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RESEARCH ARTICLE

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First isolation of *Flavobacterium psychrophilum* from wild adult Great Lakes lake whitefish (*Coregonus clupeaformis*)

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Abstract

Lake whitefish (*Coregonus clupeaformis*; LWF) is an economically and ecologically valuable native species to the Great Lakes, but recent declines in their recruitment have generated significant concern about their future viability. Although studies have sought to identify factors contributing to declining recruitment, the potential role(s) of infectious diseases has not been thoroughly investigated. In 2018 and 2019, adult LWF were collected from Lakes Superior, Michigan, and Huron for clinical examination and bacteriological analyses. Herein, we describe the first isolation of *Flavobacterium psychrophilum*, aetiological agent of bacterial coldwater disease (BCWD) and rainbow trout fry syndrome (RTFS), from systemically infected adult LWF. Bacterial isolates were yellow-orange, Gram-negative, filamentous bacilli that were oxidase and catalase positive, and produced a flexirubin-type pigment in 3% potassium hydroxide. Isolate identity was confirmed via *F. psychrophilum*-specific PCR, and multilocus sequence typing revealed three new singleton sequence types (STs) that were distinct from all previously described *F. psychrophilum* STs. The prevalence of *F. psychrophilum* infections was 3.3, 1.7, and 0.0% in Lakes Superior, Michigan and Huron respectively. Findings illustrate the potential for *F. psychrophilum* to cause systemic infections in adult LWF and highlight the need for future studies to investigate the bacterium's potential role in declining LWF recruitment.

KEYWORDS
bacterial coldwater disease, *Flavobacterium psychrophilum*, Great Lakes, lake whitefish, recruitment

1 | 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 salmonicida* 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 *Bacteriodetes*; Family *Flavobacteriaceae*), the causative agent of bacterial coldwater 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 (Baileys 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 Schorfhaar 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 × 1.8 m tall seine with a 1.8 m × 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, Baileys 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

