INTRODUCTION

The lake whitefish (Coregonus clupeaformis; Family Salmonidae) is a culturally and ecologically valuable native fish to the Laurentian Great Lakes of North America that cycles energy through the food-web (Mohr and Nalepa, 2005) and supports highly valuable commercial fisheries (Ebener et al., 2008, 2021). Although adult lake whitefish recovered from substantial abundance declines in the 1950s and 1960s (Ebener, 1997), declines in body condition and growth in the late 1990s have persisted to present (Hoyle, 2005; Lenart & Caroffino, 2016, 2017; Mohr & Ebener, 2007; Schneeberger et al., 2005). Similarly, declines in early life-stage recruitment were observed in many sites throughout the four lower Great Lakes in the late 1990s–early 2000s and have persisted to present day (Mohr and Nalepa, 2005, Ebener et al., 2008, 2021; Brenden et al., 2010; Lenart & Caroffino, 2016, 2017), though Lake Superior recruitment
has remained stable or increased through time (Ebener et al., 2021; Lenart & Caroffino, 2017). Several abiotic and biotic factors have been hypothesized as potentially causing or contributing to recruitment declines, but the exact cause remains unknown (Ebener et al., 2021). Fish pathogens have been implicated in the declines in recruitment and research aimed at understanding the role of pathogens is considered a high priority (Ebener et al., 2021).

Infectious diseases can negatively affect wild fish populations (Faisal et al., 2012; Holey et al., 1998; Lafferty et al., 2015). Indeed, several microbial pathogens that cause systemic disease and mortality in other salmonids have been detected in adult lake whitefish from Lakes Michigan and Huron (e.g., Viral Haemorrhagic Septicaemia Virus, Renibacterium salmoninarum, Carnobacterium maltaromaticum and Aeromonas salmonica subsp. salmonicida; reviewed in Loch and Faisal, 2011) and that can be transmitted from parent to offspring via infected reproductive fluids and/or gametes.

Another bacterial fish pathogen recognized for vertical transmission in salmonids is Flavobacterium psychrophilum (Phylum Bacteroidetes; Family Flavobacteriaceae), the causative agent of bacterial cold water disease (BCWD) and rainbow trout fry syndrome (RTFS; Borg, 1948; Brown et al., 1997; Holt, 1987). As the latter name implies, F. psychrophilum causes substantial early life stage mortality in some salmonid species, where survivors can shed high loads of the bacterium (Madetoja et al., 2000; Taylor, 2004). In the Great Lakes, systemic F. psychrophilum infections are prevalent in wild, feral, and hatchery-reared Pacific salmonids (Oncorhynchus spp.), with prevalence exceeding 86% in spawning Chinook salmon (O. tshawytscha) in the Lake Michigan watershed (Van Vliet et al., 2015).

Despite its widespread prevalence in Great Lakes salmonids, F. psychrophilum has never been isolated from lake whitefish. However, during a study to elucidate the potential role infectious diseases may be playing in the declines of lake whitefish recruitment, we discovered this bacterium in spawning phase lake whitefish. Herein, we report on the first isolation of F. psychrophilum from systemically infected adult lake whitefish, a noteworthy finding in the context of poor recruitment given the disease and mortality this bacterium elicits in the early life stages of other salmonids.

2 | MATERIALS AND METHODS

2.1 | Fish sampling

Our study was designed to collect adult lake whitefish and their progeny from sites illustrating both good and poor recruitment (Mohr and Nalepa, 2005; Rennie, 2014; Fera et al., 2015; Lenart & Caroffino, 2017; Ebener et al., 2021) and to compare pathogens found in both life stages between the good and poor sites. We collected adult fish from three good recruitment sites (Whitefish Bay in Lake Superior, Menominee River in Lake Michigan and Saginaw Bay in Lake Huron) and two poor recruitment sites (Bailes Harbor in Lake Michigan and Alpena in Lake Huron) from late October through mid-November of 2018 and 2019 (Figure 1). Adult fish were collected live using commercial trap nets (see Schorfaaer and Peck, 1993 for description of the gear). To reduce sampling bias, attempts were made to collect 30 males and 30 females from each site across three size classes: <450 cm, 450–550 cm and >550 cm. These size classes roughly represented age classes at first maturity, partially mature age classes and completely mature age classes that had spawned multiple times. Captured lake whitefish were immediately placed into aerated live wells onboard fishing vessels and then transferred into live wells supplied with compressed oxygen for transportation to the Michigan State University – Aquatic Animal Health Laboratory (MSU-AAHL). Upon arrival, live lake whitefish were euthanized using 250 mg/L of MS-222 (Tricaine methanesulfonate; Syndel) buffered with 500 mg/L of sodium bicarbonate (Millipore Sigma). All euthanasia was conducted in accordance with the Michigan State University – Institutional Animal Care and Use Committee (AUF 202-100-272).

From May to July of 2019 and in June of 2021, post-larval age-0 lake whitefish were collected from sandy beaches adjacent to the wild adult spawning locations (Figure 1; Ebener et al., 2021) using a 45.7 m long x 1.8 m tall seine with a 1.8 m × 1.8 m bag in the centre, constructed with 0.3 cm delta mesh. Fish were collected from Whitefish Bay in Lake Superior, Bailes Harbor area of Lake Michigan, Alpena in Lake Huron, and Saginaw Bay in Lake Huron. Although an attempt was made to collect fish from off the Menominee River mouth in 2019, collections were not successful due to unfavourable water conditions. Multiple seine hauls were performed at each location. Post-larval lake whitefish were identified by the presence of a single dorsal fin and an adipose fin, subterminal mouth, clear fins and greenish-brown backs with silver sides (Michigan Department of Natural Resources, 2021). Collected individuals were transferred to a cooler supplied with dissolved oxygen via air pumps for transport back to the MSU-AAHL. Upon arrival, post-larval fish were euthanized as described above for adults.

