Article

Investigation of the prevalence of thermophilic *Campylobacter* species at Lake Simcoe recreational beaches

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Abstract

Thermophilic *Campylobacter* species have been implicated in human gastrointestinal infections and can occur in agricultural run off, sewage discharges, and the feces of domestic and wild animals including birds. A 2-year study was designed to investigate the occurrence of the primary thermophilic *Campylobacter* species (*C. jejuni*, *coli*, and *lari*) associated with human disease at 5 recreational beaches on Lake Simcoe, Ontario, Canada. A biweekly sampling program involved collecting water samples across 3 depth zones (sand pore water and ankle- and chest-depth waters). To identify the potential sources of contamination, samples were also collected from 4 neighboring rivers corresponding to selected beaches, a few fresh seagull and Canada geese fecal droppings on beaches, and a stormwater outfall. Water and fecal samples were processed for *Campylobacter* spp. isolation and detection using a minimum probable number culture enrichment protocol. Thermophilic *Campylobacter* spp. generally occurred infrequently and at low concentrations (≤ 30 cells L^{-1}) at all sampling locations; they were detected in 12% of water samples from beaches (n = 289) compared to 14% from rivers (n = 100). *C. jejuni* and *C. lari* were the species most commonly detected. Nine isolates identified as unknown *Campylobacter* spp. were further sequenced and shown to be more closely related to *Arcobacter* spp. At beaches, thermophilic *Campylobacter* spp. were generally detected more often in sand pore water than in ankle- or chest-depth water. The study suggests that sand, rivers, and bird droppings could be potential sources of *Campylobacter* spp. contamination at Lake Simcoe recreational beaches.

Key words: *Campylobacter*, fecal pollution, human health, Lake Simcoe, recreational water quality, waterborne pathogens

Introduction

Human pathogens including bacteria, protozoa, and viruses are associated with gastroenteritis, making them key water quality concerns responsible for infectious disease outbreaks in inland and coastal recreational waters (Yoder et al. 2008). Warm-blooded animals have the potential to carry a variety of human pathogenic bacteria (e.g., *Campylobacter*) and protozoa (e.g., *Cryptosporidium*) that may pose human health risks (Dorner et al.

2004). Because of the great diversity of pathogenic bacteria, protozoa, and viruses potentially transmitted by contaminated water, and the difficulty as well as cost for directly measuring all pathogens in environmental samples, fecal indicator bacteria are used to infer the potential occurrence of pathogens in water.

Microbial water quality guidelines and standards have been developed for recreational waters in Canada and other countries. In accordance with these guidelines, fecal indicator bacteria such as *Escherichia coli* (*E. coli*) or *Enterococcus* spp. are used for measuring and assessing the levels of fecal contamination in recreational waters. Beaches are only designated as being unsafe for recreational activities when levels of indicator bacteria exceed established standards. In Ontario, recreational water quality has to meet the requirements of provincial recreational water quality standards (OMOE 1994), which specify the microbiological parameters to be tested and the standards to be met. According to the guidelines, beaches are posted with swimming advisories when *E. coli* concentrations exceed 100 colony forming units per 100 mL, or when other adverse conditions such as algal blooms or chemical spills occur. Use of fecal indicator bacteria only enables an inference of the occurrence of waterborne pathogens at beaches, therefore, more research is needed to directly measure the presence of pathogens at beaches to better understand their patterns of occurrence.

In the last two decades, thermophilic *Campylobacter* species have been recognized as one of the leading causes of bacterial gastroenteritis in humans (Park et al. 1991, Frost 2001, Jones 2001). *Campylobacter* is now the most commonly reported agent of gastrointestinal illness across Canada (PHAC 2008). The genus *Campylobacter* is currently composed of 22 species and 8 subspecies (Debruyne et al. 2009a), but from a public health perspective the *Campylobacter* species *C. jejuni*, *C. coli*, and *C. lari* are the most frequently implicated in human infections (Butzler 2004). These three species can occur in the gastrointestinal tracts of humans and other animals including bovines, birds, and pigs.

Water can be an important source of *Campylobacter* contamination, leading to outbreaks of waterborne disease. Campylobacters from the feces of birds and domestic and wild animals, municipal sewage discharges, or agricultural runoff can contaminate water (Jones 2001). Gulls can be major contributors of fecal contamination at recreational beaches, indicated by high loadings of *E. coli* (Alderisio and DeLuca 1999). Other studies have shown that fecal droppings from waterfowl and seagulls can be important sources of fecal indicator bacteria and waterborne pathogens such as *C. jejuni* and *C. lari* (Lévesque et al. 2000, Van Dyke et al. 2010). Previous studies have suggested that *Campylobacter* infections,

which typically peak during the summer months, may be linked to recreational activities, including participation in water sports (Skirrow 1987, Kueh et al. 1995).

Pathogens and associated human health risks were identified as a primary public concern in the Lake Simcoe Protection Plan (OMOE et al. 2009) established by the 2008 Lake Simcoe Protection Act. With the exception of the Great Lakes, Lake Simcoe is the largest lake in southern Ontario, and its watershed encompasses some of the fastest growing communities in Canada (Palmer et al. 2011). Lake Simcoe is also located in close proximity to more than half of the population in the province, making it a popular tourist destination. Recreational activities on Lake Simcoe such as fishing, boating, and other water sports generate more than C$200 million in annual revenue (Palmer et al. 2011). The frequency of public health advisories, beach postings, and recreational beach closures due to elevated *E. coli* levels (i.e., above provincial standards) have increased in recent years, however, raising concerns about Lake Simcoe beach water quality (OMOE et al. 2009).