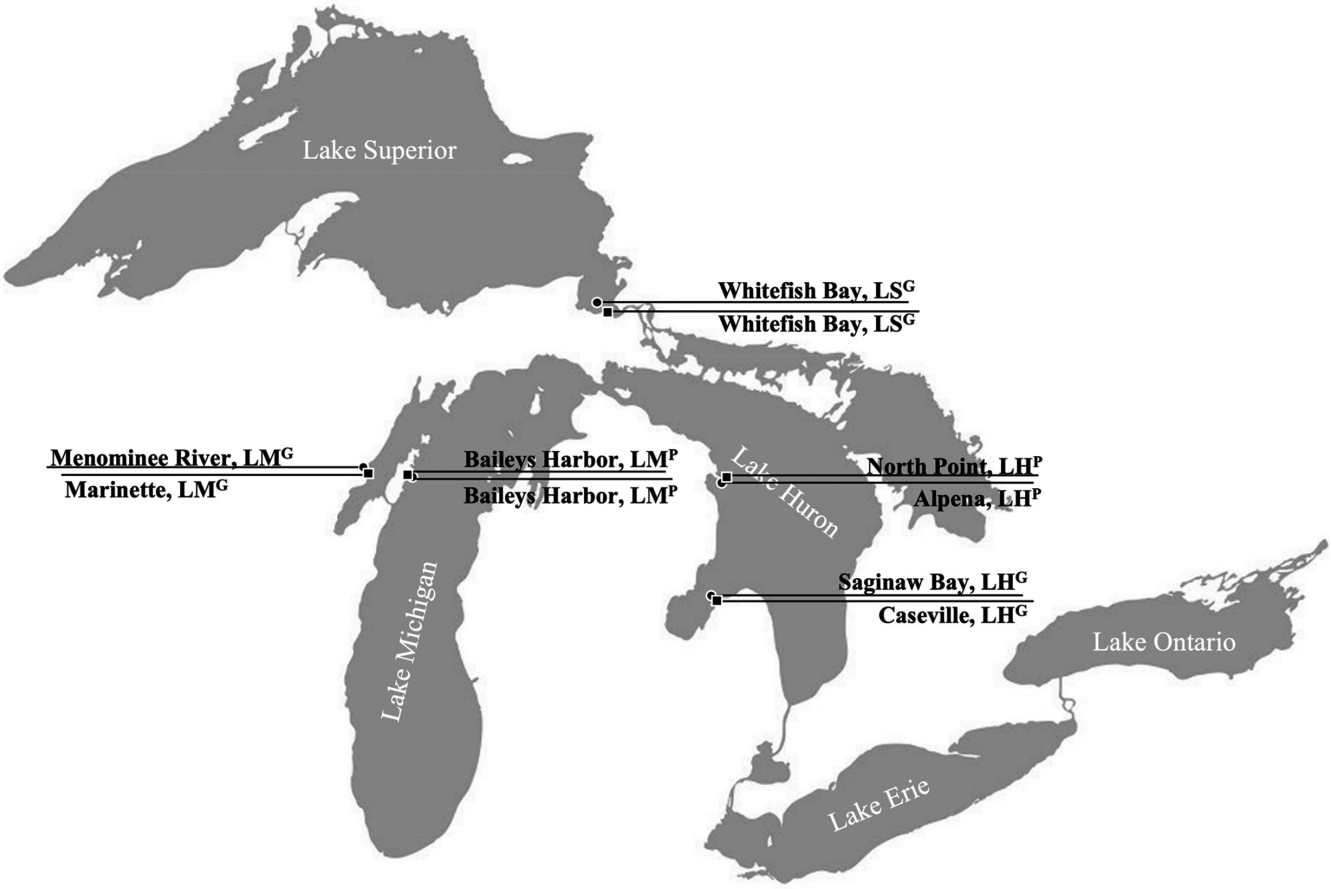

FIGURE 1 Adult and post-larval lake whitefish collection sites in Lakes Superior, Michigan and Huron. [G]Good recruitment site; [P]Poor recruitment site. Adult collection site = ●; post-larval collection site = ■

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, LS[G]	25	2.6 (0.2)	<1.0
Baileys Harbor, LM[P]	150	6.1 (0.3)	1.9 (0.3)
Marinette, LM[G]	110	3.4 (0.6)	0.3 (0.1)
North Point, LH[P]	150	3.4 (0.4)	0.3 (0.1)
Caseville, LH[G]	1	2.6	0.2

Note: Standard deviation of the data is reported in parentheses. [G]Good recruitment site; [P]Poor recruitment site.
Abbreviations: LH, Lake Huron; LM, Lake Michigan; LS, Lake Superior.

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, $MgSO_4 \cdot 7H_2O$, and $CaCl_2 \cdot 2H_2O$ 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

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.phyloviz.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/fpsychrophilum/; 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	2018	2019	Overall	2018	2019	Overall	2018	2019
Whitefish Bay, LS[G]	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, LM[P]	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, LM[G]	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[P]	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, LH[G]	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. [G]Good recruitment site; [P]Poor recruitment site.

Abbreviations: LH, Lake Huron; LM, Lake Michigan; LS, Lake Superior.

isolates were determined to be Gram-negative, filamentous bacilli that were positive for cytochrome oxidase and catalase activities and produced a flexirubin-type pigment when immersed in 3% potassium hydroxide. Based on these initial results, the bacteria were suspected of belonging to the genus *Flavobacterium* and thus subjected to molecular characterization.

Using the *F. psychrophilum*-specific endpoint PCR assay of Toyama et al. (1994), all three isolates produced amplicons of approximately 1,088 base pairs, confirming their identity as *F. psychrophilum*. All three *F. psychrophilum* isolates collected from lake whitefish were placed into three new MLST singleton sequence types (e.g. ST374, ST377 and ST378) that were distinct from all other described sequence types, including all known genotypes within the Great Lakes basin (Figure 2).

The prevalence of systemic *F. psychrophilum* infections in adult lake whitefish ranged from 0% to 3.3% across the five sampled sites (Table 2); however, the bacterium was never detected in the gonads of any adult fish, nor from any of the 436 post-larval lake whitefish. Although *F. psychrophilum* was recovered from lake whitefish collected from both Lakes Superior and Michigan, the bacterium was detected exclusively in fish collected from sites assessed to have good recruitment (Table 2).

External signs of disease that were observed in *F. psychrophilum*-infected adult lake whitefish included bilateral ocular haemorrhage, multifocal to coalescing haemorrhage along the ventrum and haemorrhage within the caudal fin. Internally, splenic congestion and swelling, hepatic pallor and friability, renal congestion, heart pallor and hyperaemia of the swim bladder vasculature were noted. In one fish, diffuse petechial haemorrhage within the swim bladder and congestion of the ovaries were also observed (Figure 3). This same fish also harboured approximately five presumptive *Cystidicola* sp. within the swim bladder lumen. In all cases, *F. psychrophilum*-infected fish harboured encysted metacercariae (presumptive *Tetracotyle* sp.) within the ventricle of the heart. No other bacteria, viruses or parasites were detected in *F. psychrophilum*-infected fish.