2.2 | Clinical examination

Following euthanasia, blood from adult fish was collected via venipuncture of caudal vertebral vessel(s) using sterile 18G needles and 5 ml sterile syringes (Beckton, Dickinson and Company). Total length in centimetres and weight in grams were measured for each lake whitefish and a thorough external and internal clinical examination was performed. During the examination, and as a proxy for nutritional status, we estimated the visceral fat index (VFI; Brown & Murphy, 1991) of each fish. Tissues (e.g., kidney and gonads) for bacteriological analyses were collected as described below. For post-larval lake whitefish, length and weight were measured, gross examinations performed, and kidney tissue collected for bacterial isolation. Due to their small sizes (Table 1), blood was not collected from post-larval lake whitefish.

As a measure for erythrocyte count (and therefore anaemia and other blood disturbances), the packed cell volume (PCV) of adult fish was determined. This was done by immediately transferring a portion of collected un-heparinized whole blood into glass capillary tubes (ThermoFisher Scientific). Blood samples were centrifuged for 2 min in a StatSpin CritSpin Microhematocrit Centrifuge (Iris Sample...
Processing). PCV measurements were then recorded using a provided card-style reader (product #HR05).

### 2.3 | Bacteriological analyses

During gross clinical examination, the external surface of each fish was disinfected with 70% ethanol prior to the coelom being opened with sterile scissors (one pair per fish) in a laminar flow hood. Tissue from reproductive organs (adult fish only) and kidneys of each fish were collected using either sterile disposable 10 μl (adults) or 1 μl (post-larval) loops and then inoculated directly onto tryptic soy agar (TSA; ThermoFisher Scientific), as well as Hsu-Shotts medium (HSU; Bullock et al., 1986) and tryptone yeast extract salts agar (TYES; Holt, 1987), both of which were supplemented with 4 mg litre⁻¹ of neomycin sulphate for semi-selectiveness for flavobacteria. During the second year of sampling, slight modifications to TYES preparation were made, as ongoing medium-optimization experiments suggested potential for improved bacterial recovery; however, further experimentation revealed no substantial differences in bacterial recovery between the two media preparation methods. Briefly, tryptone, yeast extract, MgSO₄·7H₂O, and CaCl₂·2H₂O were mixed into 250 ml water and adjusted to a pH of 7.2, filter sterilized using UltraCruz® Filter Flasks, polyethersulfone (PES; 0.22 μm), and then added to a previously autoclaved and cooled (i.e., 55°C) agar/water suspension. Following inoculation of tissues onto the three media, primary cultures were incubated at 22°C (TSA and HSU) and 15°C (TYES) for as long as 7 days and checked intermittently for visible bacterial growth. Any resultant, yellow-pigmented bacterial growth present on HSU and/or TYES was sub-cultured onto fresh analogous media and subsequently checked for purity. Once verified pure, all isolates were

### TABLE 1  Mean length (cm) and weight (g) of post-larval lake whitefish collected and sampled in 2019 and 2021 from five sites in the upper three Great Lakes

| Site                  | Number of fish collected | Mean length (cm) | Mean weight (g) |
|-----------------------|--------------------------|------------------|-----------------|
| Whitefish Bay, LS⁵    | 25                       | 2.6 (0.2)        | <1.0            |
| Baileys Harbor, LM⁴   | 150                      | 6.1 (0.3)        | 1.9 (0.3)       |
| Marinette, LM⁴        | 110                      | 3.4 (0.6)        | 0.3 (0.1)       |
| North Point, LH⁶      | 150                      | 3.4 (0.4)        | 0.3 (0.1)       |
| Caseville, LH⁶        | 1                        | 2.6              | 0.2             |

Note: Standard deviation of the data is reported in parentheses. ⁵Good recruitment site; ⁶Poor recruitment site. Abbreviations: LH, Lake Huron; LM, Lake Michigan; LS, Lake Superior.
supplemented with 20% v/v glycerol and cryopreserved at −80°C for future identification and analyses. To initially characterize recovered yellow-pigmented bacteria, 24-hr old cultures incubated at 15°C were biochemically and morphologically characterized for oxidase (BD BBL™ DrySlide™, Becton, Dickinson and Company) and catalase (hydrogen peroxide solution, 3%; Millipore Sigma) activity, presence of flexirubin-type pigments via 3% potassium hydroxide (Reichenbach et al., 1974), the string test (AFS-FHS, 2016) and Gram-stain reactions (Remel™, ThermoFisher Scientific). All translucent yellow pigmented bacterial isolates that were recovered on TYES at 15°C and were Gram-negative, oxidase and catalase positive, and produced a flexirubin-type pigment were selected for further molecular analyses.

2.4 Molecular identification

Bacterial genomic DNA was extracted from 7-day-old bacterial cultures using the DNeasy Blood and Tissue kit (Qiagen Inc.) according to the manufacturer’s protocol for Gram-negative bacteria. Nucleic acids were then quantified using the Quant-iT dsDNA Assay kit and a Qubit fluorometer (Life Technologies) and diluted to 20 ng/µl using nuclease-free water (ThermoFisher Scientific). Yellow-pigmented bacteria suspected of being F. psychrophilum were assayed using the F. psychrophilum-specific endpoint PCR assay of Toyama et al. (1994) as previously described (Van Vliet et al., 2015). The template for negative control reactions consisted of nuclease-free water, whereas positive control template was derived from a previously sequenced-confirmed F. psychrophilum isolate. Resultant PCR products were electrophoresed in a 1.5% agarose gel for 30 min (100 V) and then visualized under UV transillumination. The presence of a ~ 1,088 base pair-sized amplicon was considered confirmatory for bacterial identification as F. psychrophilum (Toyama et al., 1994).

