Journal of Water & Health

IWA PUBLISHING

Journal of Water and Health Vol 20 No 4, 657 doi: 10.2166/wh.2022.285

Occurrence of *Naegleria fowleri* and faecal indicators in sediments from Lake Pontchartrain, Louisiana

Shalina A. Shahin[a], Joshua H. Alarcon[a], Hanna N. Brosky[a], Jennifer G. Lamori[a], Ayokunle C. Dada[b], Jia Xue[a], Pradip Gyawali[c] and Samendra P. Sherchan[a,d,*]

[a] Department of Environmental Health Sciences, School of Public Health and Tropical Medicine, Tulane University, New Orleans, Louisiana 70112, USA
[b] QMRA Data Experts, Hamilton, New Zealand
[c] Institute of Environmental Science and Research Ltd, Porirua 5240, New Zealand
[d] Department of Biology, Morgan State University, 1700 East Cold Spring Lane, Baltimore, MD 21251, USA
*Corresponding author. E-mail: sshercha@tulane.edu

SPS, 0000-0002-0092-7928

ABSTRACT

The occurrence of amoeba, *Naegleria fowleri*, in sediment samples from Lake Pontchartrain in Louisiana was investigated. This amoeba is pathogenic and can cause primary amoebic meningoencephalitis. In this study, quantitative polymerase chain reaction methods were used to test for the prevalence of *Naegleria fowleri*, *HF183*, and *E. coli*. *N. fowleri* was detected in 51.25% of our sediment samples. Illumina sequencing of sediment samples revealed ten different phyla, with *Cyanobacteria* being the most predominant at sites that generally presented with the highest median *N. fowleri* concentrations. *N. fowleri* was however strongly negatively correlated with HF183 ($r = -0.859$, $p < 0.001$). Whenever sediment *E. coli* concentrations were below 1.54 Log GC/g, there was only a 37.5% chance that *N. fowleri* would be detected in the same sample. When sediment *E. coli* concentrations exceeded 2.77 Log GC/g, the chances of detecting *N. fowleri* in the same sample increased to 90%, potentially suggesting predatory activity by the amoeba. The effect of temperature was observed to be different in relation to observed *N. fowleri* concentrations and detection rates. Although sediment samples collected during periods of higher temperatures had significantly lower mean *N. fowleri* concentrations (2.7 Log GC/g) compared to those collected at lower temperatures (3.7 Log GC/g, $t(39) = 4.167$, $p < 0.001$), higher *N. fowleri* detection rates in the overall samples were observed at higher temperatures (>19.1 °C) than at lower temperatures (<19.1 °C).

Key words: *Escherichia coli*, Lake Pontchartrain, *Naegleria fowleri*, primary amoebic meningoencephalitis (PAM), qPCR

HIGHLIGHTS

- The occurrence of free-living amoeba, *Naegleria fowleri* in sediment samples from Lake Pontchartrain in Louisiana was investigated.
- *N. fowleri* was detected in 51.25% of our sediment samples. *N. fowleri* was, however, strongly negatively correlated with HF183 ($r = -0.859$, $p < 0.001$).
- Higher *N. fowleri* detection rates were observed at higher temperatures than at lower temperatures.

1. INTRODUCTION

Naegleria fowleri, also known as 'the brain-eating amoeba', is naturally found in hot springs, warm waters, and sediments. *N. fowleri* can cause primary meningoencephalitis (PAM), most often leading to death within 4–6 days if inhaled or forced into the nasal passages when swimming or diving. It is fairly resistant to chlorine-based disinfection (Bartrand *et al.* 2014). It has a three-stage lifecycle. *N. fowleri* exists in soil in a cystic form and excysts to a flagellate stage when in contact with warm water and a source of food (bacteria). The flagellate stage feeds on bacteria at the air–water interface in hot springs, surface water, and other water systems that may remain still for prolonged periods (Xue *et al.* 2018).

In 2011, two people died of PAM caused by *N. fowleri* in DeSoto Parish and St. Bernard Parish, Louisiana. Both cases involved the use of a neti-pot. In 2013, there was a second death in St. Bernard Parish (a 4-year-old boy) caused by PAM and confirmed to be an *N. fowleri* infection. Testing conducted in 2017 by the Louisiana Department of Health and Hospitals (DHH) found this amoeba in North Monroe, Schriever Water Systems. To date, a total of seven of Louisiana's public water

systems have tested positive for *N. fowleri* (Bartrand *et al.* 2014; Cope *et al.* 2015). In 2003, a case of PAM was also recorded in New Zealand after an individual swam in a geothermal pool near Lake Rotoma (Cursons *et al.* 2003). More recently, another local case of fulminant PAM due to *N. fowleri* linked to an individual who had a history of significant exposure to untreated fresh water (McLaughlin & O'Gorman 2019).

Lake Pontchartrain is an inland bay located in the southeast portion of Louisiana and its southern shore abuts the city of New Orleans. It is part of a large and productive estuarine system that supports agriculture and aquaculture, a vital shipping route, and one of the most important fisheries in the United States. The lake provides essential habitat for countless species of fish, birds, mammals, reptiles, and plants, as well as generating a multi-million-dollar fishing, tourism, sailing, and recreation industry (Xue *et al.* 2018). An increase in reported cases of PAM caused by *N. fowleri* in lakes in Florida, Georgia, Texas, Virginia and other countries has further triggered local and international responses, as well as increased investigation of its incidence (Jamerson *et al.* 2009).

The presence of *N. fowleri* in recreational water is currently not regulated in the United States. Although there has been increased research and interest in *N. fowleri* in water samples, studies that adopt a quantification approach to link concentrations of established indicator organisms to the occurrence of *N. fowleri* in recreational waters can help provide policy decision makers with science-backed information to support risk management efforts. Compared to water samples, there has been limited research evaluating sediment samples for the presence of *N. fowleri*. There is, however, some indication of the presence of *N. fowleri* in sediments underlying water samples that test positive for *N. fowleri* (Jamerson *et al.* 2009). Furthermore, lake sediments may act as deposits of *N. fowleri*, which could be resuspended back into the water column. The purpose of this study was to determine the occurrence and concentration of *N. fowleri*, in sediments from Lake Pontchartrain. As bacterial abundance is an important factor in relation to *N. fowleri* growth (i.e., as a food source), this study also investigates the microbial diversity of sediments collected from the lake. Additionally, correlation between the concentrations of *N. fowleri*, faecal indicators (*E. coli* and HF183 markers) and other physico-chemical parameters was also explored.

2. MATERIALS AND METHODS

2.1. Sampling sites information

Eight sites accessible to the public for recreational use were selected for this study. Sediment samples were collected using an Ekman sampling device from the top 20-cm layer. A total of 80 sediment samples were collected from eight sites (Site LP1, LP2, and LP5–LP10) along Lake Pontchartrain from December 2016 to August 2017 (Figure 1). Samples were transferred into sterile 50 ml tubes, stored at 4 °C in the field and immediately transported to the laboratory.

Physical and chemical water quality parameters, such as pH, temperature, dissolved oxygen, salinity, and specific conductance were measured *in situ* using a YSI Pro2030 Meter (YSI, Yellow Springs, OH, USA).

2.2. DNA extraction from sediments

On the day of sample arrival, 0.5 g of sediment was used for DNA extraction. Genomic DNA was isolated using the PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. DNA was quantified with a NanoDrop ND-2000 UV spectrophotometer (Thermo Scientific, Wilmington, USA). The DNA samples were stored at −20 °C prior to use.