The purpose of this study was to investigate the prevalence of thermophilic *Campylobacter* spp. at Lake Simcoe beaches and identify potential sources of contamination. Our specific objectives were to (1) quantify the occurrence of thermophilic *Campylobacter* species at Lake Simcoe beaches; (2) investigate potential sources of *Campylobacter* contamination, including bird fecal droppings and nearby rivers as well as stormwater outfalls that can carry wastes from humans, livestock, and pets; and (3) identify thermophilic *Campylobacter* species present using a species-specific triplex polymerase chain reaction (PCR) amplification assay and DNA sequence analysis of other campylobacters. To our knowledge, this is the first study to investigate the occurrence of thermophilic *Campylobacter* species and their potential sources in Lake Simcoe.

Materials and methods

Study sites

The study sites were located along the southeastern shore of Lake Simcoe and represented a range in the issuance frequency of public health advisories (Fig. 1). Beaches were selected from the southeastern shore because this area had the greatest number of beach postings between 2006 and 2008 (Young et al. 2010).

Beach descriptions and sampling

Four beaches (Beaverton Beach North, Holmes Point Beach, Bonnie Park Beach, and Claredon Beach) were sampled in 2010 and 2011, while Balfour Beach was sampled only in 2011. Similar to many beaches along
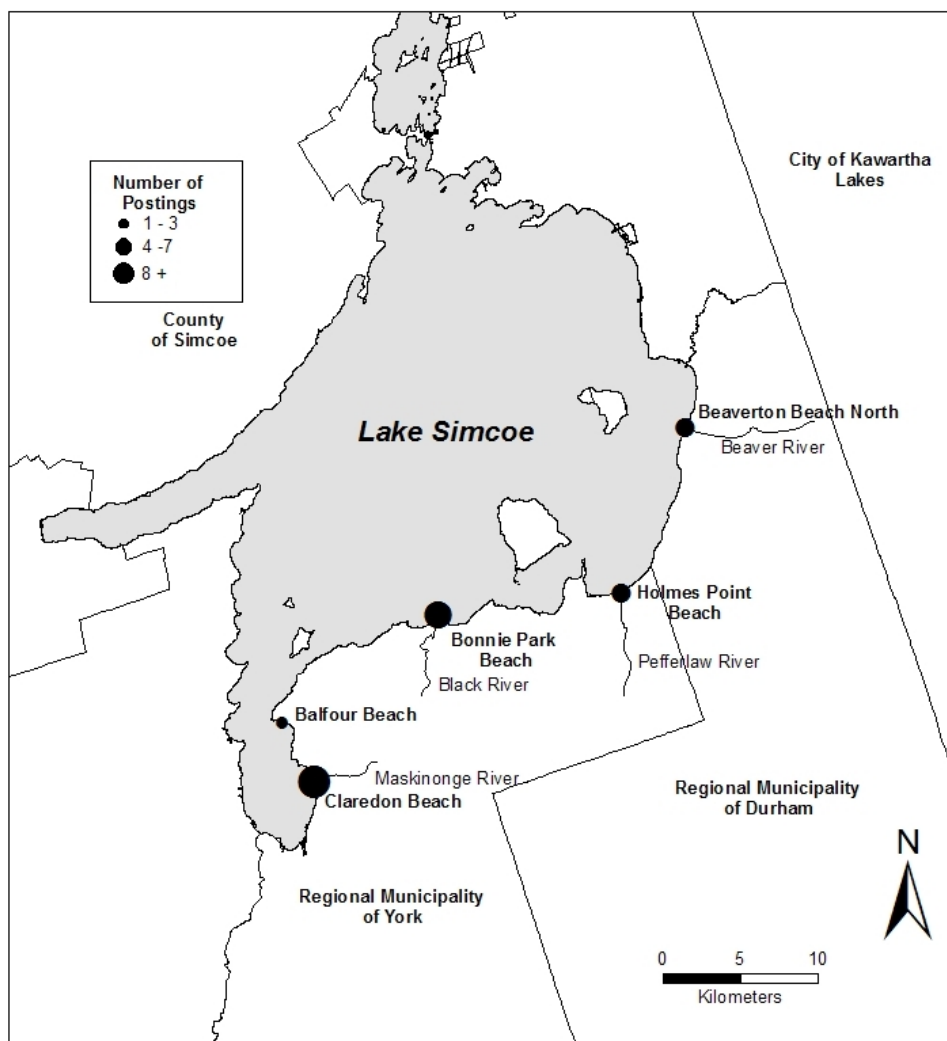
Fig. 1. Location of Lake Simcoe study beaches and nearby rivers including the total number of times swimming advisories were posted at each of the study beaches between 2006 and 2008.

Lake Simcoe, the study beaches were easily accessible and were a mix of sand beach area and maintained parkland. Rivers neighboring 4 of the study beaches were also sampled in 2010−2011 to investigate whether tributaries are a source of fecal pollution to recreational beaches and Lake Simcoe (Fig. 1). Balfour Beach was not associated with a source river in this study because the area adjacent to the beach was devoid of inflowing tributaries.

Study sites were sampled every other week between early July and November in 2010 and between May and November in 2011, with the exception of Balfour Beach where sampling started at the end of June 2011. In 2010 and 2011, water samples for *Campylobacter* analysis were collected from a single station near the mouth of each river and from 2 depths including ankle (<0.1 m) and chest (~1.2–1.5 m) depth at each beach. To assess the prevalence of *Campylobacter* species in beach sand, sand pore water was added to the sampling regime in 2011. Sand pore water was sampled by digging a hole in the wet foreshore sand about 1 m inland from the water's edge, and the water was collected as it seeped into the hole. After a significant rainfall event, one additional water sample was collected from a stormwater outfall near Claredon Beach. To identify possible avian sources of *Campylobacter* contamination at the beaches, fresh seagull (n = 1) and Canada goose (n = 7) fecal droppings were collected from the foreshore sand within 2 m of the water line. At the time of sample collection, chest-depth and river water temperatures, the number and type of birds

present on the beach and offshore on the lake and rivers, and the number of seagull and Canada goose fecal droppings within the immediate beach and river area (~5 m of shoreline) were recorded. Water samples were collected in 2 L sterile bottles while fresh bird fecal droppings were collected aseptically and suspended in 3 mL sterile phosphate buffer saline solution with a pH of 7.5. The samples were returned on ice to the laboratory and analyzed within 24 h of collection.

