	1.25	0.15	5HEX	126–186	Turgeon <i>et al.</i> , 1999
2b	<i>Cocl23</i>	1.5	0.3	56FAM	245–287	Bernatchez, 1996
3c	<i>CoclLav4</i>	2	0.2	5HEX	139–159	Rogers <i>et al.</i> , 2004
4d	<i>CoclLav45</i>	1.25	0.08	56FAM	236–258	Rogers <i>et al.</i> , 2004
4d	<i>CoclLav52</i>	1.25	0.1	5HEX	94–156	Rogers <i>et al.</i> , 2004
5c	<i>CoclLav6</i>	1.4	0.1	5HEX	123–163	Rogers <i>et al.</i> , 2004
3c	<i>CoclLav68</i>	2	0.1	NED	167–183	Rogers <i>et al.</i> , 2004
6e	<i>CoclLav8</i>	1.5	0.2	5HEX	199–251	Rogers <i>et al.</i> , 2004
7e	<i>Sfo23</i>	2	0.25	56FAM	147–207	Angers <i>et al.</i> , 1995
8e	<i>Sfo8*</i>	2	0.25	NED	205–259	Angers <i>et al.</i> , 1995

*More than one locus amplified with the primer set.

^a 95°C for 3 min; 15 cycles of 94°C for 1 min, 60°C for 45 s, 72°C for 10 s, then 20 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 10 s, and a final elongation at 72°C for 5 min.

^b 95°C for 3 min; 15 cycles of 94°C for 1 min, 55°C for 45 s, 72°C for 10 s, then 20 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 10 s, then a final elongation at 72°C for 5 min.

^c 95°C for 3 min; 35 cycles of 95°C for 1 min, 60°C for 30 s, 72°C for 1 min, then a final elongation at 72°C for 5 min.

^d 95°C for 3 min; 35 cycles of 95°C for 1 min, 62°C for 30 s, 72°C for 1 min, then a final elongation at 72°C for 5 min.

^e 94°C for 2 min; 35 cycles of 94°C for 45 s, 56°C for 45 s, 72°C for 1.5 min, then a final elongation at 72°C for 5 min.

GENECLASS. Confidence in assignment to a reference population was also calculated using GENECLASS. A similar analysis was performed across two simulations in Structure by setting the number of assumed populations at $K=4$ (the four populations described above) and then at $K=2$ (Lake Erie vs. Lake Huron). At $K=2$, we could also estimate the number of individuals with a parent from each lake, *i.e.*, an inter-lake hybrid. An individual was classified as an inter-lake hybrid if its q-score for the cluster associated with Lake Erie was between 0.4 and 0.9. The Bayesian analysis was performed over 10 runs, each with a burn-in period of 100,000 iterations followed by 100,000 iterations. We selected the “admixture model” as our ancestry model and used the population information (USEPOPINFO=1) to determine the origins of the samples from the Detroit River going back one generation (GENSBACK=1). Individual membership coefficients (q) from each run were averaged and summarized by year and sampling location.

We examined the impact of temperature on the estimated number of hybrids each year by comparing the percent hybrids to the temperature in each lake. We performed a linear regression using the day of the year in the water temperature first fell below 8°C and the percent of hybrids for each lake (NOAA, 2023) for each year from 2009 to 2018.

We used the program COLONY 2.0.6.8 (Wang, 2009; Jones and Wang, 2010) to estimate the number of families and effective number of breeding adults at each site for each year when sample sizes were greater than 20. COLONY uses a maximum likelihood method to assign samples to full and half-sibling families and to determine the minimum number of individuals that contributed to sampled genotypes (Wang, 2004; Wang and Santure, 2009). One hundred runs were

performed for each set of samples and input parameters were: male and female polygamy without inbreeding and clonality, diploid and dioecious species, medium run using the full likelihood analysis method with high precision, allele frequencies were not updated, sib-ship sizes were scaled with no sibship size prior, all parameters involving maternity and paternity were unknown, and a genotyping error rate of 0.01. We ran the analysis for samples collected at each site in each year to look at changes in number of families and spawners using the site. We also ran samples collected at all sites in a year to look at patterns of reef use.