2.3. Quantitative PCR assays

All qPCR assays were performed using the Applied Biosystems StepOne Real-Time PCR system (Applied Biosystems, NY, USA) using 1x PerfeCTa qPCR ToughMix (Quanta Biosciences, Beverly, MA). The reaction mixture (20 μl) contained 1x PerfeCTa qPCR ToughMix (Quanta Biosciences, Beverly, MA), 0.2 μM of each primer, and 2.5 μl of the template DNA. qPCR reactions for *N. fowleri* were performed in duplicate and amplification protocols consisted of a hold at 95 °C for 3 min, followed by 45 cycles of 95 °C for 10 s, 63 °C for 30 s (60 °C for 10 s and 72 °C for 10 s for *E. coli*), and 72 °C for 30 s. A calibration curve with concentrations spanning a range from 10^1 to 10^6 gene copies per reaction, with two replicates, was prepared. Duplicate no-template controls (NTCs) were included in each run. A summary of qPCR target organisms, primer/probe name, and sequences is provided in Table 1.

A TaqMan-based quantitative real-time PCR assay targeting a 153 base-pair (bp) fragment of the multi-copy 18S rRNA gene in *N. fowleri* was used in this study (Qvarnstrom *et al.* 2006). qPCR assays targeting the faecal indicator bacteria *E. coli* and

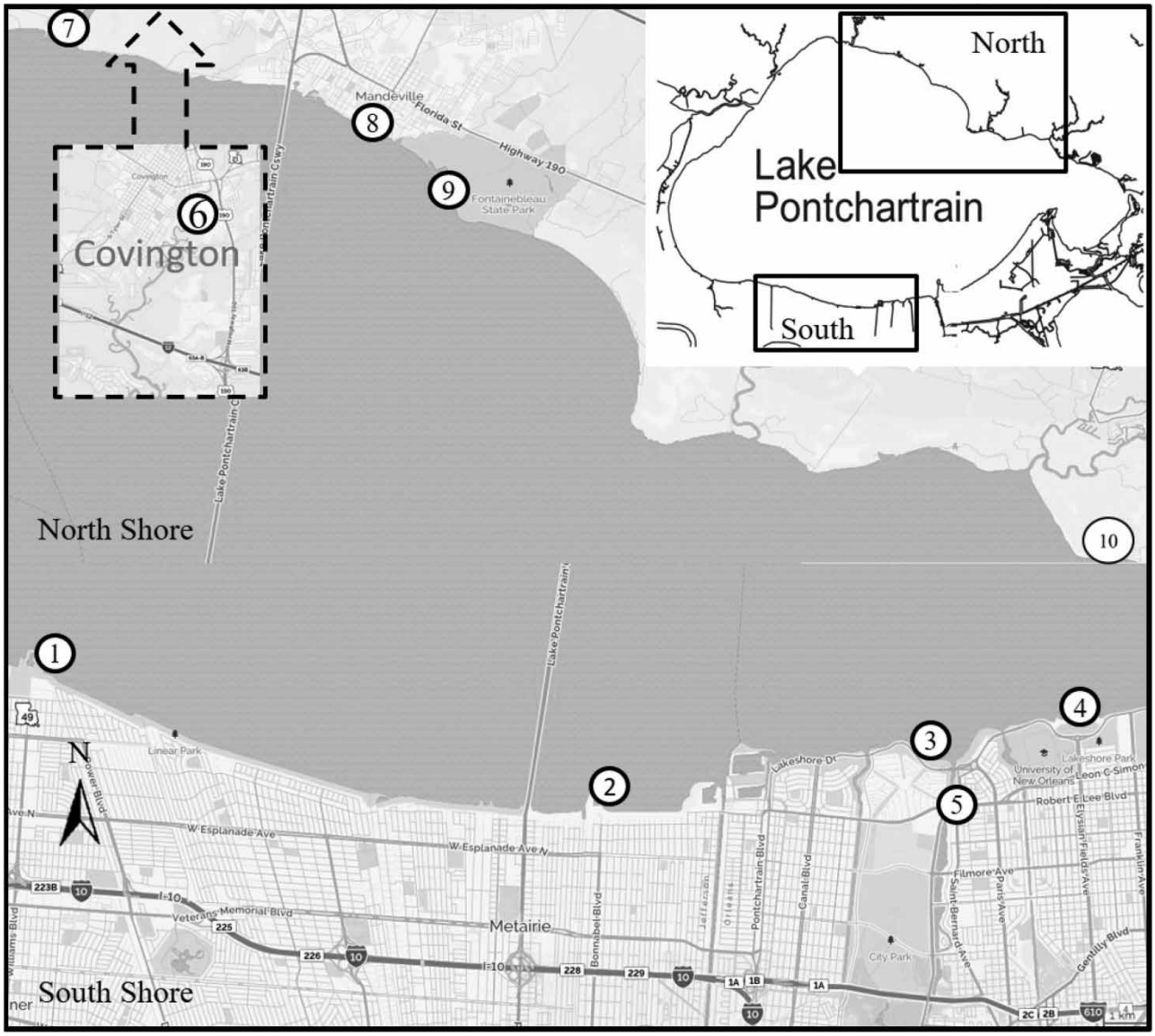

Figure 1 | Lake Pontchartrain sampling sites.

HF183 markers were used to measure faecal pollution. An *E. coli* qPCR assay targeted an 83 bp fragment of the single copy uidA gene codes for the enzyme β-D-glucuronidase (Frahm & Obst 2003), and SYBR green-based qPCR assays for HF183 targeted an 82 bp fragment of the human-associated Bacteroidales 16S rDNA gene (Bernhard & Field 2000; Bernhard *et al.* 2003).

2.4. 16S Metagenomic gene sequencing

The extracted DNA was processed for 16S ribosomal RNA gene sequencing using the Illumina MiSeq System (Illumina, Inc., San Diego, CA, USA). 16S metagenomic sequencing library preparation and sequencing were conducted as follows. Amplicon PCRs were conducted to amplify templates out of DNA samples targeting the V3 and V4 regions of the 16S rRNA gene. Illumina adapter overhang nucleotide sequences were added to the gene-specific sequences. The full-length primer sequences were 16S amplicon PCR forward: 5′-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3′, 16S amplicon PCR reverse: 5′-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3′. The 25 μl amplicon PCR reaction included 2.5 μl (5 ng/μl) microbial genomic DNA, amplicon PCR primers (Forward and reverse 1 μM) 5 μl each, 2x KAPA HiFi HotStart Ready Mix (Kapa Biosystems, Manufacturing,

Table 1 | Summary of qPCR assay conditions in this study

Target organism	Primer/ Probe	Sequence	Annealing Temperature (°C)	Size (bp)	Reference
E. coli	784F 866R Ec807	GTG TGA TAT CTA CCC GCT TCG C AGA ACG GTT TGT GGT TAA TCA GGA FAM -TCG GCA TCC GGT CAG TGG CAG T-TAMRA	60	83	Frahm & Obst (2003)
N. fowleri	NaeglF192 NaeglR344 NfowlP	GTG CTG AAA CCT AGC TAT TGT AAC TCA GT CAC TAG AAA AAG CAA ACC TGA AAG G HEX-ATA GCA ATA TAT TCA GGG GAG CTG GGC-BHQ1	63	153	Qvarnstrom *et al.* (2006)
Human *Bacteriodales*	HF183F HF265R	ATCATGAGTTCACATGTCCG TACCCCGCCTACTATCTAATG	60	82	Bernhard & Field (2000)

R&D Cape Town, South Africa) 12.5 μl. PCR was performed in a 96-well thermal cycler (Applied Biosystems, USA) under the following thermocycling steps: 95 °C for 3 min, 25 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, with a final extension at 72 °C for 5 min, then hold at 4 °C. The resulting PCR product was cleaned up following instructions using AMPure XP beads (Beckman Coulter, Agencourt Bioscience Corporation, MA, USA). After purification, the Illumina sequencing adapters and dual-index barcodes to the amplicon targets were added using the full complement of Nextera XT indices (Illumina, Inc., San Diego, CA, USA) through a limited cycle PCR: thermocycling steps included 95 °C for 3 min, 8 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, with a final extension at 72 °C for 5 min, then hold at 4 °C. The resulting PCR product was cleaned up again following instructions using AMPure XP beads. The PCR products were validated by using Bioanalyzer DNA 1000 chip (Agilent, Santa Clara, CA, USA) and the expected size of the final library was ~630 bp. The pooled final DNA library was sequenced using the Illumina MiSeq System to generate high-quality, full-length reads of the V3 and V4 region in a single 65-hour run.