2.5 Multilocus sequence typing of F. psychrophilum and data analysis

Multilocus sequence typing (MLST) is a well-established method for characterizing strain diversity of bacterial pathogens, including F. psychrophilum (Knupp et al., 2019; Nicolas et al., 2008). Indeed, MLST-based analyses of F. psychrophilum have revealed useful information as to the bacterium’s genetic diversity and its relationship to geographic distribution, host specificity and virulence. We conducted MLST analyses on isolates collected as part of this study due to F. psychrophilum having never been previously isolated from lake whitefish in the Great Lakes and to be able to elucidate the relatedness of the newly recovered isolates to those that are widespread in the Great Lakes basin (Knupp et al., 2019; Van Vliet et al., 2016).

The partial sequences of seven genes (trpB, gyrB, dnaK, fumC, murG, tuf and atpA; Nicolas et al., 2008) were PCR amplified, after which the resulting products were electrophoresed and the appropriate size of the amplicon verified as previously described (Knupp et al., 2019). Amplicons were then purified using ExoSAP-IT (ThermoFisher Scientific) and bidirectionally sequenced at the Michigan State University – Research Technology Support Facility using the same primers used for PCR amplification of each of the housekeeping genes. The quality of chromatograms was verified using an in-house script as previously described (Nicolas et al., 2008) prior to allele and sequence type assignment. We used GoeBURST (www.phylodiv.net/goeburst; Francisco et al., 2009) to visualize phylogenetic relationships, where sequence types were dichotomized into clonal complexes or singletons based on locus variations in their allelic profiles (Feil et al., 2004). All 1,545 F. psychrophilum isolates present in the pubMLST database (https://pubmlst.org/psychrophilum/; Jolley et al., 2018) were included in the analysis.

2.6 Data analyses

Because of low F. psychrophilum prevalence overall, statistical testing was not performed to infer differences among sampling locations. A Kruskal-Wallis test was used to determine whether median PCV values of adult lake whitefish were equal among collection locations. For VFI values, which are ordinal variables, a one-way permutation test of independence was used to determine whether values were equal among the collection locations. For both tests, if the null hypothesis of no differences among collection locations was rejected, follow-up pairwise tests were undertaken to determine which collection locations may have differences in PCVs or VFIs. For PCV, follow-up analyses consisted of Dunn’s (1964) multiple comparison tests. For VFIs, follow-up analyses consisted of pairwise permutation tests of independence. For both follow-up analyses, Bonferroni corrections were used to protect the Type-1 error rate of the tests. All statistical testing was conducted in R (4.1.2 GUI 1.77 High Sierra build 8007) using the Fisheries Stock Analysis (FSA; Ogle et al., 2021), coin (Hothorn et al., 2006), and rcompanion (Mangiafico, 2021) packages.

3 RESULTS

A total of 600 adult lake whitefish were collected from five sites in Lakes Superior, Michigan and Huron (Figure 1; Table 2) during 2018 and 2019, as were 436 post-larval lake whitefish (Figure 1; Table 1). The overall mean ± standard error of the mean) length and weight of collected adult lake whitefish were 51.7 ± 0.2 cm and 1382.4 ± 22.4 g, with some variation noted by site (Table 2). The overall mean length and weight of collected post-larval lake whitefish were 4.3 ± 0.1 cm and 0.9 ± 0.0 g (Table 1).

Two kidney cultures derived from adult lake whitefish collected from Whitefish Bay (Lake Superior) and one kidney culture derived from an adult lake whitefish collected from the Menominee River (Lake Michigan) yielded yellow-orange, semi-translucent, low convex colonies with slightly undulate margins. In all three cases, these isolates yielded one colony forming unit (CFU) per 10 µl of kidney inoculum. Following subculture, the three yellow-pigmented bacterial
TABLE 2  Mean length (cm), weight (g), visceral fat index, packed cell volume (PCV) and Flavobacterium psychrophilum infection prevalence in the kidneys of adult lake whitefish collected from five sites in the upper three Great Lakes during 2018 and 2019

| Site                  | Mean length (cm) | Mean weight (g) | Mean visceral fat index | Mean packed cell volume | F. psychrophilum prevalence |
|-----------------------|------------------|-----------------|-------------------------|-------------------------|-----------------------------|
|                       | 2018             | 2019            | 2018                    | 2019                    | Overall                     | 2018 | 2019 | Overall | 2018 | 2019 | Overall | 2018 | 2019 | Overall | 2018 | 2019 | Overall |
| Whitefish Bay, LS⁵    | 50.7 (5.1)       | 52.3 (4.9)      | 1163.8 (380.7)          | 1414.7 (549.3)          | 1.7 (0.8)                  | 1.9 (1.0)                  | 1.8 (0.9) | 38.5 (9.6) | 41.9 (9.8) | 40.2 (9.8) | 0/60 | 2/60 |
| Baileys Harbor, LM⁷   | 55.2 (6.3)       | 56.3 (4.7)      | 1658.7 (454.8)          | 1511.4 (462.0)          | 0.2 (0.4)                  | 1.3 (1.0)                  | 0.9 (0.9) | 44.3 (10.6) | 39.5 (7.6) | 41.9 (9.5) | 0/60 | 0/60 |
| Menominee, LM⁵        | 45.8 (3.9)       | 46.4 (3.3)      | 756.8 (222.0)           | 798.6 (178.4)           | 1.1 (0.8)                  | 1.6 (0.9)                  | 1.4 (0.9) | 48.0 (7.8) | 54.4 (14.6) | 51.2 (12.0) | 0/60 | 1/60 |
| Alpena, LH⁲           | 57.2 (4.6)       | 56.8 (4.0)      | 1779.9 (506.4)          | 1629.9 (448.6)          | 1.0 (0.7)                  | 1.5 (1.1)                  | 1.8 (0.9) | 42.8 (11.0) | 41.5 (11.2) | 42.1 (11.1) | 0/60 | 0/60 |
| Saginaw Bay, LH⁵      | 56.4 (4.3)       | 56.7 (3.9)      | 1710.3 (475.7)          | 1658.0 (436.7)          | 1.1 (0.9)                  | 1.5 (1.0)                  | 1.3 (1.0) | 46.0 (10.2) | 44.4 (7.5) | 45.2 (9.0) | 0/60 | 0/60 |

Note: Mean PCVs exclude fish infected with *F. psychrophilum*. Standard deviation of the data is reported in parentheses. ⁵Good recruitment site; ⁷Poor recruitment site.