Isolation and culture conditions for thermophilic *Campylobacter* species

Samples were processed according to the protocol developed and described by Khan and Edge (2007). Briefly, 1 L of each water sample was centrifuged at 14 000g for 20 min (Beckman, Indianapolis, IN, USA), and the pellet was resuspended in 3 mL of 0.85% saline solution to concentrate *Campylobacter* cells. The resuspended pellet was analyzed by a minimum probable number (mpn) method using a 10-fold serial dilution approach for a semiquantitative analysis of *Campylobacter* spp. occurrence. From each suspended pellet and fecal sample, 1 mL was inoculated in a single set of 9 mL Bolton broth (Oxoid, Lenexa, KS, USA) tubes containing a selective antibiotic (cefoperazone, cyclohexamide, trimethoprim, and vancomycin) supplement. The inoculum was further serially diluted and incubated at 42 °C under microaerophilic conditions (5% O_2, 85% N_2, and 10% CO_2) for 48 h in an MCO-18M multi-gas incubator (Sanyo, Tokyo, Japan). The enumeration of mpn L^{-1} was carried out by assessing turbidity and subculture confirmation. The cultures from each tube were further streaked with a sterile loop on Modified Karmali Agar (MKA; Oxoid, Lenexa, KS, USA) containing a selective supplement including antibacterial and antifungal (amphotericin B, cefoperazone, sodium pyruvate, and vancomycin) agents, and plates were incubated at 42 °C under microaerophilic conditions for 24–48 h. The putative *Campylobacter* cultures were selected based on colony morphology (i.e., smooth, shiny, and convex with defined or flat edges, transparent or translucent colorless to grayish or light cream in color; Karmali et al. 1986); samples that contained such colonies were further analyzed by DNA extraction and PCR assays.

Nucleic acid-based identification of *Campylobacter* species

Nucleic acid extraction: Nucleic acid extraction was carried out for putative *Campylobacter* culture isolates obtained by scraping an MKA plate with a sterile loop. The cells were resuspended in a sterile 1.5 mL microfuge tube containing 100 µL TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0), and were gently mixed and boiled for 10 min according to the procedure described previously by Hamelin et al. (2007). The tube was then centrifuged at high speed for 1 min, and the supernatant containing purified DNA was further quantified by an N-1000 spectrometer and 0.8% agarose (Fisher Scientific, Ottawa, ON, Canada) gel electrophoresis using 1x TAE (0.04 M Tris-acetate, 0.001 M EDTA, pH 7.8) buffer. The DNA extract was stored at −20 °C for further *Campylobacter* genus- and species-specific PCR as well as DNA sequence analyses.

Campylobacter **genus-specific PCR amplification:** For the confirmation of putative culture isolates, a DNA-based PCR amplification assay was performed using *Campylobacter* genus-specific oligonucleotide primers (Table 1) and a previously described PCR protocol (Linton et al. 1996). The PCR amplification reaction was carried out in a Mastercycler Gradient PCR system (Eppendorf, Hauppauge, NY, USA) with a 25 µL reaction mixture containing 50–70 ng of each target template DNA, 1.25 units of *Ex Taq* DNA polymerase (Takara, Mountain View, CA, USA), 1X *Ex Taq* buffer with MgCl₂, 200 µM each of the dNTPs, and 50 ng of the forward and reverse primers. The volume was adjusted with sterile distilled water to yield 25 µL. The PCR reaction was performed using an initial template denaturation step at 94 ºC for 3 min followed by 30 cycles of amplification (denaturation at 94 ºC for 60 s, annealing at 55 ºC for 60 s, and extension at 72 ºC for 60 s) ending with a 5 min final extension at 72 ºC. The PCR amplicons were electrophoresed on a 1% agarose gel matrix with 1x TAE buffer using 100 base pair (bp) DNA size marker (PGC Scientifics, Palm Desert, CA, USA). The gels were stained in ethidium bromide (0.5 µg mL⁻¹), visualized on an ultraviolet (UV) transilluminator and photographed using an Ingenius Syngene Bioimaging (Perkin Elmer, Waltham, MA, USA) gel documentation system.

Species-specific PCR amplification: A species-specific PCR amplification was further performed for the confirmation of culture isolates to species level by a triplex PCR assay using species-specific oligonucleotide primer pairs for thermophilic *Campylobacter* species including *C. jejuni*, *C. coli*, and *C. lari* and a PCR protocol, as described by Khan and Edge (2007). The triplex PCR amplification reaction was carried out in the Mastercycler Gradient PCR system as described above, but with 80 ng of each forward and reverse pair of primers (Table 1). The volume was adjusted with sterile distilled water to yield 25 µL. The PCR reaction was performed using an initial template denaturation step at 94 ºC for 3 min followed by 30 cycles of amplification (denaturation at 94 ºC for 30 s, annealing at 46 ºC for 30 s, and extension at 72 ºC for

Table 1. Oligonucleotide PCR primers for amplification of the genus- and species-specific genes encoding 16S rRNA gene and 16S-23S rDNA internal transcribed spacer (ITS) region of thermophilic *Campylobacter* species.