2.5. Data analyses

Pearson correlation coefficients (r) were calculated to illustrate the relationships between *N. fowleri*, HF183, *E. coli* concentrations, and physical parameters. Pearson coefficients were interpreted as strong, moderate, and weak correlation if their values lay between ± 0.50 and ± 1, ± 0.30 and ± 0.49, and ± 0.0 and ± 0.29, respectively. One-way analysis of variance (ANOVA) was used to determine whether significant differences ($p < 0.05$) existed in *N. fowleri*, HF183, and *E. coli* concentrations recorded across sites for each sampling day. Cross-tabulations and stacked plots presenting side-by-side frequency distributions of the dependent variable for every category of the independent variable were used to visualize associations between the detection of a parameter and the occurrence of another parameter at levels higher than a defined threshold. Thresholds were based on percentile calculations of observed concentrations (Supplementary Material, Table S1). Combinations tested were: *N. fowleri* detection versus *E. coli* concentrations, *N. fowleri* detection versus HF183 concentrations, and HF183 versus *E. coli* concentrations. Chi-squared and Cramer's V analysis were used to test whether the associations were statistically significant. Statistical analyses were performed using statistics software (SPSS version 17.0, SPSS) for Microsoft Windows. Bacterial compositions of the sediment samples were categorized in distinct phylum and family levels and the results were analyzed using stacked plots in Excel.

3. RESULTS

3.1. Physico-chemical parameters of water at the corresponding sediment sample site

Surface water temperature ranged from 8.5 to 31.5 °C, with an overall median of 19.1°C. Observed water temperatures were generally higher in the months of April, May, June, and July (ranging from 23.4 to 31.5 °C) compared to other months of the year when the temperature was generally lower and highly variable (ranging from 8.5 to 23.7 °C, Figure 3(a)). Conversely, observed water dissolved oxygen were generally lower in the warmer months (Figure 3(c)). Median temperatures across sites were comparable across sites (Figure 2(a)). Median dissolved oxygen was lowest at Site LP7 (Supplementary Material, Figure S1). With the notable exemption of Site LP6, which had comparably low conductivity (<60 mS), median conductivity at all the tested sites ranged from 152.5 to 2686.5 mS (Supplementary Material, Figure S1), the highest being for Site LP10

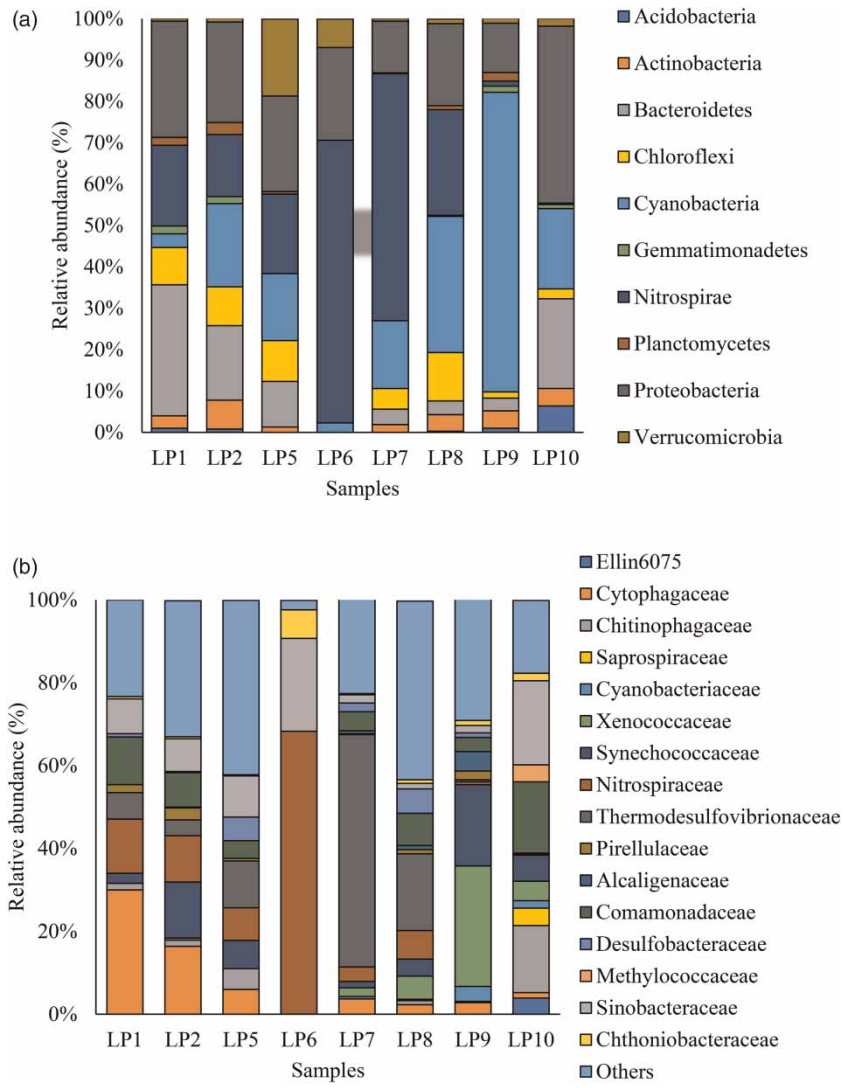

Figure 2 | Taxonomic classification of sequences from bacterial communities of the sediment samples. (a) Relative abundance of the dominant phyla in each sample. (b) Relative abundance of the dominant families in each sample.

(Supplementary Material, Figure S1). Median salinity was generally low across all tested sites, ranging from 0.03 to 1.6 ppt (Supplementary Material, Figure S1).

3.2. Quantification of *N. fowleri*, *E. coli*, and HF183 marker in sediments

A total of 80 sediments were analyzed by *qPCR* over 9 months from December 2016 to August 2017. The *N. fowleri* target DNA was detected in 51.2% of the sediment samples (41/80), *E. coli* in 93.75% (75/80), and HF183 human marker in 46.25% (37/80). Concentrations of *E. coli*, *HF183*, and *N. fowleri* among positive samples ranged from 1.3 to 3.4 Log copies/g, 2.8–5.4 Log copies/g and 1.3–5.3 Log copies/g, respectively (Figure 3(e)–3(g); Supplementary Material, Figure S1). *N. fowleri* was detected in all months except for the month of February (Figure 3(g)). The highest sediment concentrations of *N. fowleri* were detected in the month of March at Site 8. Sites LP7, LP8, and LP9 generally presented with the highest median *N. fowleri* concentrations (Figure 3(g)). The highest rates of *N. fowleri* detection were observed in March to June, when up to seven out of the eight sampled sites tested positive for the pathogen (Supplementary Material, Figure S2). In contrast, *E. coli* was detected throughout the study period (Figure 3(e)). Overall, one-way ANOVA indicated no statistically significant difference in *N. fowleri* (F (7,31) = 0.474, $p = 0.85$), *E. coli* (F (7,68) = 1.01, $p = 0.43$), and HF183(F (7,28) = 0.0811, $p = 0.99$) concentrations recorded across all the tested sites.