3 Results

Genotype data for 15 adult lake whitefish and 824 larvae from the Detroit River were collected for the analysis. All larvae were genetically confirmed to be lake whitefish. The samples were grouped by site and year. Samples from sites directly on or near the constructed reef habitat were grouped together because fish spawn on both sites (Fischer *et al.*, 2018). The area where the samples were collected was not recorded for 128 samples, therefore analyses summarized by reef areas could only be made for 711 of the samples and the remaining samples were classified as coming from the Detroit River. Reference populations for the population assignment of the larvae included 2,534 lake whitefish from Lake Huron and 380 from Lake Erie. The reference data set was missing 0.9% of data across all loci; missing data by population ranged from 0.7% (Lake Huron-Main Basin) to 1.0% (Lake Erie) and by locus from 0% (*CoclLav68*) to 2.8% (*CoclLav8*). The larval and adult samples collected from the Detroit River were

Table 3. Summary of assignments (as percent of sample) lake whitefish (*Coregonus clupeaformis*) eggs collected from the Detroit River to populations in lakes Huron and Erie in each sampling year using GENECLASS and percent of inter-lake hybrids observed using results from Structure. *N*=sample size.

Year	<i>N</i>	Lake huron-georgian bay	Lake huron-main basin	Lake huron-north channel	Lake erie	Inter-lake hybrid
2005	1	0.0	0.0	0.0	100.0	0.0
2006	9	0.0	0.0	0.0	100.0	0.0
2009	55	12.7	10.9	3.6	72.7	12.7
2010	204	5.9	5.9	2.0	86.3	8.8
2013	140	2.1	17.9	0.7	79.3	2.9
2014	141	4.3	14.2	0.0	81.6	1.4
2015	68	0.0	14.7	1.5	83.8	0.0
2016	69	1.4	11.6	0.0	87.0	1.4
2017	17	0.0	23.5	0.0	76.5	5.9
2018	120	2.5	13.3	1.7	82.5	2.5

missing 5.3% of data across loci which ranged by locus from 0.6% (*C2-157*) to 13.2% (*CoclLav52*).

The accuracy of assignment of a fish to a source population was tested using GENECLASS and we found that 66% of the reference samples could be correctly assigned to their actual source population. The largest mis-assignments occurred among the Lake Huron populations, with Georgian Bay samples being classified as North Channel samples 18.2% of the time and as Main Basin samples 16.8% of the time. When we repeated the analysis using lake as a source (*i.e.*, $K=2$), samples were assigned correctly 91.0% of the time. In all years sampled, most of the hatched eggs were assigned to the group associated with Lake Erie, ranging from 76.5% of the samples in 2017 to 100% of the samples in 2005 and 2006 (Tab. 3). Samples assigned to Lake Huron populations were most frequently assigned to the Main Basin followed by Georgian Bay. Similar trends were observed when samples were categorized by collection area (Belle Isle and Fighting Island). Adults captured in the Detroit River (Belle Isle, Fighting Island, Trenton Channel) produced similar results, 11 of the 14 samples were classified as Lake Erie fish and three were assigned to the Georgian Bay population of Lake Huron (Tab. 1).

The Bayesian analysis using the Structure software was conducted using $K=4$ to explore differences among genetic groups and at $K=2$ (Lake Huron and Lake Erie) to further explore differences among sites and years. Consistent with the GENECLASS results, more than 90% of the reference samples could be assigned correctly to their lake of origin and most samples were classified as having Lake Erie origins (Fig. 2). The number of inter-lake hybrids varied during the sampling period (Tab. 3) from a high of 23.6% in 2009 to a low of 0% in 2015, with fewer hybrids in later years (Fig. 2; Tab. 3). We tested if water temperature had an impact on the number of hybrids each year. Lake whitefish spawning begins when water temperature is between 5 and 8 °C in Lake Erie (Lawler, 1965; Edsall and Rottiers, 1976). We saw little evidence of a relationship between the proportion of hybrids and first day in fall and winter when water temperatures in Lake Huron ($R^2=0.14$, $F(1, 6)=1.00$, $p=0.355$) and Lake Erie ($R^2=0.005$, $F(1, 6)=0.033$, $p=0.861$) fell below 8 °C (Fig. 3).