Species	Primers	Sequences (5´-3´)	Length (nt)	PCR products (bp)
Campylobacter genus-specific	16S-UP	GGA TGA CAC TTT TCG GAG C	19	816
	16S-DN	CAT TGT AGC ACG TGT GTC	18	
C. jejuni	ICJ-UP	CTT AGA TTT ATT TTT ATC TTT AAC T	25	349
	ICJ-DN	ACT AAA TGA TTT AGT CTC A	19	
C. lari	ICL-UP	CTT ACT TTA GGT TTT AAG ACC	21	279
	ICL-DN	CAA TAA AAC CTT ACT ATC TC	20	
C. coli	ICC-UP	GAA GTA TCA ATC TTA AAA AGA TAA	24	72
	ICC-DN	AAA TAT ATA CTT GCT TTA GAT T	22	

30 s) ending with a 5 min extension at 72 °C. This reaction is able to detect single or multiple species including *C. jejuni*, *C. coli*, and *C. lari* in a single sample. Due to the expectedly small amplicon fragment sizes, ranging from 349 to 72 bp, the PCR amplicons were electrophoresed on a 2% agarose gel matrix (Fisher Scientific, Ottawa, ON, Canada) as described in the preceding section.

PCR-based DNA sequencing of unknown *Campylobacter* species: The putative *Campylobacter* isolates that yielded a positive PCR amplification reaction with the genus-specific PCR assay but did not show a PCR amplification reaction with species-specific triplex PCR assay were further analyzed at species-level by a DNA sequence analysis. The genus-specific amplified PCR products were purified by using a QIAquick PCR purification kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The quality and concentration of purified PCR amplicons were confirmed by using an N-1000 spectrometer, and 0.8% agarose (Fisher Scientifics, Ottawa, ON, Canada) gel electrophoresis using 1x TAE (0.04 M Tris-acetate, 0.001 M EDTA, pH 7.8) buffer.

The PCR amplicons were sequenced using an ABI 3730 Sanger sequencer (Applied Biosystem, Carlsbad, California, USA) at McMaster University (Mobix DNA Core Laboratory, Hamilton, ON, Canada). The sequence data were analyzed using a BLAST search against the global database to identify species-level designations. The DNA sequences for all samples were further analyzed and compared by multiple sequence alignment and phylogenetic analysis with reference *Campylobacter* species and other closely related genera including *Arcobacter* and *Helicobacter* using MegAlign 1993−2006 (DNASTAR Inc., Madison, WI, USA). Multiple sequence alignments and tree construction were carried out using Clustal-V and Clustral-W pairwise and neighbor-joining (version 7.1.0) methods, respectively. Bootstrapping was performed by creating 1000 trials.

Nucleotide sequence accession numbers: Sequences for the 9 genus-specific positive PCR amplicons identified as unknown *Campylobacter* species were submitted to GenBank with the accession numbers JQ863066-JQ863074.

Statistical analysis

To compare the prevalence of *Campylobacter* at the genus and species level, Chi-square and Fisher's exact tests were applied including beaches versus rivers, beaches versus birds, and rivers versus birds as well as seasons. Fisher's exact test was applied in place of a Chi-square test when at least one cell in a 2 by 2 site and season comparison table was <5 (Motulsky 1995). Moreover, the estimated 95% confidence intervals (upper and lower limits) were plotted as error bars in data summary figures.

Results

Campylobacter species recovered from beaches, rivers, and fecal droppings were typically detected at the lowest mpn (3–30 cells L^{-1}), ranging up to a maximum of 300 cells L^{-1} (data not shown). All putative *Campylobacter* culture isolates showed typical growth patterns on MKA media and were further confirmed as *Campylobacter* species by the 16S rRNA genus-specific PCR assay with an expected amplicon size of 816 bp. Overall, *Campylobacter* was detected infrequently in the 289 beach, 100 river, and 8 bird fecal dropping samples collected during the 25 sampling events. *Campylobacter* was not detected in the single stormwater outfall sample. *Campylobacter* putative culture isolates yielded typical PCR amplification signals at the genus level for only 12% (33/289 samples) of beach water samples (including sand pore and ankle- and chest-depth water samples), 14% (14/100) of riverwater samples, and 25% (2/8) of bird fecal dropping samples (Fig. 2A). The prevalence of *Campylobacter* (at
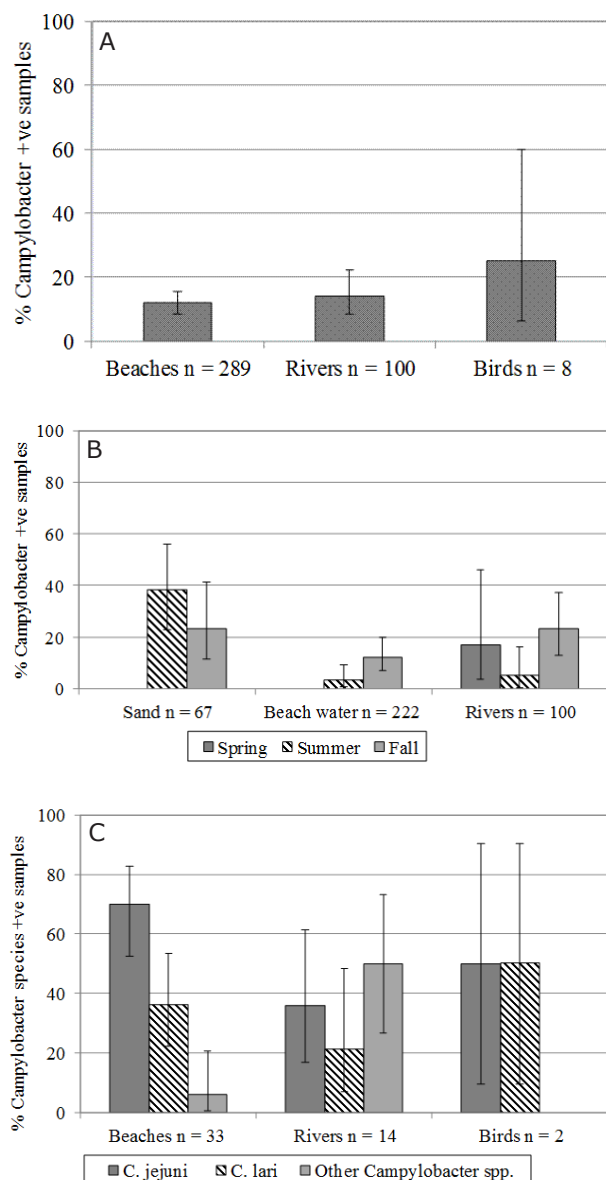
Fig. 2. Percent occurrence of (A) *Campylobacter* in beach water (sand pore, ankle- and chest-depth water samples data combined), river water, and bird fecal droppings (n indicates the total number of samples from 2010 to 2011); (B) *Campylobacter* in sand pore, beach (ankle- and chest-depth water samples data combined) and river water samples during the spring (May), summer (Jun–Aug) and fall (Sep–Nov) sampling periods (n indicates the total number of samples from 2010 to 2011); and (C) *Campylobacter* species *C. jejuni*, *C. lari*, and unidentified species in beach water (sand pore and ankle- and chest-depth water samples data combined), river water, and bird fecal droppings that were positive for *Campylobacter* (n indicates the total number of samples from 2010 to 2011 that were positive for *Campylobacter* spp.; percentage may not equal 100% because more than one *Campylobacter* species was detected in some water samples). Error bars indicate the estimated 95% confidence intervals (upper and lower limits) to the genus and species levels between sites including beaches vs. rivers, beaches vs. birds, and rivers vs. birds, and seasons.