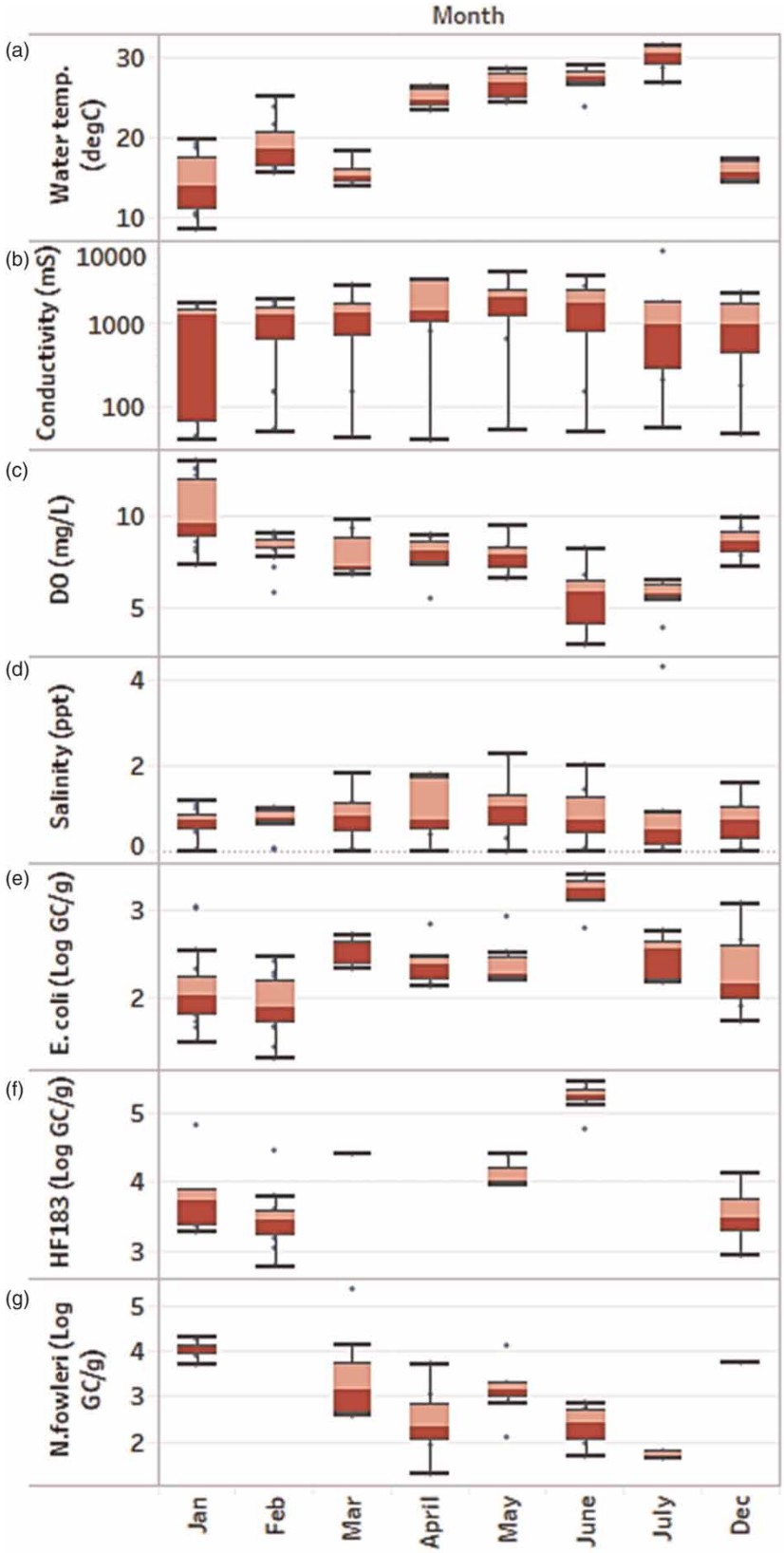

Figure 3 | Monthly water (a) temperature, (b) dissolved oxygen, (c) conductivity, (d) salinity and sediment, (e) HF183, (f) *E. coli*, and (g) *N. fowleri* QPCR concentrations (Log GC/g) recorded for samples collected from Lake Pontchartrain (December 2017–July 2018). Logarithmic axis applied for conductivity.

3.3. Sequencing of microbial communities in water samples

Illumina sequencing of sediment samples collected from Lake Pontchartrain indicated that microbial diversity in the sediment samples consisted of 10 different phyla and over 17 different family groups. Among the 10 different phyla, *Cyanobacteria* was the most predominant at Sites LP9 (72.4%) and LP8 (32.9%), the sites which generally presented with the highest median *N. fowleri* concentrations (Supplementary Material, Figure S2). *Nitrospirae* was predominant at Sites LP6 (68.3%) and LP7 (59.7%). Proteobacteria ranged from 12.5% at Site LP7 to 42.8% at Site LP10 (Figure 5). At the family level, *Nitrospiraceae* and *Thermodesulfovibrionaceae* were the most predominant, respectively, at Sites LP6 (63.1%) and LP7 (56.1%) in LP6. The Shannon diversity index ranged from 10.219 to 11.917. The highest reported value was found in Site LP6 and the lowest value was discovered in LP2 (Supplementary Material, Table S1).

3.4. Correlations between *N. fowleri*, *E. coli*, HF183, and environmental factors

Analysis of Pearson coefficients indicated that HF183 concentrations were strongly positively correlated with water temperature ($r = 0.588$, $p < 0.001$), moderately negatively correlated with dissolved oxygen ($r = -0.48$, $p = 0.003$) but not correlated with conductivity and salinity (Table 2). *E. coli* concentration was strongly correlated with HF183 concentrations ($r = 0.795$, $p < 0.001$, Table 2). *E. coli* concentration was moderately positively correlated with water temperature ($r = 0.389$, $p = 0.001$), moderately negatively correlated with dissolved oxygen ($r = -0.413$, $p < 0.001$), but poorly correlated with salinity and conductivity (Table 2). *N. fowleri* concentration was strongly negatively correlated with water temperature ($r = -0.588$, $p < 0.001$), strongly positively correlated with dissolved oxygen ($r = 0.501$, $p = 0.001$), but not correlated with conductivity, salinity, or *E. coli*. *N. fowleri* was, however, strongly negatively correlated with HF183 ($r = -0.859$, $p < 0.001$, Table 2).

Using the overall median temperature (19.1 °C) as a threshold, sediment samples collected during higher temperatures showed significantly lower mean *N. fowleri* concentrations (2.7 Log GC/g) compared to those collected during lower temperatures (3.7 Log GC/g, $t(39) = 4.167$, $p < 0.001$; Supplementary Material, Figure S3(a)). Conversely, sediment samples collected during higher temperatures had significantly higher mean HF183 concentrations (4.6 Log GC/g) compared to those collected during lower temperatures (3.6 Log GC/g, $t(35) = 4.654$, $p < 0.001$; Supplementary Figure S3(b)). Sediment samples collected during higher temperatures presented with significantly higher mean *E. coli* concentrations (2.5 Log GC/g) than those collected during lower temperatures (2.2 Log GC/g, $t(81) = 2.389$, $p = 0.0181$; Supplementary Material, Figure S3(c)). During the summer months when higher temperatures prevail, the results indicate a higher level of pollution at the lake considering that mean conductivity (1711.2 mS) was significantly higher than during lower temperatures (1067.7 mS, $t(75) = 2.389$, $p = 0.0194$; Supplementary Material, Figure S3(d)). Dissolved oxygen was also significantly lower during higher temperatures than during lower temperatures ($t(78) = 5.212$, $p < 0.0001$; Supplementary Material, Figure S3(e)).