Overall, the mean packed cell volume (PCV) of adult lake whitefish was 44.1 (Table 2), with some variation among collection sites. The mean PCV in good and poor recruitment sites was 45.5. and 42.0 respectively. The null hypothesis of no difference in median PCV values of uninfected adult lake whitefish among the sampling locations was rejected in both 2018 ($\chi^2 = 31.2$, $df = 4$, $p < .001$) and 2019 ($\chi^2 = 20.0$, $df = 4$, $p < .001$). We were unable to reject the null hypothesis of no difference in median PCV values for uninfected and infected adult fish among sampling locations in 2018 and 2019 ($\chi^2 = 4.6$, $df = 3$, $p = .200$). Based on pairwise comparisons for 2018, Whitefish Bay median PCVs were significantly different from all other locations except for the Menominee River (Tables S1–S3). In 2019, Baileys Harbor and the Menominee River median PCVs were significantly different from all other locations (Tables S1–S3). There were also differences in PCV between sexes. Overall, the mean PCV of male and female lake whitefish was 46.2 and 41.2 respectively. The PCVs of *F. psychrophilum*-infected fish collected from Whitefish Bay, Lake Superior (female) were 32.0 and 34.0 compared to the mean PCV of 41.9 for uninfected fish collected from the same site

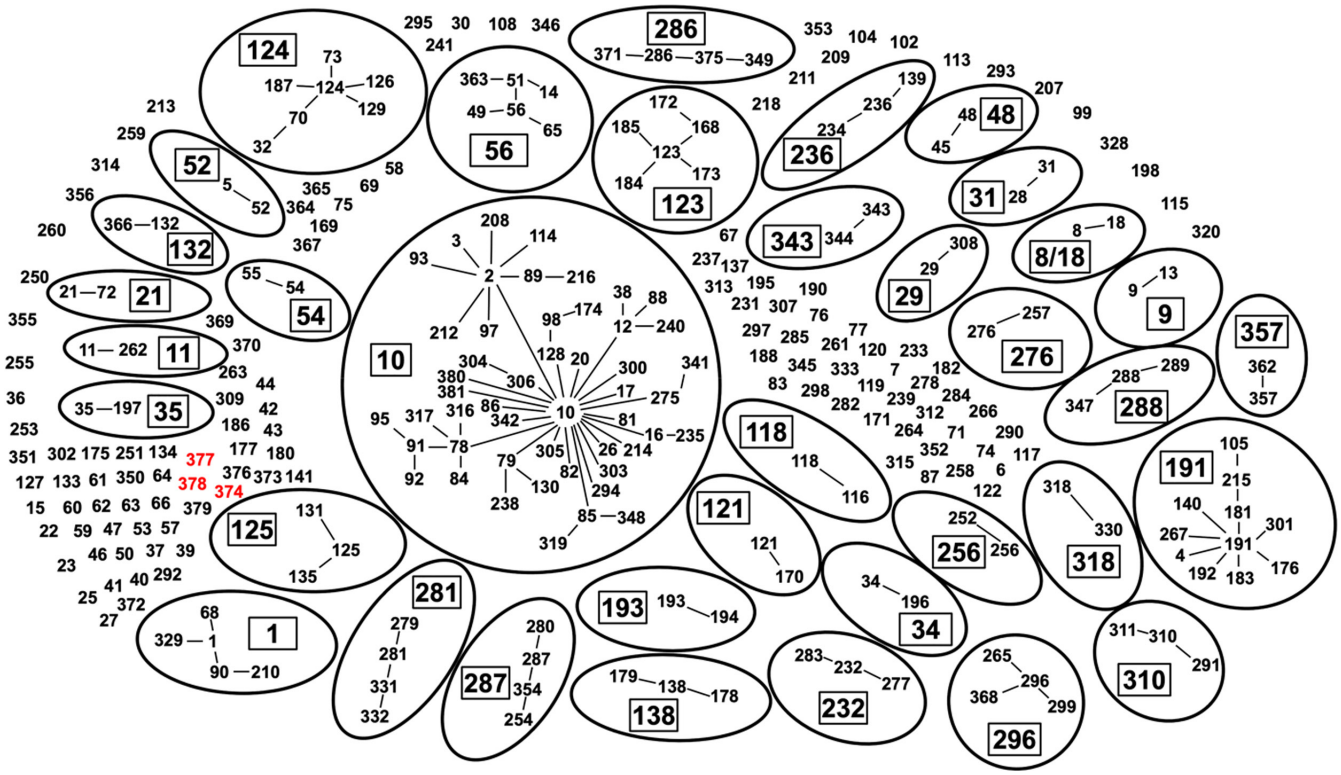

FIGURE 2 goeBURST diagram depicting the genetic relatedness (as determined via multilocus sequence typing) of the three *F. psychrophilum* isolates recovered from adult lake whitefish (shown in red) when compared with all 1,545 previously typed *F. psychrophilum* isolates. Clonal complexes (CC), defined as groups of isolates connected by single-locus variant (SLV) links, are depicted by circles. A CC is named after the most likely founding ST identified as the ST with the highest number of SLVs. Founding STs are named after the ST that has the highest number of SLVs. If STs have the same number of isolates, the CC is named after the earliest ST. The founding ST of a CC is depicted in a box

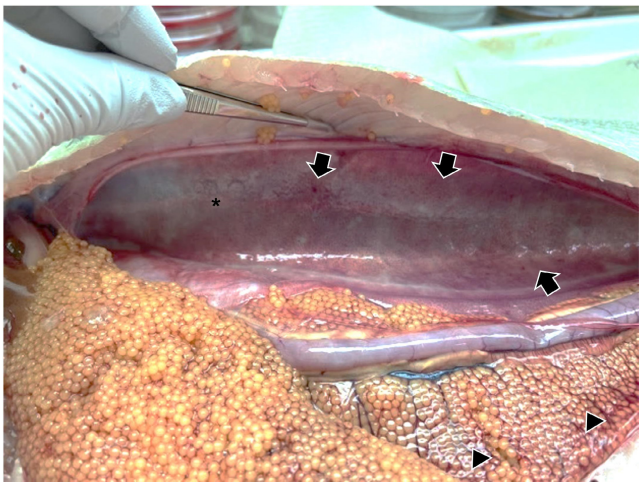

FIGURE 3 *Flavobacterium psychrophilum*-infected adult lake whitefish with diffuse ecchymosis throughout the swim bladder (arrows) and concurrent congestion of the ovaries (arrowhead). Also note the presence of the swim bladder nematode (*), presumptive *Cystidicola* sp., within the swim bladder lumen

(Table 2). 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 ($p < .001$).

4 | 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., 1988; Starliper, 2011). It is also noteworthy that some uninfected adult lake whitefish also showed similar clinical signs, though no such signs were observed in juvenile fish.