The genotypes of lake whitefish collected from a reef area were used to estimate the number of individuals that spawned,

the effective population size, and the number of families produced. Estimates of all metrics were similar for the two years of sampling at Belle Isle while all metrics increased for estimates from samples from Fighting Island until 2010 and then they appear to have declined (Tab. 4). We looked for evidence of use of both sites in 2010 and 2014 as those were the only years where >20 samples were collected from both sites. In 2010, 24 parents were estimated to have offspring at both sites with no full-sibling pairs and 24 half-sibling pairs. In 2014, the estimated number of parents was 26 and there was 1 full-sib and 26 half-sib pairs.

4 Discussion

Detroit River reef restoration projects have re-established spawning habitat for several species, including lake whitefish (Fischer *et al.*, 2018), thus providing greater availability and diversity of spawning habitat and, in turn, a healthier ecological portfolio. Analysis of microsatellite DNA data showed that lake whitefish from both Lake Huron and Lake Erie used the reefs to spawn between 2009 and 2018, although more fish from Lake Erie used them, consistent with our hypothesis. In the early period of our study, we observed an increase in the use of spawning areas in the Detroit River based on increases in numbers of parents observed at Fighting Island, however these numbers decreased in later years.

Lake whitefish appear to have resumed use of historical spawning areas in the Detroit River, despite the massive loss of spawning habitat early in the 20th century. Lake whitefish may have been migrating into the river throughout the twentieth century, but may have not been able to successfully spawn until recently. The last commercial landing of lake whitefish in the Detroit River was reported in 1925 (Roseman *et al.*, 2007), but small numbers of lake whitefish were reported in other surveys (*e.g.*, Wright, 1955). Lake whitefish eggs have been captured in areas throughout the Detroit River, but catch-per-unit effort is higher in areas near the constructed spawning reefs (Prichard *et al.*, 2017); not surprising given reef placement was optimized to match the hydrodynamic preferences of the target species (lake sturgeon, lake whitefish, walleye) (Manny *et al.*, 2015).

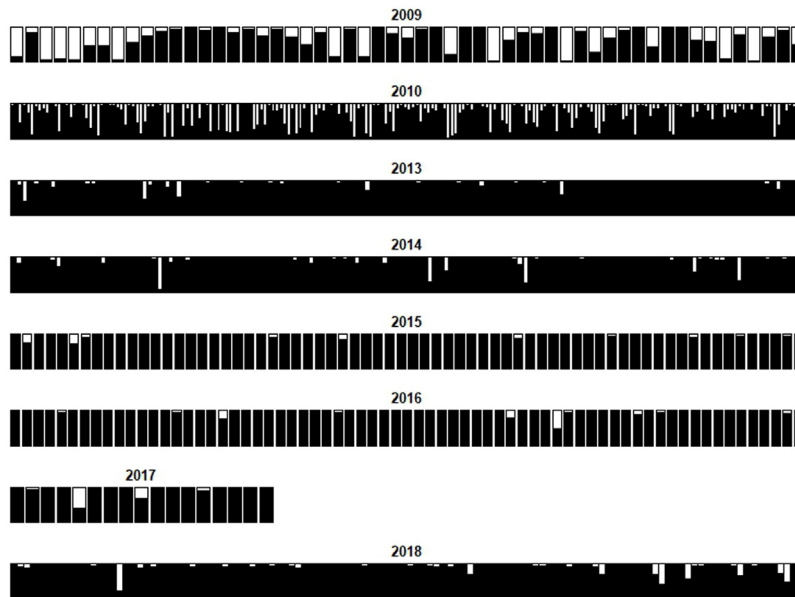


Fig. 2. Genetic assignment of lake whitefish (*Coregonus clupeaformis*) eggs collected from the Detroit River between 2009 and 2018 calculated using the software package Structure. Each bar represents an individual fish and shows the proportion of its genotype associated with Lake Erie (black portion) and Lake Huron (white portion).