Significantly lower *N. fowleri* concentrations during higher temperatures (i.e., >19.1 °C). However, further investigation showed that higher *N. fowleri* detection rates were observed at higher temperatures (i.e., >19.1 °C) than at lower temperatures (i.e., <19.1 °C; Supplementary Material, Figure S4). Using a reference water temperature of 25 °C as published by Chalmers (2014), similar results were obtained, i.e., higher *N. fowleri* detection rates at temperatures >25 °C (Supplementary Material, Figure S5). Significantly higher HF183 concentrations during higher temperatures, but lower HF183 detection rates were observed at higher temperatures (i.e., >19.1 °C) than at lower temperatures (i.e., <19.1 °C; Supplementary Material, Figure S4). *E. coli* detection rates were comparable during conditions of high and low temperatures (Supplementary Material, Figure S4). Chi-squared analysis showed that there was a statistically significant association between *N. fowleri* detection rates and overlying water column temperature but not between *E. coli* or HF183 detection rates and elevated temperatures (Supplementary Material, Table S2). There was no statistically significant association even when other temperature categories were tested (0–10, 10–20, 20–30 °C, etc., results not included).

3.5. Test of association between *N. fowleri*, *E. coli*, and HF183 detection

Figure 4(a) presents stacked bar graphs based on the cross-tabulated distribution of *N. fowleri* detection, by HF183. There was a higher probability that *N. fowleri* would be detected in sediment samples where HF183 was not detected. For instance, when HF183 was not detected, a higher *N. fowleri* detection rate (58.7%) was observed in the sediment samples, compared to when HF183 was detected (41.2%, Figure 4(a)). Similarly, when *E. coli* was not detected, a higher *N. fowleri* detection rate (60%) was observed in the sediment samples, compared to when *E. coli* was detected (50.7%, Figure 4(b)). If *E. coli* was detected, the chances of detecting or not detecting HF183 in the same sediment sample were roughly equal (Figure 4(c)).

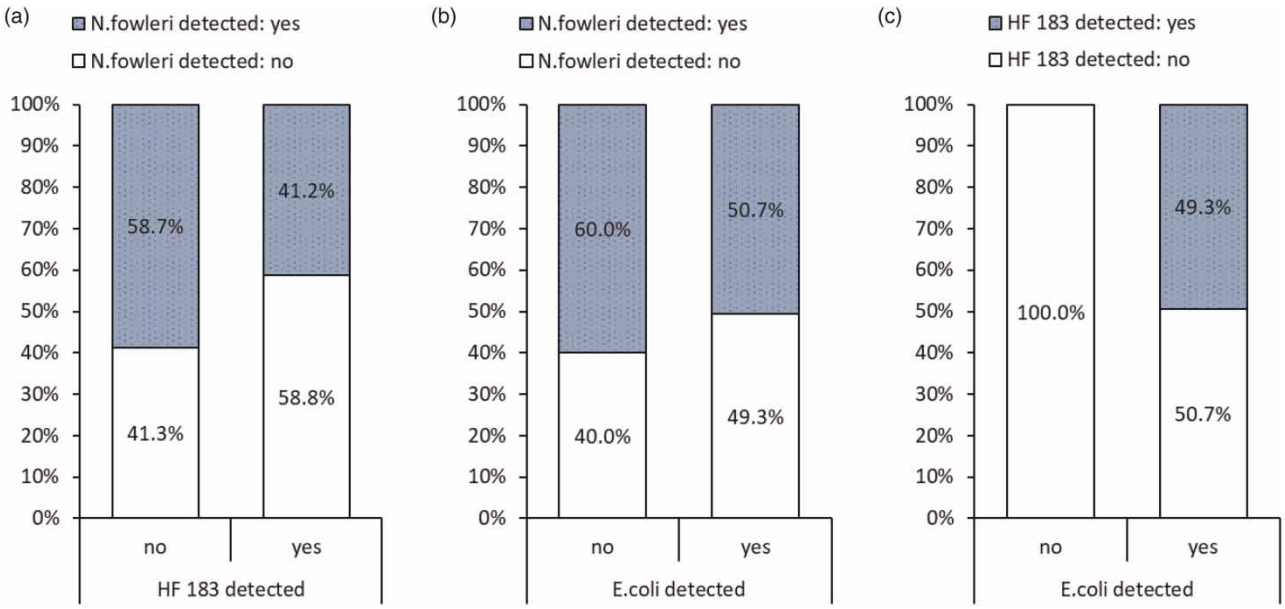

Figure 4 | Stacked bar graph, distribution of (a) *N. fowleri* detection, by HF183 (b) *N. fowleri* detection, by *E. coli* detection, and (c) HF183 detection, by *E. coli* detection.

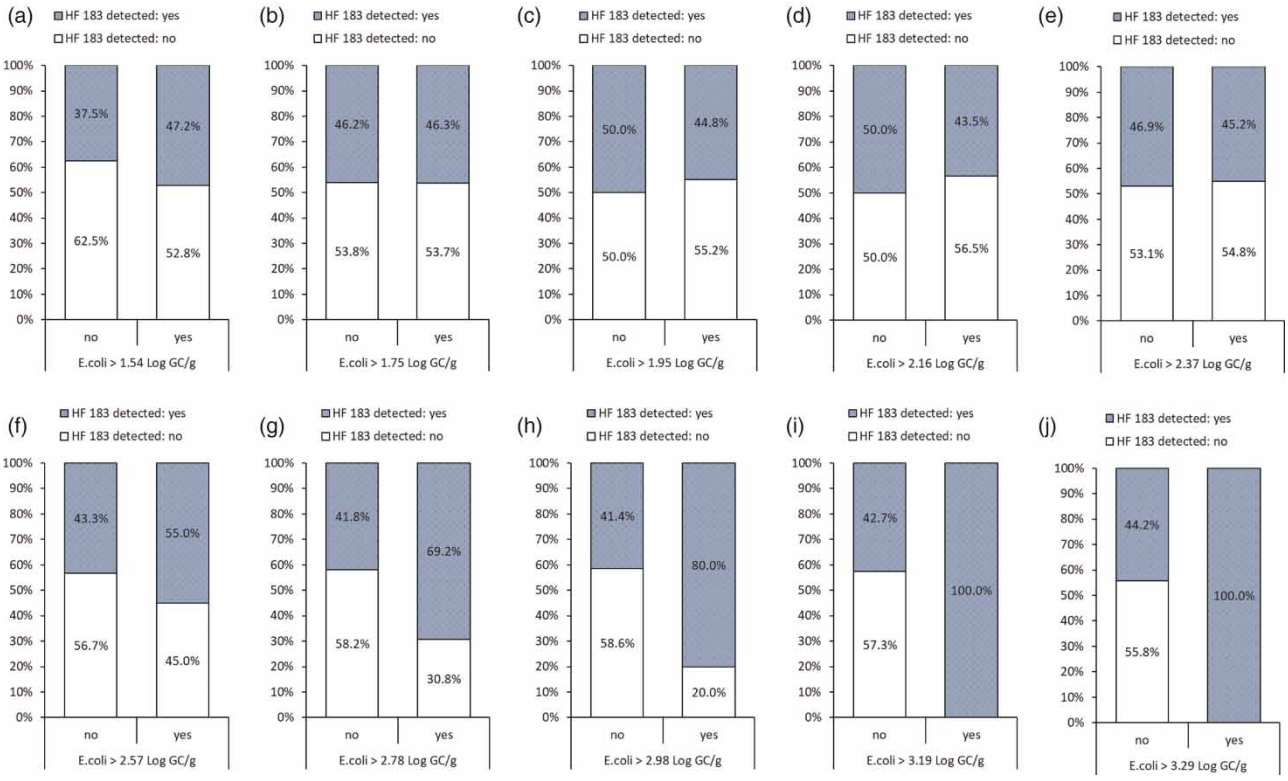

Figure 5 | Stacked bar graph, distribution of *N. fowleri* detection, by *E. coli* detection at various percentile thresholds: (a) 10th perc., (b) 20th perc., (c) 30th perc., (d) 40th perc., (e) 50th perc., (f) 60th perc., (g) 70th perc., (h) 80th perc., (i) 90th perc., and (j) 95th perc. concentrations.