Abbreviations: LH, Lake Huron; LM, Lake Michigan; LS, Lake Superior.
The PCV of the *F. psychrophilum*-infected fish (male) from the Menominee River in Lake Michigan was 56.0 compared to the mean PCV of 54.4 from uninfected fish (Table 2). The visceral fat index (VFI) of adult lake whitefish ranged from 0 (lowest possible score) to 4 (highest possible score) throughout this study, with an overall mean of 1.4 (Table 2). Mean VFI varied by collection site, with adult fish collected from Whitefish Bay having the highest mean VFI across both years and Baileys Harbor having the lowest (Table 2). Mean VFI did not vary substantially among fish that were collected from good (1.5) versus poor (1.4) recruitment sites (Table 2). Interestingly, the mean VFI of *F. psychrophilum*-infected fish was 3.0 compared to the means of 1.8 and 1.5 in Whitefish Bay and the Menominee River respectively. The null hypothesis of no differences in VFI scores for uninfected fish among sampling locations was rejected for 2018 (*p* < .001) but not for 2019 (*p* = .059). For 2018, VFI scores of uninfected fish from Baileys Harbor were significantly different from all other locations (Tables S1–S3). When testing for differences in VFI scores between infected and uninfected across years, the null hypothesis of no overall difference was rejected (*p* < .001).

**DISCUSSION**

Herein, *Flavobacterium psychrophilum*, the cause of substantial salmonid mortality around the globe (Starliper, 2011), was recovered from the kidneys of systemically infected adult lake whitefish,
marking the first time that this bacterial pathogen has been isolated from *C. clupeaformis* populations in the Great Lakes. This bacterium was recovered from lake whitefish collected from both Lakes Superior and Michigan that concurrently showed gross signs of systemic disease (e.g., haemorrhage, visceral pallor, swelling, etc.). Although it is not possible to attribute the observed disease signs to the *F. psychrophilum* infections, similar signs are frequently reported in other fish species suffering from BCWD (Bernardet et al., 1995; Rangdale et al., 1996; Taylor, 2004), thereby potentially cause disease (Hedrick, 1998). If fish in some sites are healthier, for example, fish collected from Whitefish Bay had higher VFIs (Table 2), which is indicative of good nutritional status (Adams, 1999), it may be that fish have higher survival rates leading to a higher probability of infected individuals being captured. Conversely, *F. psychrophilum* infection at sites with poor recruitment could have led to significantly higher mortalities and subsequently a low probability of detection. Again, the role of *F. psychrophilum* as a contributing factor in poor recruitment remains unknown but given that *F. psychrophilum* is associated with mortality of Atlantic salmon (*Salmo salar*) eggs (Cipriano, 2015). Although *F. psychrophilum* was not detected in the gonads of any lake whitefish in the current study, the bacterium was recovered from the kidneys. The kidney excretory function of fish is such that bacteria can be shed with urine, thus contaminating eggs (Perry, 2011). Thus, in conjunction with poor recruitment of juvenile lake whitefish, systemic infection of adult lake whitefish during spawning creates a potential pathway for the bacterium to be transmitted from infected parent to offspring.

Following morphological and molecular analyses that confirmed the identity of the recovered bacterium as *F. psychrophilum*, MLST revealed that the three lake whitefish *F. psychrophilum* isolates each belonged to newly identified singleton sequence types that were distinct from the >1,500 isolates that were genotyped using the same MLST scheme and originated from five different continents (Apablasa et al., 2013; Einarsdottir et al., 2020; Fujimurana-Nagata et al., 2013; Knupp et al., 2019; Nicolas et al., 2008; Nilsen et al., 2014; Sebastião et al., 2020; Siekoula-Nguedia et al., 2012; Strepparava et al., 2013; Van Vliet et al., 2016). This “distinctness” from all other MLST-genotyped *F. psychrophilum* isolates is notable for several reasons. Based on currently available data, this finding suggests that other Great Lakes salmonids are not the putative transmission source of the detected lake whitefish and vice versa. Indeed, studies have shown that some *F. psychrophilum* MLST genotypes show strong preference for a particular host species (Knupp et al., 2021; Van Vliet et al., 2016). Whether the *F. psychrophilum* strains recovered in this study preferentially infect lake whitefish remains to be determined. Nevertheless, the prospect of lake whitefish hatchery propagation within the Great Lakes basin is being increasingly considered (Bence et al., 2019; Ebener et al., 2021), and if pursued, the risk of *F. psychrophilum* transmission from infected broodstock to hatchery stocks is a concern that hatchery managers must recognize. All *F. psychrophilum* isolates in this study have been cryopreserved and can serve as a resource for developing BCWD prevention and control strategies, including the development of autogenous vaccines, should lake whitefish hatchery propagation begin.

In the context of good versus poor recruitment locations of this study, we detected *F. psychrophilum* infections exclusively in spawning aggregations of adult lake whitefish that have a history of good recruitment (Table 2), leaving the role of *F. psychrophilum* in declining recruitment unknown. However, complex interactions between a pathogen, host and the environment must occur for pathogens to cause disease (Hedrick, 1998). If fish in some sites are healthier, for example, fish collected from Whitefish Bay had higher VFIs (Table 2), which is indicative of good nutritional status (Adams, 1999), it may be that fish have higher survival rates leading to a higher probability of infected individuals being captured. Conversely, *F. psychrophilum* infection at sites with poor recruitment could have led to significantly higher mortalities and subsequently a low probability of detection. Again, the role of *F. psychrophilum* as a contributing factor in poor recruitment remains unknown but given that *F. psychrophilum* has the capacity to kill early life stages of other salmonids, studies should investigate the virulence of lake whitefish-associated isolates.