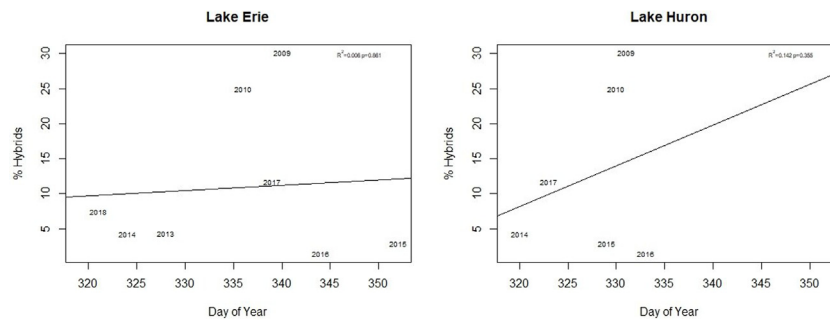


Fig. 3. Relationship between water temperatures of Lake Huron and Lake Erie and rate of inter-lake hybrids. Each graph shows the day of the year in the fall when the water temperature first falls below 8 °C for each year in the study versus the estimated proportion of inter-lake hybrids in each year. Each point is represented by the year in which data were collected (NOAA, 2023; <https://coastwatch.glerl.noaa.gov/marobs/marobs.html>).

Table 4. Lake whitefish (*Coregonus clupeaformis*) effective population size, number of adults, and number of full-sib pairs found at sites on the Detroit River estimated using genotypes from eggs collected in the Detroit River and COLONY. N = number of larvae included in simulation, N_e = effective population size, N_{ad} = estimated number of breeding adults, N_{fs} = estimated number of full-sib pairs, CI = confidence interval.

Site	Year	N	N_e	95% CI of N_e	N_{ad}	N_{fs}
Belle Isle	2010	26	16	8, 35	19	16
Belle Isle	2014	21	12	6, 30	15	16
Fighting Island	2009	55	42	27, 68	44	54
Fighting Island	2010	183	118	89, 155	134	158
Fighting Island	2013	138	82	59, 112	94	121
Fighting Island	2014	115	70	50, 99	77	110
Fighting Island	2015	56	29	18, 50	42	53
Fighting Island	2016	63	30	18, 50	44	49

The observation that most lake whitefish using the reefs in the Detroit River are from Lake Erie is consistent with historical observations. Lake whitefish from northern and eastern parts of Lake Erie used the Detroit River for spawning and may have also used sites in the St. Clair River (Milner, 1874; Reighard, 1910; Smith, 1915). Lake whitefish migrated each year from the central and eastern basins of Lake Erie to reach spawning grounds in the western basin of Lake Erie and the Detroit and St. Clair rivers (Milner, 1874; Wright, 1955; Goodyear *et al.*, 1982a,b). The microsatellite DNA loci we used could not reliably distinguish among populations in Lake Erie (Stott *et al.*, 2013) to determine whether different populations used a specific reef area in the Detroit River. However, a recent analysis of Lake Erie population structure using SNPs (Euclide *et al.*, 2022) identified two populations with high rates of gene flow between them; one associated with reefs in the southern part of the western basin and one composed of fish from the northwestern, central, and eastern basins of the lake. The central/eastern population identified by Euclide *et al.* (2022) may enter the Detroit River to spawn, while the southwestern population may use shoals and reefs that run from Port Clinton (41.5170 N, -82.9366 W) to South Bass Island (41.6481 N, -82.8262 W) and Kelleys Island (41.6060 N, -82.7080 W) as suggested by historical and recent lake whitefish spawning assessments (Amidon *et al.*, 2021). Population structure in Lake Erie may have been more pronounced historically, due to differences in migratory behavior. Some fish from the central and eastern basins stayed in Lake Erie to spawn, while others made a longer migration into the Detroit River. This could be related to differences in migration patterns due to age and/or genetics, for example, larger lake whitefish are more likely to move longer distances in Lake Huron (Li *et al.*, 2017). Also, the chromosomal inversion that characterized the lake whitefish from the western Lake Erie population identified using genomic markers (Euclide *et al.*, 2022) may explain a genetic basis for the differences in migratory behavior. Chromosomal inversions have been associated with life history differences in other species, including the migration and spawning behavior of Atlantic cod (*Gadus morhua*) (Puncher *et al.*, 2019). Despite changes in availability in spawning habitat which may have increased gene flow between the two populations in Lake Erie or reduced the abundance of the northern population, the chromosomal inversion could have protected the region from recombination, helped maintain genetic differentiation between the two populations, and preserved the migratory behavior. Additional genotyping using genomics of lake whitefish captured in the Detroit River and fish from other lakes is needed to further explore this idea.