A stacked bar graph showing the distribution of *N. fowleri* detection, by *E. coli* detection, at various percentile thresholds revealed that as *E. coli* concentrations increased from 10th to 95th percentile values, the chances of detecting *N. fowleri* increased (Figure 5(a)–5(g)). For instance, when *E. coli* concentrations in the sediment sample were below 1.54 Log GC/g

Table 2 | Correlation analysis of water quality parameters, HF183 and *N. fowleri*

Parameter	Statistics	Water temperature (°C)	Dissolved Oxygen (mg/L)	Conductivity (mS)	Salinity (ppt)	HF183 (Log GC/g)	*E. coli* (Log GC/g)
Dissolved Oxygen	Pearson Correlation	−**0.692****					
	Sig. (two-tailed)	0.000					
	N	80					
Conductivity	Pearson Correlation	0.377**	−0.056				
	Sig. (two-tailed)	0.001	0.628				
	N	77	77				
Salinity	Pearson Correlation	0.285*	−0.025	**0.992****			
	Sig. (two-tailed)	0.010	0.826	0.000			
	N	80	80	77			
HF183 (Log GC/g)	Pearson Correlation	**0.588****	−0.482**	0.216	0.103		
	Sig. (two-tailed)	0.000	0.003	0.220	0.544		
	N	37	37	34	37		
E. coli (Log GC/g)	Pearson Correlation	0.389**	−0.413**	0.149	0.112	**0.795****	
	Sig. (two-tailed)	0.001	0.000	0.212	0.337	0.000	
	N	75	75	72	75	37	
N. fowleri (Log GC/g)	Pearson Correlation	−**0.588****	**0.501****	−0.244	−0.197	−**0.859****	−0.296
	Sig. (two-tailed)	**0.000**	**0.001**	0.129	0.217	0.000	0.071
	N	**41**	**41**	40	41	14	38

*p < 0.05, **p < 0.01.
Bold values are correlation coefficients >0.5 or <−0.5.

(the 10th percentile of the *E. coli* concentrations recorded in the entire study), there was only a 37.5% chance that *N. fowleri* would be detected in the same sample (Figure 5(a)). Conversely, when *E. coli* concentrations in the sediment sample exceeded 2.77 Log GC/g (the 70th percentile of the *E. coli* concentrations recorded in the entire study), the chances of detecting *N. fowleri* in the same sample increased to 90% (Figure 5(h)). Chi-squared and Cramer's V analysis also indicated a statistically significant association between the detection of *N. fowleri* and exceedance of 2.77 Log GC/g *E. coli* concentrations in a sediment sample ($\chi^2(1, N = 80) = 10.473$, $p = 0.001$, $\phi_C = 0.36$). *N. fowleri* was significantly more likely to be detected in a sediment sample when the HF183 concentrations exceed 4.91 Log GC/g (100%) than when the same sediment sample contained HF183 concentrations below that threshold (46.6%), $\chi^2(1, N = 80) = 7.297$, $p = 0.007$, $\phi_C = 0.30$ (Table 3; Supplementary Material, Figure S6). HF183 were significantly more likely to be detected in a sediment sample when the *E. coli* concentrations exceed 3.19 Log GC/g (100%) than when the same sediment sample contained *E. coli* concentrations below that threshold (42.7%), $\chi^2(1, N = 80) = 5.237$, $p = 0.022$, $\phi_C = 0.28$ (Table 3; Supplementary Material, Figure S7).

4. DISCUSSION

The current study assessed the occurrence of *N. fowleri* in sediments collected from Lake Pontchartrain. The *N. fowleri* target DNA sequence was detected in more than half of the 80 samples analysed, indicating that sediment from the lake can be a reservoir for pathogenic amoeba. Results of microbial diversity of sediments collected from the lake showed that *Cyanobacteria* were predominant at sites that showed the highest median *N. fowleri* concentrations. It appears that the elevated *N. fowleri* concentrations at these sites with predominantly cyanobacteria concentrations may simply be because cyanobacteria were acting as a food source. This observation is consistent with previous studies. *N. fowleri*, like other free-living amoebae (FLA), are predatory heterotrophic protozoa, feeding as trophozoites on bacteria, cyanobacteria, fungi, and algae through phagocytosis while adhering to surfaces (Scheid 2019). Studies have posited that grazing is a major factor that regulates cyanobacterial population dynamics, as FLA feed efficiently on cyanobacteria (Urrutia-Cordero *et al.* 2013; Maciver *et al.* 2020). Predation experiments in another study revealed a *Naegleria* sp. strain W2, which readily consumed cyanobacteria with clearance rates ranging from 0.332 to 0.513 nL amoeba^{-1} h^{-1} (Xinyao *et al.* 2006). In a study comparing growth of *N. fowleri* in the presence of bacteria, it was found that increased cyanobacteria levels were associated with higher reports of isolated amoebae (Jamerson *et al.* 2009). In another study where qPCR techniques were used for *N. fowleri* enumeration, it was

Table 3 | Chi-squared test of association between HF183 and *E. coli* threshold concentrations (Log GC/g) and *N. fowleri* detection

Test	Independent (*x*), dependent variable (*y*)	Pearson Chi-Square	No. of cases	Sig. (two-sided)	Cramer's V
E. coli vs. *N. fowleri* detection	*E. coli* >1.54 Log GC/g vs. *N. fowleri*	0.673	80	0.412	0.092
	E. coli >1.74 Log GC/g vs. *N. fowleri*	2.606	80	0.106	0.180
	E. coli >1.95 Log GC/g vs. *N. fowleri*	4.586	80	0.032**	0.239
	E. coli >2.15 Log GC/g vs. *N. fowleri*	11.287	80	0.001***	0.376
	E. coli >2.36 Log GC/g vs. *N. fowleri*	3.565	80	0.059*	0.211
	E. coli >2.57 Log GC/g vs. *N. fowleri*	8.822	80	0.003***	0.332
	E. coli >2.78 Log GC/g vs. *N. fowleri*	10.473	80	0.001***	0.362
	E. coli >2.98 Log GC/g vs. *N. fowleri*	6.869	80	0.009***	0.293
	E. coli >3.18 Log GC/g vs. *N. fowleri*	5.073	80	0.024**	0.252
	E. coli >3.29 Log GC/g vs. *N. fowleri*	2.965	80	0.085*	0.193
E. coli vs. HF183 detection	*E. coli* >1.54 Log GC/g, vs. HF183	0.274	80	0.601	0.058
	E. coli >1.74 Log GC/g, vs. HF183	0.000	80	0.994	0.001
	E. coli >1.95 Log GC/g, vs. HF183	0.172	80	0.679	0.046
	E. coli >2.15 Log GC/g, vs. HF183	0.334	80	0.563	0.065
	E. coli >2.36 Log GC/g, vs. HF183	0.024	80	0.877	0.017
	E. coli >2.57 Log GC/g, vs. HF183	0.821	80	0.365	0.101
	E. coli >2.78 Log GC/g, vs. HF183	3.298	80	0.069*	0.203
	E. coli >2.98 Log GC/g, vs. HF183	5.237	80	0.022**	0.256
	E. coli >3.19 Log GC/g, vs. HF183	6.198	80	0.013**	0.278
	E. coli >3.29 Log GC/g, vs. HF183	3.622	80	0.057*	0.213
HF183 vs. *N. fowleri* detection	HF183 >3.04 Log GC/g vs. *N. fowleri*	2.402	80	0.121	0.173
	HF183 >3.31 Log GC/g vs. *N. fowleri*	1.204	80	0.273	0.123
	HF183 >3.58 Log GC/g vs. *N. fowleri*	0.011	80	0.916	0.012
	HF183 >3.84 Log GC/g vs. *N. fowleri*	1.564	80	0.211	0.140
	HF183 >4.11 Log GC/g vs. *N. fowleri*	2.009	80	0.156	0.158
	HF183 >4.38 Log GC/g vs. *N. fowleri*	3.187	80	0.074*	0.200
	HF183 >4.65 Log GC/g vs. *N. fowleri*	5.750	80	0.016**	0.268
	HF183 >4.91 Log GC/g vs. *N. fowleri*	7.297	80	0.007***	0.302
	HF183 >5.18 Log GC/g vs. *N. fowleri*	6.170	80	0.013**	0.278
	HF183 >5.31 Log GC/g vs. *N. fowleri*	1.951	80	0.162	0.156

***Correlation is significant at the 0.01 level.
**Correlation is significant at the 0.05 level.
*Correlation is significant at the 0.1 level.

discovered that cyanobacteria may provide a substrate for attachment as a food source, and *N. fowleri* thrives under these conditions because of the abundance of bacteria (Painter *et al.* 2013).