Findings from this study have contributed to potentially establishing baseline haematological (e.g., PCV) values for lake whitefish from the Great Lakes, although not a primary goal of our study. A handful of toxicological and dietary studies in hatchery-reared adult lake whitefish have reported PCVs of 36.7–42.3 (Pedlar et al., 2002), 36.8–43.3 (Cooley et al., 2000) and 38.5–40.1 (Ptashynski et al., 2002). In our study, the overall PCV was 44.1 and ranged from 40.2 to 54.4, which is higher than previously reported. Although a notable trend in PCVs of *F. psychrophilum*-infected fish was not observed, nor were significant differences in PCVs between
F. psychrophilum-infected and uninfected fish detected, there were significant differences in PCVs of uninfected fish among both years. In 2018, fish collected from Whitefish Bay had median PCVs that were significantly different from all other locations except for the Menominee River. In 2019, the Baileys Harbor and the Menominee River sites in Lake Michigan had significantly different median PCVs from all other locations. However, the factors behind these differences remain to be determined.

In conclusion, this study represents the first report of systemic F. psychrophilum infections in wild adult lake whitefish from Lakes Superior and Michigan. The presence of this bacterium, when combined with the observed clinical signs in infected fish, suggest F. psychrophilum is capable of causing systemic disease in lake whitefish. The bacterium's ability to not only be transgenerationally transmitted in other salmonids, but also cause substantial early life stage mortality, highlights a need to better understand the effects that F. psychrophilum has on lake whitefish health and survival. Importantly, results from the molecular analyses performed herein indicate the F. psychrophilum variants infecting lake whitefish in the Great Lakes are distinct from the variants that are widespread in other Great Lakes salmonids and may represent strains with a preference for lake whitefish. With these isolates now available, the ability of lake whitefish-associated F. psychrophilum strains to cause disease and/or mortality in the early life stages of this invaluable Great Lakes fish species can now be elucidated.

ACKNOWLEDGEMENTS
This research was funded by the Great Lakes Fishery Trust (Grant # 2018.1806). The authors thank Gary Whelan and Seth Herbst (Michigan Department of Natural Resources Fisheries Division), Sam McMurry (Big Stone Bay Fishery, Inc.), Tod Williams (Bay Port Fish Company), Tony LeBlanc (Big Abe Fisheries), Wilcox & Sons Fishery personnel, Todd Stuth (Baileys Harbor Fish Company), Don Tagderson, Chris Olds (USFWS, Alpena Fish and Wildlife Conservation Office), Ted Tresa and Sharon Rayford (USFWS, Green Bay Fish and Wildlife Conservation Office), Paul Ripple (Bay Mills Indian Community Biological Services) and Kris Dey (Little Traverse Bay Bands of Odawa Indians) for their excellent assistance in wild fish collection and/or transport. The authors also thank Dr. Pierre Nicolas (Université Paris-Saclay, INRAE) for his assistance in AT and ST assignments, and past and present members of the Michigan State University – Aquatic Animal Health Laboratory who provided excellent technical expertise and assistance during fish collections and clinical examination.

CONFLICT OF INTEREST
The authors declare no conflicts of interest associated with this study.

DATA AVAILABILITY STATEMENT
The MLST sequence data has been deposited in the Flavobacterium psychrophilum MLST Database (https://pubmlst.org/organisms/flavobacterium-psychrophilum). All other data presented in this study are available on request from the corresponding author.

ORCID
Courtney E. Harrison https://orcid.org/0000-0003-1685-1128
Christopher K. Knupp https://orcid.org/0000-0003-1292-4596
Thomas P. Loch https://orcid.org/0000-0002-6985-2477

REFERENCES
Adams, S. M. (1999). Ecological role of lipids in the health and success of fish populations. In M. T. Arts, & B. C. Wainman (Eds.), Lipids in Freshwater Ecosystems (pp. 132–160). Springer.

AFS-FHS (American Fisheries Society-Fish Health Section) (2016). FHS Blue Book: Suggested procedures for the detection and identification of certain fish and shellfish pathogens, 2016th ed. AFS-FHS.

Apablaza, P., Løland, A. D., Brevik, Ø. J., Ilardi, P., Battaglia, J., & Nylund, A. (2013). Genetic variation among Flavobacterium psychrophilum isolates from wild and farmed salmonids in Norway and Chile. Journal of Applied Microbiology, 114, 934–946. https://doi.org/10.1111/jam.12121

Becker, G. (1983). Fishes of Wisconsin. The University of Wisconsin Press.

Bence, J. R., Brenden, T. O., & Liljestrand, E. M. (2019). Feasibility of rehabilitating and supplementing fisheries by stocking lake whitefish in the upper Great Lakes. Great Lakes Fishery Trust. https://www.canr.msu.edu/qfc/publications/pdf-techreports/2019-techreports/LWF%20stocking%20white%20paper%20GLFT_revised%20November_final.pdf

Bernardet, J., Baudin-Laurencin, F., & Tiexerant, G. (1988). First identification of Cytophaga psychrophila in France. Bulletin of the European Association of Fish Pathologists, 8, 104–105.

Borg, A. F. (1948). Studies on myxobacteria associated with diseases in salmonid fishes. University of Washington.