the genus and species level) did not differ among beaches, rivers, and bird fecal droppings (Chi-square and Fisher's exact test p-values >0.05; Fig. 2). Among positive beach water samples (Table 2), sand pore water showed a higher frequency of occurrence of campylobacters (27% of the 67 samples) than ankle- or chest-depth samples (10 and 5%, respectively, of the 111 samples).

Campylobacter was detected in 17% of the Beaverton Beach North and Claredon Beach water samples (11/64), 12% of the Balfour Beach water samples (4/33), 7% of the Holmes Point Beach water samples (4/64), and 5% of the Bonnie Park Beach water samples (3/64). Similarly, of the 25 samples collected from each river, *Campylobacter* was more frequently detected in Beaver River (28%) and Maskinonge River (12%), which neighbor Beaverton Beach North and Claredon Beach, respectively, than in Pefferlaw River (8%) and Black River (7%), which neighbor Holmes Point Beach and Bonnie Park Beach, respectively.

Although *Campylobacter* detection did not statistically differ among seasons (Chi-square and Fisher's exact tests p-values >0.05), there was a tendency toward seasonal changes in *Campylobacter* occurrence (Fig. 2B). In the river samples, *Campylobacter* was more frequently detected during the fall and spring when it was present in 23% of the 44 fall samples and 17% of the 12 spring samples as compared to summer, when it was present in 5% of the 44 samples. *Campylobacter* was not detected in sand pore and beach water samples in the spring but was present in the summer and fall. In the sand pore water samples, *Campylobacter* was detected in 11 of the 29 summer samples (38%) and 7 of the 30 fall samples (23%). In the beach water samples, including both ankle and chest depths, *Campylobacter* was less prevalent in summer than fall, occurring in 3% of the 98 summer samples, but 12% of the 100 fall samples (Fig. 2B). Greater *Campylobacter* prevalence in the spring and fall compared to the summer may be related to water temperature. River water temperature in the spring and fall ranged from 4 to 22 °C but increased to a maximum of 26 °C during the summer sampling events. Similarly, chest-depth water temperature ranged from 3 to 20 °C in spring and fall but increased to a maximum of 29 °C in summer.

The occurrence of thermophilic *Campylobacter* species including *C. jejuni*, *C. coli*, and *C. lari* was investigated using the 16S-23S rDNA internal transcribed spacer (ITS) region triplex PCR assay (Table 2). Of 33 beach and sand pore water samples positive for campylobacters at the genus level, 58% (n = 19) were positive for *C. jejuni*, 24% (n = 8) were positive for *C. lari*, and 12% (n = 4) were positive for both *C. jejuni* and *C. lari* (Fig. 2C). Two *Campylobacter* cultures isolated from sand pore and

Table 2. Number (and percentage) of positive water and fecal samples for thermophilic *Campylobacter* species using genus- and species-specific triplex PCR assays.

Campylobacters	Beach water n = 289			River water N = 100	Bird fecal N = 8
	Sand pore n = 67	Ankle n = 111	Chest n = 111		
Campylobacter spp.	18 (27)	10 (9)	5 (5)	14 (14)	2 (25)
C. jejuni	12 (36)	9 (27)	0 (0)	5 (36)	1 (50)
C. lari	7 (21)	2 (6)	2 (6)	3 (21)	1 (50)
C. coli	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Unknown spp.	1 (3)	0 (0)	1 (3)	7 (50)	0 (0)

chest-depth waters did not amplify with the triplex PCR assay and were classified as "unknown" *Campylobacter* species. None of the beach water samples showed a positive PCR amplification reaction for *C. coli* (Table 2). Among beach samples, *C. jejuni* was present in 36% of sand pore water samples, 27% of ankle-depth, and none of the chest-depth water samples. *C. lari* was present in 21% of sand pore water samples and 6% of each ankle-depth and chest-depth water samples (Table 2). Of the 14 river samples that showed a positive *Campylobacter* amplification reaction at genus level, *C. jejuni* and *C. lari* were detected at relatively low frequency (36 and 21%, respectively), and 50% of isolates were identified as unknown *Campylobacter* species. The 2 *Campylobacter* isolates from bird fecal droppings were identified as *C. jejuni* and *C. lari* (Table 2; Fig. 2C).