In this study, we also assessed relationships between environmental factors and *N. fowleri* concentrations. We had previously found a positive correlation between *N. fowleri* concentrations in water samples and water temperature (Xue *et al.* 2018). An interesting finding in the current study was the strong negative correlation between sediment *N. fowleri* concentration and overlying water temperature that resulted in lower *N. fowleri* concentrations in sediment samples collected during higher temperatures compared to those collected during lower temperatures. This might be because *N. fowleri* exists in colder sediment in a cystic form which is more or less a formation of a resting cyst from the vegetative cell. Following disturbances that stir up sediments into warmer water column above sediment, it excysts to a flagellate stage where growth resumes in the presence of sources of food (bacteria). This hypothesis is in line with previous studies (Delaquis *et al.* 2016; Hoseinzadeh *et al.* 2021). Considering that *N. fowleri* concentration was also positively correlated with dissolved oxygen, it could be that primary production linked to increased dissolved oxygen concentrations and associated grazing population (Burns *et al.* 2005; Matsuzaki *et al.* 2018) may be driving *N. fowleri* concentrations at the sites where the lake sediments were collected. While our study would be the first to report on negative correlations between *N. fowleri* sediment concentration and overlying water temperature, future research would need to evaluate the effect of stratification on *N. fowleri* concentrations in the lake. Existing literature on the effect of temperature on *N. fowleri* seem to present varying results. While it is established that *N. fowleri* infections are usually a result of swimming in warmer rather than cooler waters (Painter *et al.* 2013), it has also been shown that the pathogen grows more frequently in water temperatures above 30 °C but less than

45–46 °C (Bartrand *et al.* 2014). Previous research also suggests a similar trend; that *N. fowleri* thrives in warmer waters, with temperatures of 30 °C and over (Jamerson *et al.* 2009; Garcia *et al.* 2013). Contrasting with these previous findings, there is other evidence that suggests that environmental variables such as water temperature are not significant factors in *N. fowleri* prevalence. Others have reported that water characteristics such as conductivity, dissolved oxygen, temperature, and pH do not affect the presence of *N. fowleri* or correlate with detection of the amoeba (Laseke *et al.* 2010; Mushtaq & Shinwari 2016). Another contrasting study, conducted in Arizona, suggests that *N. fowleri* has been collected more frequently in locations with lower temperatures and organic levels (Sifuentes *et al.* 2014).

Given these correlation results in our study and the published literature, it would be expected that higher detection rates would be the case in lower temperatures, but our findings reveal the reverse (i.e., higher *N. fowleri* detection rates were observed at higher temperatures and in months characterized by high temperatures, e.g., April–June). Our findings indicate that the effect of temperature produces different effects in relation to observed *N. fowleri* concentrations and detection rates. Our observation of higher detection rates of *N. fowleri* at higher temperatures, regardless of whether a median temperature of 19.1 or 25 °C is applied as a defining threshold, is consistent with previous studies. Chalmers (2014) stated that water that seasonally exceeds 30 °C or that continually exceeds 25 °C can potentially support the growth of *N. fowleri*. Also, in another study, *N. fowleri* was detected in water samples with temperatures ranging from 29 to 47 °C, suggesting the thermo-tolerance of this microorganism (Laseke *et al.* 2010).

There is a dearth of published studies that address whether correlations exist between the concentrations of *N. fowleri* and indicators (*E. coli* and HF183 markers) in sediment samples. In this study, we found negative correlations between *N. fowleri* concentrations and marker concentrations of well-established indicators (HF183 and *E. coli*). HF183 has long been established as a human wastewater-associated Bacteroides marker and has been applied to detect sewage pollution in environmental waters (Bernhard & Field 2000; Bernhard *et al.* 2003; Gourmelon *et al.* 2007; Ahmed *et al.* 2008, 2010, 2012; McQuaig *et al.* 2009; Sercu *et al.* 2011). Ahmed *et al.* (2012) reported that more than 90% of 79 composite human wastewater and individual human faecal DNA samples tested positive for the HF183 marker, unlike more than 90% of 214 animal faecal DNA samples, which were found to be negative for the HF183 marker. The negative correlation between *N. fowleri* and human faeces-specific HF183 concentrations in our study is therefore consistent with the host-specificity of the HF183 marker. Two possible inferences could be drawn from these results. The first inference is that it seems that *N. fowleri* in Lake Pontchartrain may be more related to natural occurrence or pollutants from non-human sources than to human activity. The weak negative correlation between *N. fowleri* and *E. coli* concentrations observed in our study also supports the environmental nature of the amoeba and seem to indicate that traditional *E. coli* guidelines cannot be relied upon as an indicator for the presence or absence of *N. fowleri* (Chalmers 2014). It thus appears that the prevalence of *N. fowleri* at these sites is not directly related to human activity, but can be, if human activity results in the modification of temperature or the promotion of bacterial (food source) production; for instance, anthropogenic activities that promote nutrient loading which ultimately may cause the proliferation of cyanobacteria (Fastner *et al.* 2016), or disturbance of the water surface by boating (Mushtaq & Shinwari 2016). Another inference that could be drawn from the study relates to the changing proportions of *N. fowleri* detections with increasing *E. coli* concentrations (as reported in Figure 5(a)–5(j)). Increasing *N. fowleri* detection rate with increasing *E. coli* concentrations in sediment samples may suggest predatory activity by the amoeba as studies have linked the occurrence of *N. fowleri* to nutrient sources and large numbers have been detected where coliforms or eubacteria are present as food sources (Marshall *et al.* 1997; Painter *et al.* 2013; Scheid 2019).

In the future, it will be beneficial to continue to research patterns of *N. fowleri* occurrence in lake sediment water to determine what influencing factors are contributing to the amoeba's growth. The negative correlation with temperature but increased detection rate during higher temperatures observed in our study also warrants future research. Due to the detrimental impact of *N. fowleri* on human health, it is imperative to undertake research on other environmental conditions that can potentially foster the growth of this amoeba, with a view to informing management options aimed at reducing the prevalence of *N. fowleri*.

5. CONCLUSION

The present study is a positive step forward towards understanding the prevalence of *N. fowleri* in environmental samples. We have shown that lake sediment can be a reservoir for the amoeba as *N. fowleri* was detected in the tested samples. We also showed that *N. fowleri* was strongly negatively correlated with HF183 and temperature but lacked correlation with *E. coli*.

Statistically significant associations were, however, observed between the detection of *N. fowleri* and the presence/absence of indicators (*E. coli* and HF183 markers), when certain thresholds were exceeded.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Ahmed, W., Stewart, J., Powell, D. & Gardner, T. 2008 Evaluation of Bacteroides markers for the detection of human faecal pollution. *Letters in Applied Microbiology* **46** (2), 237–242.

Ahmed, W., Yusuf, R., Hasan, I., Goonetilleke, A. & Gardner, T. 2010 Quantitative PCR assay of sewage-associated Bacteroides markers to assess sewage pollution in an urban lake in Dhaka, Bangladesh. *Canadian Journal of Microbiology* **56** (10), 838–845.