Only one previously published study, which utilized molecular pyrosequencing techniques, has detected *F. psychrophilum* in *C. clupeaformis*, which were collected from Cliff, Indian, East and Webster Lakes within the St. John River drainage, Québec (Canada) and Maine (United States; Sevellec et al., 2014). Importantly, pyrosequencing is a sensitive means of detecting genetic traces of bacteria (Ronaghi, 2001), which may or may not be indicative of a true infection status (Nocker et al., 2010). Thus, our study provides the first evidence that lake whitefish in the Great Lakes are indeed susceptible hosts for *F. psychrophilum* and can suffer from systemic infections as they approach spawning. Only one other study has documented that *F. psychrophilum* may be capable of infecting fish within the genus *Coregonus*; Lorenzen et al. (1997) recovered a phenotypically similar bacterium from skin lesions of muksun (*C. muksun*) in Finland, but no further description of disease was given.

Although the prevalence of *F. psychrophilum* infection was low in the adult lake whitefish that were analysed in this study (Table 2), the discovery of these infections is potentially significant for several reasons. During spawning, lake whitefish congregate over rocky shoals in shallow water (Becker, 1983), thus providing an opportunity for horizontal transmission of *F. psychrophilum*. Indeed, *F. psychrophilum* can be transmitted either directly (fish-to-fish) or indirectly (through the water column; Madetoja et al., 2000). Perhaps most importantly, *F. psychrophilum* is known to be vertically transmitted in other salmonids from infected parents to offspring via reproductive fluids and within infected eggs (Cipriano, 2005; Cipriano et al., 1995; Rangdale et al., 1996; Taylor, 2004), thereby potentially leading to substantial mortality in the early life stages. For example, Decostere et al. (2001) found that clinical disease signs and mortality were more severe in 10-week-old rainbow trout (*O. mykiss*) than in 15-week-old rainbow trout, and there is also evidence that the bacterium 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 contacting 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 (Apablaza et al., 2013; Einarsdottir et al., 2020; Fujiwara-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.

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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.

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