In this study, 9 DNA sequences from unknown *Campylobacter* species, including 7 from rivers and 2 from beaches, were generated using the *Campylobacter* genus-specific 16S rRNA gene PCR assay. DNA sequences were aligned and analyzed, and a phylogenetic tree was generated (Fig. 3). The BLAST search results for the 9 sequences indicated that 4 sequences from Beaver River obtained from 4 different samples were more closely related to *C. peloridis* or *C. lari*; however, one isolate from Holmes Point Beach chest-depth water showed little similarity to *Campylobacter* species. The remaining 4 isolates from 3 river samples and 1 sand pore water sample showed closer relatedness to *Arcobacter* species including *A. butzleri*, *A. cryaerophilus*, and *A. cibarius* than to known *Campylobacter* species.

Discussion

This study provides the first evidence of thermophilic *Campylobacter* species at Lake Simcoe recreational beaches and rivers. The presence of campylobacters in beach and river water samples suggests the presence of fecal contamination (Jones 2001, 2005). *Campylobacter*

spp. were detected infrequently, however, and the maximum estimated number of *Campylobacter* cells was about 30 mpn L^{-1} from water samples at Lake Simcoe beaches and rivers and ~300 mpn L^{-1} from sand pore water samples. These levels of contamination are below the infective dose of ~500 cells of *Campylobacter* (e.g., *C. jejuni*) estimated to cause human illness (Black et al. 1988, Health Canada 2012) and suggest relatively low health risks at the study beaches over the period of investigation. The mpn of *Campylobacter* spp. detected at Lake Simcoe beaches and rivers were lower than results from other Canadian, German, and UK river studies where *Campylobacter* was detected at <10 mpn cells 100 mL^{-1} by culture-based assays, and the median concentration ranged from 4 to 8 to a maximum of 130 mpn cells 100 mL^{-1} by quantitative PCR-based assays, respectively (Bolton et al. 1987, Stelzer and Jacob 1991, Van Dyke et al. 2010).

The occurrence of *Campylobacter* species (*C. jejuni* and *C. lari*) was found to be greater in foreshore sand and declined moving offshore to ankle- and chest-depth waters. This sand to water gradient is similar to that frequently observed for *E. coli* at beaches (Edge and Hill 2007). *Campylobacter* presence in sand has been noted at UK beaches (Bolton et al. 1999). These results suggest that beach sand may act as a reservoir of fecal indicator bacteria and pathogenic organisms like *Campylobacter* species. The sand may enable *Campylobacter* to survive for longer periods due to aspects like protection from light (particularly UV radiation), which is potentially lethal to campylobacters and other bacteria in the aquatic environment (Thomas et al. 1999, Edge and Hill 2007, Khan et al. 2009). Sand may also act as a filter and retain pathogenic organisms when beach water recedes during low tide (Bolton et al. 1999).

Our results indicate that avian fecal droppings are a potential source of *Campylobacter* at Lake Simcoe beaches. Waterfowl are carriers of a wide range of pathogenic viral, bacterial, fungal, and parasitic microor-
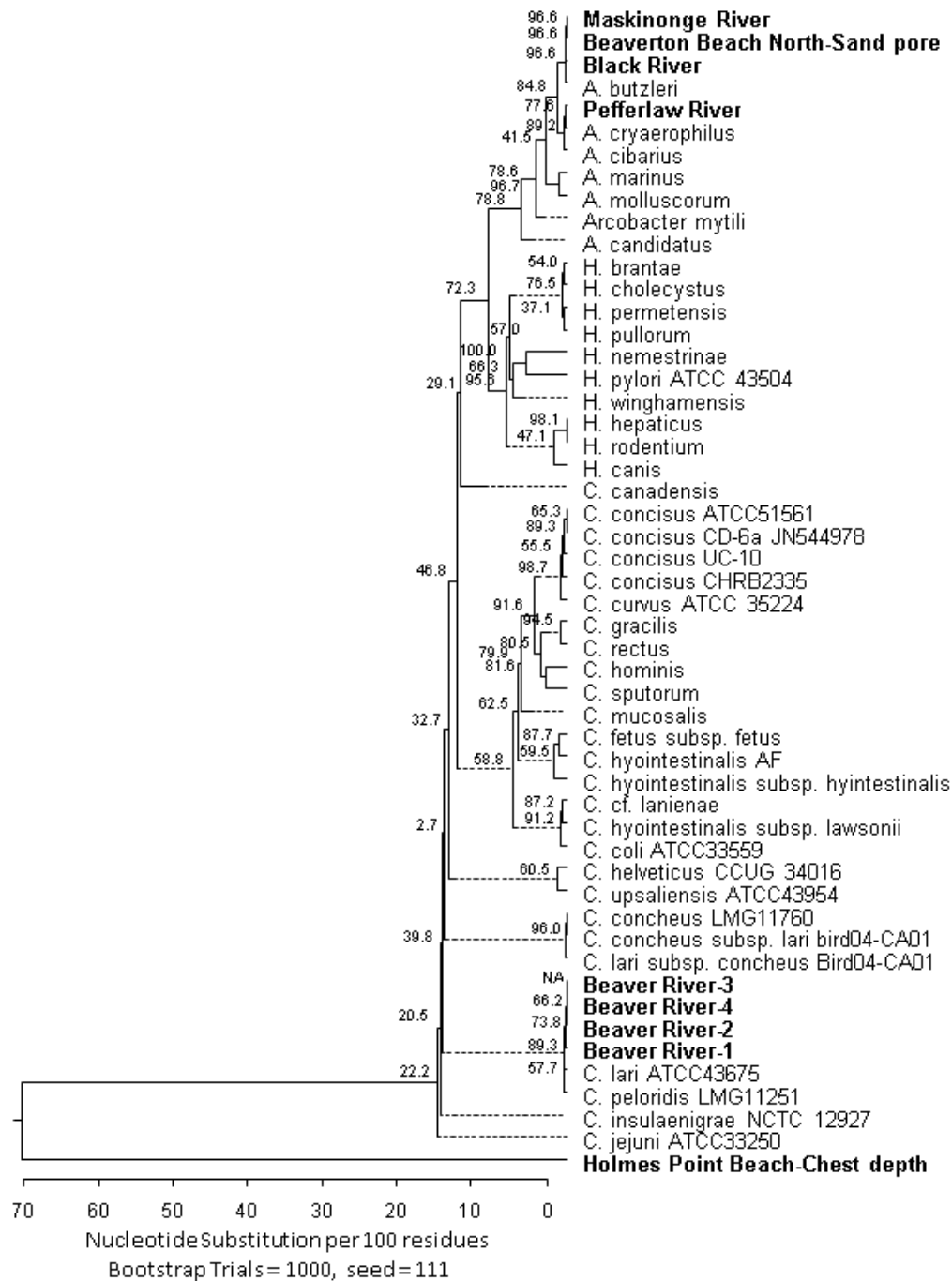
Fig. 3. Neighbor-joining phylogenetic tree of 9 "unknown" *Campylobacter* sequences (bold) based on 16S rRNA gene sequences obtained from beaches and rivers. Bootstrapping values obtained from 1000 bootstrap replicates were analyzed as percentages.