Ahmed, W., Masters, N. & Toze, S. 2012 Consistency in the host specificity and host sensitivity of the Bacteroides HF183 marker for sewage pollution tracking. *Letters in Applied Microbiology* **55** (4), 283–289.

Bartrand, T. A., Causey, J. J. & Clancy, J. L. 2014 *Naegleria fowleri*: an emerging drinking water pathogen. *Journal-American Water Works Association* **106** (10), E418–E432.

Bernhard, A. E. & Field, K. G. 2000 A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA. *Applied and Environmental Microbiology* **66** (10), 4571–4574.

Bernhard, A. E., Goyard, T., Simonich, M. T. & Field, K. G. 2003 Application of a rapid method for identifying fecal pollution sources in a multi-use estuary. *Water Research* **37** (4), 909–913.

Burns, N. M., Rockwell, D. C., Bertram, P. E., Dolan, D. M. & Ciborowski, J. J. 2005 Trends in temperature, Secchi depth, and dissolved oxygen depletion rates in the central basin of Lake Erie, 1983–2002. *Journal of Great Lakes Research* **31**, 35–49.

Chalmers, R. M. 2014 Naegleria. In: Persival S. L. (ed.). *Microbiology of Waterborne Diseases*, 2nd edn. Elsevier, Amsterdam, pp. 407–416.

Cope, J. R., Ratard, R. C., Hill, V. R., Sokol, T., Causey, J. J., Yoder, J. S., Mirani, G., Mull, B., Mukerjee, K. A., Narayanan, J. & Doucet, M. 2015 The first association of a primary amebic meningoencephalitis death with culturable *Naegleria fowleri* in tap water from a US treated public drinking water system. *Clinical Infectious Diseases* **60** (8), e36–e42.

Cursons, R., Sleigh, J., Hood, D. & Pullon, D. 2003 A case of primary amoebic meningoencephalitis: North Island, New Zealand. *The New Zealand Medical Journal (Online)* **116** (1187), U712.

Delaquis, P., Brassard, J. & Gajadhar, A. 2016 Pathogenic viruses and protozoa transmitted by soil. *Manual of Environmental Microbiology* pp. 3–3.

Fastner, J., Abella, S., Litt, A., Morabito, G., Vörös, L., Pálffy, K., Straile, D., Kümmerlin, R., Matthews, D., Phillips, M. G. & Chorus, I. 2016 Combating cyanobacterial proliferation by avoiding or treating inflows with high P load – experiences from eight case studies. *Aquatic Ecology* **50** (3), 367–383.

Frahm, E. & Obst, U. 2003 Application of the fluorogenic probe technique (TaqMan PCR) to the detection of *Enterococcus* spp. and *Escherichia coli* in water samples. *Journal of Microbiological Methods* **52** (1), 123–131.

Garcia, A., Goñi, P., Cieloszyk, J., Fernandez, M. T., Calvo-Beguería, L., Rubio, E., Fillat, M. F., Peleato, M. L. & Clavel, A. 2013 Identification of free-living amoebae and amoeba-associated bacteria from reservoirs and water treatment plants by molecular techniques. *Environmental Science & Technology* **47** (7), 3132–3140.

Gourmelon, M., Caprais, M. P., Ségura, R., Le Mennec, C., Lozach, S., Piriou, J. Y. & Rincé, A. 2007 Evaluation of two library-independent microbial source tracking methods to identify sources of fecal contamination in French estuaries. *Applied and Environmental Microbiology* **73** (15), 4857–4866.

Hoseinzadeh, E., Rostamian, A., Razaghi, M. & Wei, C., 2021 Waterborne transmission of protozoan parasites: a review of water resources in Iran. An update 2020. *Desalination and Water Treatment* **213**, 91–105.

Jamerson, M., Remmers, K., Cabral, G. & Marciano-Cabral, F. 2009 Survey for the presence of *Naegleria fowleri* amebae in lake water used to cool reactors at a nuclear power generating plant. *Parasitology Research* **104** (5), 969–978.

Laseke, I., Korte, J., Lamendella, R., Kaneshiro, E. S., Marciano-Cabral, F. & Oerther, D. B. 2010 Identification of *Naegleria fowleri* in warm ground water aquifers. *Journal of Environmental Quality* **39** (1), 147–153.

Maciver, S. K., Piñero, J. E. & Lorenzo-Morales, J. 2020 Is *Naegleria fowleri* an emerging parasite? *Trends in Parasitology* **36** (1), 19–28.

Marshall, M. M., Naumovitz, D., Ortega, Y. & Sterling, C. R. 1997 Waterborne protozoan pathogens. *Clinical Microbiology Reviews* **10** (1), 67–85.

Matsuzaki, S. i. S., Suzuki, K., Kadoya, T., Nakagawa, M. & Takamura, N. 2018 Bottom-up linkages between primary production, zooplankton, and fish in a shallow, hypereutrophic lake. *Ecology* **99** (9), 2025–2036.

McLaughlin, A. & O'Gorman, T. 2019 A local case of fulminant primary amoebic meningoencephalitis due to *Naegleria fowleri*. *Rural and Remote Health* **19** (2), 175.

McQuaig, S. M., Scott, T. M., Lukasik, J. O., Paul, J. H. & Harwood, V. J. 2009 Quantification of human polyomaviruses JC virus and BK virus by TaqMan quantitative PCR and comparison to other water quality indicators in water and fecal samples. *Applied and Environmental Microbiology* **75** (11), 3379–3388.

Mushtaq, S. & Shinwari, Z. K. 2016 *Naegleria fowleri, the Causative Agent of Primary Amoebic Meningoencephalitis*.

Painter, S. M., Pfau, R. S., Brady, J. A. & McFarland, A. 2013 Quantitative assessment of *Naegleria fowleri* and *Escherichia coli* concentrations within a Texas reservoir. *Journal of Water and Health* **11** (2), 346–357.

Qvarnstrom, Y., Visvesvara, G. S., Sriram, R. & da Silva, A. J. 2006 Multiplex real-time PCR assay for simultaneous detection of *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Naegleria fowleri*. *Journal of Clinical Microbiology* **44** (10), 3589–3595.

Scheid, P. L. 2019 *Vermamoeba vermiformis* – a free-living amoeba with public health and environmental health significance. *The Open Parasitology Journal* **7** (1), 40–47.

Sercu, B., Van De Werfhorst, L. C., Murray, J. L. & Holden, P. A. 2011 Sewage exfiltration as a source of storm drain contamination during dry weather in urban watersheds. *Environmental Science & Technology* **45** (17), 7151–7157.

Sifuentes, L. Y., Choate, B. L., Gerba, C. P. & Bright, K. R. 2014 The occurrence of *Naegleria fowleri* in recreational waters in Arizona. *Journal of Environmental Science and Health, Part A* **49** (11), 1322–1330.

Urrutia-Cordero, P., Agha, R., Cirés, S., Lezcano, M. Á., Sánchez-Contreras, M., Waara, K.-O., Utkilen, H. & Quesada, A. 2013 Effects of harmful cyanobacteria on the freshwater pathogenic free-living amoeba *Acanthamoeba castellanii*. *Aquatic Toxicology* **130**, 9–17.

Xinyao, L., Miao, S., Yonghong, L., Yin, G., Zhongkai, Z., Donghui, W. & Chencai, A. 2006 Feeding characteristics of an amoeba (Lobosea: Naegleria) grazing upon cyanobacteria: food selection, ingestion and digestion progress. *Microbial Ecology* **51** (3), 315–325.

Xue, J., Lamar, F. G., Zhang, B., Lin, S., Lamori, J. G. & Sherchan, S. P. 2018 Quantitative assessment of *Naegleria fowleri* and fecal indicator bacteria in brackish water of Lake Pontchartrain, Louisiana. *Science of the Total Environment* **622**, 8–16.

First received 16 November 2021; accepted in revised form 9 February 2022. Available online 11 March 2022