The proportion of lake whitefish from the study reefs with parents from Lake Huron was low and fairly constant through the sampling period. Historical reports indicate that lake whitefish from Lake Huron were less likely to move into the St. Clair River, although such movement was observed after heavy winds and storms originating from the north (Milner, 1874). Some of the fish could have been mis-assigned Lake Erie fish. However, the proportion of fish identified as Lake Huron in each year was usually greater than the assignment error rate, therefore some of those fish were likely from Lake Huron. Telemetry studies on walleye also noted limited movement between Lake Erie and Lake Huron. Walleye that

migrated from Lake Erie would remain in the system longer than those from Lake Huron (Wang *et al.*, 2007; Hayden *et al.*, 2019). Future efforts to assess and include other lake whitefish spawning locations, such as those in the St. Clair River, could continue in order to monitor the contribution of Lake Huron and Lake Erie fish to this spawning population, and ultimately the Lake Erie adult lake whitefish population.

We observed more inter-lake hybrids in 2009 and 2010 than in subsequent years. However, the relative proportion of fish from Lake Huron remained relatively constant. Therefore something else may be related to the differences in the rate of hybridization by changing encounter probabilities between lake whitefish from each lake. For example, if the timing of when lake whitefish from each lake were using the reefs changed so they were not on reefs at the same time, then inter-lake hybrid rates would be lower in some years. If the timing of spawning was different in each lake, then there may not have been as much overlap in when lake whitefish from each lake moved into or through the Detroit River. However, we saw little evidence of a relationship between the proportion of hybrids and first day in fall and winter when water temperatures in Lake Huron and Lake Erie fell below 8 °C (Fig. 3). However, even if the use of the spawning reefs by lake whitefish from the two lakes did not change over time, changes in the dates when egg mats were set and retrieved may have influenced the results, so that in some years the egg mats were deployed at times when fish from both lakes were using the reefs while in other years they were not.

Lake whitefish spawn on the constructed reefs throughout the Detroit River. However, higher numbers of eggs were captured at sites directly upstream or downstream of the constructed reefs (DeBruyne *et al.*, 2022a), typically in natural substrate with slightly smaller rock sizes than the reef rock (Manny *et al.*, 2015). The numbers of families found at Fighting Island each year varied (73 on average) and were generally greater than the estimates for Belle Isle (19 and 15). These estimates should be treated as lower end estimates because all adults were likely not detected given the small number of samples and markers used. The numbers of families and effective number of breeder estimates were lower in recent collections, consistent with decreases in abundance possibly due to a decrease in sampling effort at sites within the reef areas where lake whitefish spawned and an increase in sedimentation at sites (Fischer *et al.*, 2020). Changes are most notable at the Fighting Island reef area where the number of 'off-reef' sites sampled decreased from 12 in 2009 to 2 in 2015. The reduced numbers of sampling sites in some years (e.g., 2015 and 2016, Fighting Island, Tab. 4) may have also contributed to the lower number of spawners using COLONY because these estimates can be correlated with sample size (Wang and Santure, 2009; Hunter *et al.*, 2020).

We found offspring from the same parents at different sites in 2010 and 2014. A similar result was found in a study of lake sturgeon on some of the same reefs (Hunter *et al.*, 2020). They found eggs from the same parents at different sites and speculated that eggs may drift downstream after being spawned. We do not know if our results were also related to egg drift downstream (DeBruyne *et al.*, 2022b) or if parents used more than one site; both explanations are possible in this fast-flowing river system.

Continued successful use of the Detroit River as a lake whitefish spawning area may help increase species genetic diversity and population stability in the Great Lakes. A healthy ‘portfolio’ of diversity in a species can provide stability at larger regional scales through a buffering effect that allows populations to respond to stresses that may have different impacts at different geographic scales (Schindler *et al.*, 2010; DuFour *et al.*, 2015). Effective habitat restoration projects provide a diversity in habitat that can help rebuild a healthy portfolio (Bouska *et al.*, 2023) and possibly maintain multiple genetic populations. Multiple spawning stocks with restricted gene flow among them will result in greater overall genetic diversity relative to single populations of the same size (Whitlock and Barton, 1997). Our results provide additional evidence that restoration efforts in the Detroit River are providing spawning habitat for lake whitefish and that reef use is consistent with historical observations. Continued monitoring can help to determine whether spawners continue to use the reefs and to whether lake whitefish produced on the reefs survive.

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Author contributions statement

WLS and ER conceptualized the study. WLS coordinated genetic data collection and analyzed genetic data. WLS wrote the manuscript and revised it with input from all coauthors.

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