ganisms (Hubálek 2004), and *Campylobacter* have frequently been reported in healthy waterfowl without obvious symptoms of infection (Luechtefeld et al. 1980, Kapperud and Rosef 1983, Waldenström et al. 2002). Birds were often observed at the study beaches and in the water immediately offshore of the beaches in numbers ranging from 5 to >100 birds (Table 3). Based on observa-

tions during sampling events, Canada geese were common at Claredon Beach, ring-billed gulls were common at Bonnie Park Beach, and both species were common at Beaverton Beach North and Holmes Point Beach. Mallard ducks were also observed, particularly at Beaverton Beach North and Balfour Beach. Birds were particularly prevalent during the summer and early fall, coinciding

Table 3. Total number of birds and bird fecal droppings recorded over 2 years (2010−2011) of field study at beaches and corresponding river sampling locations.

Sampling sites	Birds				Fecal droppings			
	Gulls	Canada Geese	Swans	Ducks	Gulls	Canada Geese	Swans	Ducks
Balfour Beach	2	330	0	0	4	460	0	0
Beaverton Beach North	308	702	23	131	106	13	0	0
Beaver River	0	5	0	0	0	136	0	0
Claredon Beach	72	270	0	142	0	506	0	0
Maskinonge River	0	21	0	0	0	0	0	0
Holmes Point Beach	78	25	0	4	11	111	0	0
Pefferlaw River	0	0	0	0	1	0	0	0
Bonnie Park Beach	186	257	0	6	253	785	0	0
Black River	0	65	0	4	0	0	0	0

with the period of peak *Campylobacter* detection at beaches. Arvanitidou et al. (1995) similarly found that the occurrence of thermophilic *Campylobacter* species at beaches was greater in the summer and fall than in the spring. The presence of bird fecal matter provided further evidence of avian influence at Lake Simcoe beaches. Ring-billed gull and Canada geese fecal droppings were observed along beach shorelines (Table 3) and tended to increase in quantity over the summer despite regular beach grooming. Although the number of bird droppings observed during each sampling event ranged from 10 to >100 droppings per comparable length of beach shoreline, only a few fresh fecal droppings could be analyzed for the presence of *Campylobacter* because most droppings were either dried or mixed with beach sand. Despite the low number of droppings tested, *C. jejuni* and *C. lari* were detected in seagull and Canada goose droppings, respectively, suggesting that birds are a potential source of *Campylobacter* at Lake Simcoe beaches. Our results are consistent with the occurrence of *Campylobacter* species, including *C. jejuni* and *C. lari* isolated from bird droppings, reported by other researchers (Abulreesh et al. 2006, Van Dyke et al. 2010, Lu et al. 2011).

Rivers may also act as a source of *Campylobacter* to Lake Simcoe. Runoff from urban and agricultural areas may transport fecal contamination and associated bacteria to rivers and beaches around the lake. Interestingly, the frequency of *Campylobacter* detection was highest at Beaver River and Maskinonge River, which neighbor the beaches with the highest *Campylobacter* detection, while the frequency of detection was lowest at Pefferlaw River and Black River, which neighbor the beaches with the lowest *Campylobacter* detection. These results suggest beaches could reflect the related contaminant loading from the nearby area and/or rivers that are contributing to

Campylobacter occurrence in Lake Simcoe. Differences in land use and runoff among the subwatersheds may explain the different *Campylobacter* detection rates reported here. Detection of *Campylobacter* in river water tended to be greater during spring and fall when river water temperatures were cooler, suggesting that lower water temperatures may support greater survival and prevalence of *Campylobacter* (Waldenström et al. 2002).