Brenden, T. O., Ebener, M. P., & Sutton, T. M. (2010). Assessing the health of lake whitefish populations in the Laurentian Great Lakes. Journal of Great Lakes Research, 36, 1–5. https://doi.org/10.1016/j.jglr.2010.02.006

Brown, L. L., Cox, W. T., & Levine, R. P. (1997). Evidence that the causal agent of bacterial coldwater disease Flavobacterium psychrophilum is transmitted within salmonid eggs. Diseases of Aquatic Organisms, 29, 213–218. https://doi.org/10.3354/dao029213

Brown, M. L., & Murphy, B. R. (1991). Relationship of relative weight (Wr) to proximate composition of juvenile striped bass and hybrid striped bass. Transactions of the American Fisheries Society, 120, 509–518.

Bullock, G. L., Hsu, T. C., & Shotts, E. B. (1986). Columbaris disease of salmonids (p. 72). U.S. Fish and Wildlife Service fish disease leaflet 72.

Cipriano, R. C. (2005). Intraovum infection caused by Flavobacterium psychrophilum among eggs from captive Atlantic salmon broodfish. Journal of Aquatic Animal Health, 17, 275–283. https://doi.org/10.1577/H05-003.1

Cipriano, R. C. (2015). Bacterial analysis of fertilized eggs of Atlantic salmon from the Penobscot, Naragansett, and Machias Rivers. Maine. Journal of Aquatic Animal Health, 27(3), 172–177. https://doi.org/10.1080/08997659.2015.1050127

Cipriano, R. C., Ford, L. A., & Teska, J. D. (1995). Association of Cytophaga psychrophila with mortality among eyed eggs of Atlantic salmon (Salmo salar). Journal of Wildlife Diseases, 51, 166–171. https://doi.org/10.7589/0090-3558-31.2.166

Cooley, H. M., Evans, R. E., & Klaverkamp, J. F. (2000). Toxicology of dietary uranium in lake whitefish (Coregonus clupeaformis). Aquatic Toxicology, 48(4), 495–515. https://doi.org/10.1016/S0166-445X(99)00057-0

Decostere, A., D’Haese, E., Lammens, M., Nelis, H., & Haesebrouck, F. (2001). In vivo study of phagocytosis, intracellular survival and multiplication of Flavobacterium psychrophilum in rainbow trout, Oncorhynchus mykiss (Walbaum), spleen phagocytes. Journal of Fish Diseases, 24, 481–487. https://doi.org/10.1046/j.1365-2761.2001.00322.x
Dunn, O. J. (1964). Multiple comparisons using rank sums. Technometrics, 6, 241–252. https://doi.org/10.1080/00401706.1964.10482090

Ebener, M. P. (1997). Recovery of lake whitefish populations in the Great Lakes. Fisheries, 22(7), 18–20.

Ebener, M. P., Dunlop, E. S., & Muir, A. M. (2021). Declining recruitment of lake whitefish to fisheries in the Laurentian Great Lakes: Management considerations and research priorities. www.glfc.org/pubs/misc/2021-01.pdf

Ebener, M., Kinnunen, R., Mohr, L., Schneeeberger, P., Hoyle, J., & Peeters, P. (2008). Management of commercial fisheries for lake whitefish in the Laurentian Great Lakes of North America. In M. Schechter, W. Taylor, & N. Leonard (Eds.). International governance of fisheries ecosystems: Learning from the past, finding solutions for the future (pp. 99–143). American Fisheries Society.

Einarsdottir, T., Guttormsdottir, G., Connaghan, D., & Hjartardottir, S. (2020). Longitudinal survey of Flavobacterium species in Icelandic salmonid fish farms. Diseases of Aquatic Organisms, 141, 15–24. https://doi.org/10.3354/dao03058

Faisal, M., Shavalier, M., Kim, R. K., Millard, E. V., Gunn, M. R., Winters, A. D., Schulz, C. A., Eissa, A., Thomas, M. V., Wolgaamood, M., Whelan, G. E., & Winton, J. (2012). Spread of the emerging viral hemorrhagic septicemia virus strain, genotype IVb, in Michigan, USA. Viruses, 4(1), 734–760. https://doi.org/10.3390/v4050734

Feil, E. J., Li, B. C., Aaenensen, D. M., Hanage, W. P., & Spratt, B. G. (2004). eBURST: Inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. Journal of Bacteriology, 186, 1518–1530. https://doi.org/10.1128/JB.186.5.1518-1530.2004

Fera, S. A., Rennie, M. D., & Dunlop, E. S. (2015). Cross-basin analysis of long-term trends in the growth of lake whitefish in the Laurentian Great Lakes. Journal of Great Lakes Research, 41, 1138–1149. https://doi.org/10.1016/j.jglr.2015.08.010

Francisco, A. P., Bugalho, M., Ramirez, M., & Carrico, J. A. (2009). Global optimal eBURST analysis of multilocus typing data using a graphic matroid approach. BMC Bioinformatics, 10, 152. https://doi.org/10.1186/1471-2105-10-152

Fujiwara-Nagata, E., Chantry-Darmon, C., Bernardet, J. F., Eguchi, M., Duchaufour, E., & Nicolas, P. (2013). Population structure of the fish pathogen Flavobacterium psychrophilum at a whole country and model river levels in Japan. Veterinary Research, 44, 34. https://doi.org/10.1186/1297-9716-44-34

Hedrick, R. P. (1998). Relationships of the host, pathogen, and environment: Implications for diseases of cultured and wild fish populations. Journal of Aquatic Animal Health, 10(2), 107–111

Holey, M. E., Elliot, R. F., Marczynski, S. V., Hnath, J. G., & Smith, K. D. (1998). Chinook salmon epizootics in Lake Michigan, possible contributing factors and management implications. Journal of Aquatic Animal Health, 10(2), 202–210.

Holt, R. A. (1987). Cytophaga psychrophila, the causative agent of bacterial cold water disease in salmonid fish. Oregon State University.