Increased land runoff during ice melt and precipitation events may also contribute to higher *Campylobacter* presence during the spring and fall. Similar seasonal trends reported for rivers in Norway and the United Kingdom were also attributed to enhanced *Campylobacter* input from sewage and agricultural surface runoff (Bolton et al. 1987, Brennhovd et al. 1992). *Campylobacter* was not detected in the single sample collected from a stormwater outfall adjacent to the north side of Claredon Beach. This sample was collected following a storm event, suggesting enhanced runoff; however, high flow volume may have diluted any *Campylobacter* present. Because no significant precipitation events occurred during our sampling events, only one stormwater outfall sample was collected from Claredon Beach. A more comprehensive study is needed on the occurrence of *Campylobacter* species in point and nonpoint fecal pollution sources like stormwater outfalls to help identify sources of *Campylobacter* contamination in Lake Simcoe beaches and neighboring rivers. Additional work is also needed to understand how climate, land use, and other factors affect bacteria levels at Lake Simcoe beaches and rivers.

Some of the *Campylobacter* isolates identified as unknown campylobacters in our study showed close similarity to *Campylobacter* species such as *C. peloridis* or *C. lari*. *C. peloridis* and *C. lari* have previously been isolated from healthy and diseased human feces and

shellfish (Debruyne et al. 2009b). Interestingly, a significant number of unknown *Campylobacter* sequences seemed more closely related to *Arcobacter* species than *Campylobacter* species. *Arcobacter* spp. have frequently been isolated from the intestinal tracts and fecal samples of different farm animals, pets, and wild animals, but this genus apparently only has the capacity to cause disease in some of them. The most serious effects of *Arcobacter* in animals include abortions, diarrhea, and mastitis. *A. butzleri* and *A. cryaerophilus* have been associated with human gastrointestinal diseases (Vandenberg et al. 2004, Wybo et al. 2004) and have been isolated from untreated water, meat processing equipment surfaces, and environmental samples (Vytrasová et al. 2003). *A. butzleri* has been associated with enteritis and diarrhea in pigs, cattle, and horses, whereas *A. skirrowii* has been associated with diarrhea and haemorrhagic colitis in sheep and cattle (Collado and Figueras 2011). *A. cibarius* was first isolated in 2005 from the skin of chicken carcasses and piggery effluents (Houf et al. 2005, Chinivasagam et al. 2007) but has not yet been linked to animal illnesses.

Because we obtained only partial 16S rRNA gene sequences (752 bp PCR product), it is possible that the BLAST analyses were not able to provide sufficient taxonomic resolution to clearly identify these unknown campylobacters. Alternatively, the unknown species may, as yet, be undescribed and potentially novel species within the *Campylobacter* genus. Also possible is that the *Campylobacter* genus-specific primers developed by Linton et al. (1996) are not specific to *Campylobacter* species; rather, they may also amplify other closely related species from genera such as *Arcobacter*. While we confirmed the specificity of the Linton et al. (1996) oligonucleotide primer set using RDP and NCBI BLAST searches, the BLAST results only show the first 100 highly conserved matching results, which included *Campylobacter* spp. and ~40% uncultured bacteria. Additional research on these novel *Campylobacter*-like species is warranted to confirm their identity and assess any potential for human health risks. The DNA sequences obtained in this study could be used to further refine PCR assays to identify a wide range of *Campylobacter* species and determine the distribution of campylobacters in sand and water sources. Investigations of other available *Campylobacter* genus-specific primers are needed to establish optimal detection methods for this bacterium because it has the potential to impact human health.

Recovery methods for isolation of thermophilic *Campylobacter* spp. typically use an incubation temperature of 42 °C, which has been optimal for detecting *C. jejuni* and *C. coli*, the more common causative agents of human illness. In recent years, however, there has been growing evidence that other *Campylobacter* spp. may also be human pathogens, and that their detection may require an incubation temperature of 37 °C for successful recovery and growth (Koenraad et al. 1997, Engberg et al. 2000), suggesting that more than one incubation temperature should be used for the recovery and growth of *Campylobacter* spp. (Pearson et al. 2000). In future studies, investigations will be carried out to comparatively evaluate 37 and 42 °C incubation temperatures for studying the occurrence of thermophilic and unknown *Campylobacter*-like species in recreational waters.

This study provided information on *Campylobacter* occurrence at recreational freshwater beaches along the shoreline of Lake Simcoe, an economically important lake in southern Ontario. *Campylobacter* species are frequently associated with waterborne disease; therefore, it is important to assess the potential for this pathogen to impact the health of the hundreds of thousands of residents and tourists who visit Lake Simcoe beaches. This study showed that thermophilic *Campylobacter* species, including *C. jejuni* and *C. lari* associated with human health impacts, were detected in beach water samples, river water samples, and bird fecal droppings at beaches; however, these thermophilic campylobacters were generally detected infrequently and occurred in relatively low concentrations. Interestingly, *C. coli* was not detected. The unknown *Campylobacter* species isolated were less likely to be species shown to be associated with human disease and could be undescribed species of *Campylobacter* or species from similar genera like *Arcobacter*. The latter case would indicate the Linton et al. (1996) PCR primers are not specific to *Campylobacter* species. These unknown *Campylobacter*-like species are poorly characterized and are of unknown human health risk. Further characterization of these isolates is needed by using advanced molecular subtyping techniques, which may enhance understanding of pathogen dynamics and contaminant sources in the Lake Simcoe area. Our results indicate that the foreshore sand reservoir, migratory birds, and nearby rivers could act as a source of *Campylobacter* contamination to Lake Simcoe beach waters. Future work on pathogen source dynamics is needed to elucidate factors such as climate and land use that control contaminant loading to Lake Simcoe.

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