Hothorn, T., Hornik, K., van de Wiel, M. A., & Zeileis, A. (2006). A Lego system for conditional inference. The American Statistician, 60(3), 257–263. https://doi.org/10.1198/000313006X118430

Hoyle, J. A. (2005). Status of lake whitefish (Coregonus clupeaformis) in Lake Ontario and the response to the loss of Diporeia spp. L. C. Mohr, & T. F. Nalepa (Eds.). Proceeding of a workshop on the dynamics of lake whitefish (Coregonus clupeaformis) and the amphipod Diporeia spp. in the Great Lakes (pp. 47–66). Great Lakes Fishery Commission. Technical report 66.

Jolley, K. A., Bray, J. E., & Maiden, M. C. J. (2018) Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Research, 3, 124. https://doi.org/10.12688/wellcomeopenres.14826.1

Knupp, C., Klupel, M., Brenden, T. O., & Loch, T. P. (2021). Host-specific preference of some Flavobacterium psychrophilum multilocus sequence typing genotypes determines their ability to cause bacterial coldwater disease in coho salmon (Oncorhynchus kisutch). Journal of Fish Diseases, 44, 521–531. https://doi.org/10.1111/jfd.13340

Knupp, C. K., Wiens, D., Faisal, M., Call, D. R., Cain, K. D., Nicolas, P., Van Vliet, D., Yamashita, C., Ferguson, J. A., Meuninck, D., Hsu, W., Baker, B. B., Shen, L., & Loch, T. P. (2019). Large-scale analysis of Flavobacterium psychrophilum multilocus sequence typing genotypes recovered from North American salmonids indicates that both newly identified and recurrent clonal complexes are associated with disease. Applied and Environmental Microbiology, 85, e02305. https://doi.org/10.1128/AEM.02305-18

Lafferty, K. D., Harvell, C. D., Conrad, J. M., Friedman, C. S., Kent, M. L., Kuris, A. M., Powell, E. N., Rondeau, D., & Saksida, S. M. (2015). Infectious diseases affect marine fisheries and aquaculture economics. Annual Review of Marine Science, 7, 471–496. https://doi.org/10.1146/annurev-marine-010814-015646

Lenart, S. J., & Caroffino, D. C. (2016) Executive summary. In Technical fisheries committee administrative report 2016: Status of lake trout and lake whitefish populations in the 1836 treaty-ceded waters of Lakes Superior, Huron and Michigan, with recommended yield and effort levels for 2016 (pp. 4–8). Modeling Subcommittee to the Technical Fisheries Committee. https://doi.org/10.13140/RG.2.2.19714.32964

Lenart, S. J., & Caroffino, D. C. (2017). Executive summary. In Technical fisheries committee administrative report 2016: Status of lake trout and lake whitefish populations in the 1836 treaty-ceded waters of Lakes Superior, Huron and Michigan. Modeling Subcommittee to the Technical Fisheries Committee.

Loch, T. P., & Faisal, M. (2011). Infectious diseases of lake whitefish (Coregonus clupeaformis) in the Laurentian Great Lakes. In Aquatic animal health: A continuing dialogue between Russia and the United States. Proceedings of the third bilateral conference between the United States and Russia: Aquatic animal health 2009. 12–20 (July 2009). Michigan State University, East Lansing.

Lorenzen, E., Dalsgaard, I., & Bernardet, J. (1997). Characterization of isolates of Flavobacterium psychrophilum associated with coldwater disease or rainbow trout fry syndrome I: Phenotypic and genomic studies. Diseases of Aquatic Organisms, 31, 197–208. https://doi.org/10.3354/dao03119

Madetoja, J., Nyman, P., & Wiklund, T. (2000). Flavobacterium psychrophi lum, invasion into and shedding by rainbow trout Oncorhynchus mykiss. Diseases of Aquatic Organisms, 43, 27–38. https://doi.org/10.3354/dao43027

Mangiavicho, S. (2021). Rcompanion: Functions to support extension education program evaluation. R package version 2.4.6. https://CRAN.R-project.org/package=rcompanion

Michigan Department of Natural Resources (2021). Lake whitefish. https://www.michigan.gov/dnr/0,4570,7-350-79135_79218_79614_82676--,00.html

Mohr, L. C., & Ebener, M. P. (2007) Evaluation of two harvest policies for lake whitefish (Coregonus clupeaformis) populations in Laurentian Great Lake, Lake Huron. Biology and management of coregonid fishes – 2005. Proceedings of the ninth international symposium on the biology and management of coregonid fishes. Advances in Limnology, 60, 471-483.

Mohr, L. C., & Nalepa, T. F. (Eds.). (2005) Proceedings of a workshop on the dynamics of lake whitefish (Coregonus clupeaformis) and the amphipod Diporeia spp. in the Great Lakes. Great Lakes Fishery Commission. Technical report 66.

Nicolás, P., Mondot, S., Achaz, G., Bouchenot, C., Bernardet, J. F., & Duchaufour, E. (2008). Population structure of the fish-pathogenic bacterium Flavobacterium psychrophilum. Applied and Environmental Microbiology, 74, 3702–3709. https://doi.org/10.1128/AEM.00244-08

Nilsson, H., Sundell, K., Duchaufour, E., Nicolás, P., Dalsgaard, I., Madsen, L., Aspán, A., Jansson, E., Colquhoun, D. J., & Wiklund, T. (2014). Multilocus sequence typing identifies epidemic clones...
of Flavobacterium psychrophilum in Nordic countries. Applied and Environmental Microbiology, 80, 2728–2736. https://doi.org/10.1128/AEM.04233-13

Nocker, A., Richter-Heitmann, T., Montijn, R., Schuren, K., & Kort, R. (2010). Discrimination between live and dead cells in bacterial communities from environmental water samples analyzed by 454 pyrosequencing. International Microbiology, 13(2), 59–65. https://doi.org/10.2436/20.1501.01.111

Ogle, D. H., Doll, J. C., Wheeler, P., & Dinno, A. (2021). FSA: Fisheries stock analysis. R package version 0.9.1. https://github.com/droglenc/FSA

Ptashynski, M. D., Pedlar, R. M., Evans, R. E., Baron, C. L., & Klaverkamp, J. F. (2002). Toxicological effects of dietary arsenic exposure in lake whitefish (Coregonus clupeaformis). Aquatic Toxicology, 57(3), 167–189. https://doi.org/10.1016/S0166-445X(01)00198-9

Perry, S. F. (2011) Role of the kidneys. Encyclopedia of Fish Physiology, 37, 1411–1418.

Ptashynski, M. D., Pedlar, R. M., Evans, R. E., Baron, C. L., & Klaverkamp, J. F. (2002). Toxicology of dietary nickel in lake whitefish (Coregonus clupeaformis). Aquatic Toxicology, 58(3–4), 229–247. https://doi.org/10.1016/S0166-445X(01)00239-9

Rendall, R. E., Richards, R. H., & Alderman, D. J. (1996). Isolation of Cytophaga psychrophila, causal agent of rainbow trout fry syndrome (RTFS) from reproductive fluids and egg surfaces of rainbow trout (Oncorhynchus mykiss). Bulletin of the European Association of Fish Pathologists, 16, 63–67.

Reichenbach, H., Kleining, H., & Achenbach, H. (1974). The pigments of Flexibacter elegans: Novel and chemosystematically useful compounds. Archives of Microbiology, 101, 131–144. https://doi.org/10.1007/BF00455933

Rennie, M. D. (2014). Context-dependent changes in lake whitefish populations associated with dreissenid invasion. In T. F. Nalepa, & D. W. Schloesser (Eds.), In Quagga and zebra mussels biology, impacts, and control (pp. 661–680). CRC Press.

Ronaghi, M. (2001). Pyrosequencing sheds light on DNA sequencing. Genome Research, 11, 3–11. https://doi.org/10.1101/gr.150601

Schneeeberger, P. J., Ebener, M. P., Toneyes, M., & Peeters, P. J. (2005) Status of lake whitefish (Coregonus clupeaformis) in Lake Michigan. Proceedings of a workshop on the dynamics of lake whitefish (Coregonus clupeaformis) and the amphipod Diporeia spp. in the Great Lakes. M. C. Mohr, & T. F. Nalepa (Eds.). Great Lakes Fishery Commission technical (pp. 67-86). Report 66.

Schorfhaar, R. G., & Peck, J. W. (1993). Catch and mortality of non-target species in lake whitefish trap nets in Michigan waters of Lake Superior. Michigan Department of Natural Resources. Fisheries Research Report Number 1974. https://www2.dnr.state.mi.us/Publications/PDFS/IFR/IFRlibra/research/reports/1974rr.pdf

Sebastião, F. A., Loch, T. P., Knupp, C., Mukkatira, K., Veek, T., Richey, C., Adkinson, M., Griffin, M. J., & Soto, E. (2020). Multilocus Sequence Typing (MLST) analysis of California Flavobacterium psychrophilum reveals novel genotypes and predominance of CC-ST10 in California salmonid hatcheries. Aquaculture Research, 51, 2349–2358. https://doi.org/10.1111/are.14578

Sevellec, M., Pavey, S. A., Boutin, S., Filteau, M., Derome, N., & Bernatchez, L. (2014). Microbiome investigation in the ecological speciation context of lake whitefish (Coregonus clupeaformis) using next-generation sequencing. Journal of Evolutionary Biology, 27, 1029–1046. https://doi.org/10.1111/jeb.12374

Siekoula-Nguedia, C., Blanc, G., Duchaud, E., & Calvez, S. (2012). Genetic diversity of Flavobacterium psychrophilum isolated from rainbow trout in France: Predominance of a clonal complex. Veterinary Microbiology, 161, 169–178. https://doi.org/10.1016/j.vetmic.2012.07.022

Starliper, C. E. (2011). Bacterial coldwater disease of fishes caused by Flavobacterium psychrophilum. Journal of Advanced Research, 2, 97–108. https://doi.org/10.1016/j.jare.2010.04.001

Strepparava, N., Nicolas, P., Wahl, T., Segner, H., & Petrini, O. (2013). Molecular epidemiology of Flavobacterium psychrophilum from Swiss fish farms. Diseases of Aquatic Organisms, 105, 203–210. https://doi.org/10.3354/dao02609

Taylor, P. W. (2004). Detection of Flavobacterium psychrophilum in eggs and sexual fluids of Pacific salmonids by a polymerase chain reaction assay: Implications for vertical transmission of bacterial coldwater disease. Journal of Aquatic Animal Health, 16, 104–108. https://doi.org/10.1577/H03-053.1

Toyama, T., Kita-Tsukamoto, K., & Wakabayashi, H. (1994). Identification of Cytophaga psychrophila by PCR targeted 16S ribosomal RNA. Fish Pathology, 29, 271–275.

Van Vliet, D., Loch, T. P., & Faisal, M. (2015). Flavobacterium psychrophilum infections in salmonid broodstock and hatchery-propagated stocks of the Great Lakes basin. Journal of Aquatic Animal Health, 27(4), 192–202. https://doi.org/10.1080/08997659.2015.1088488

Van Vliet, D., Wiens, G. D., Loch, T. P., Nicolas, P., & Faisal, M. (2016). Genetic diversity of Flavobacterium psychrophilum isolates from three Oncorhynchus spp. in the United States, as revealed by multilocus sequence typing. Applied and Environmental Microbiology, 82, 3246–3255. https://doi.org/10.1128/AEM.00411-16

SUPPORTING INFORMATION
Additional supporting information may be found in the online version of the article at the publisher’s website.