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Results of *Naegleria fowleri* testing for Washington County, Minnesota
lake water and sediment, August 2014

Vincent R. Hill, PhD, PE
Team Lead
Phone: 404-718-4151
vhill@cdc.gov

Amy Kahler, MS
Microbiologist
Phone: 404-718-4153
akahler@cdc.gov

Water, Sanitation and Hygiene Laboratory Team
Centers for Disease Control and Prevention
National Center for Emerging and Zoonotic Infectious Diseases
Waterborne Disease Prevention Branch
1600 Clifton Rd MS/D-66
Bldg 23 Room 9-146
Atlanta, GA 30329-4018

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SAMPLE COLLECTION AND PROCESSING

In August 2014, Washington County Public Health & Environment (WSPHE) staff collaborated with scientists in the CDC Waterborne Diseases Prevention Branch (WDPB) to conduct environmental testing of samples from lakes in Washington County as part of an ongoing ecological investigation of *Naegleria fowleri* in environmental water systems. For this study, WSPHE staff collected 1-L water and sediment samples from 10 lakes in Washington County, Minnesota (Figure 1). These were the same 10 lakes that were also studied by WSPHE and WDPB in 2011, 2012, and 2013. In addition to collecting samples, the field team measured water temperature and dissolved oxygen (DO) at each sampling site. Each 1-L sample was a composite of 4, 250-mL samples collected from a bathing area at each lake. Samples were shipped priority overnight for testing at CDC by scientists in the WDPB Environmental Microbiology Laboratory. The lake samples were tested using the same methods reported in Mull, Jothikumar, and Hill, 2013*. In short, water samples were centrifuged to pellet *N. fowleri* trophozoites and cysts. After washing the sediment samples in WB saline, the supernatants were processed using the same procedures as performed for the water samples. Immunomagnetic separation (IMS) was used to separate *N. fowleri* trophozoites and cysts from other amebas and other water constituents. After IMS, each sample was assayed by real-time PCR (to detect and estimate the concentration of *N. fowleri*) and by culture (for *N. fowleri* isolation). The sample pellets were also analyzed by real-time PCR and cultured directly without IMS processing.

* Bonnie J. Mull, Jothikumar Narayanan, and Vincent R. Hill, “Improved Method for the Detection and Quantification of *Naegleria fowleri* in Water and Sediment Using Immunomagnetic Separation and Real-Time PCR,” *Journal of Parasitology Research*, vol. 2013, Article ID 608367, 8 pages, 2013. doi:10.1155/2013/608367

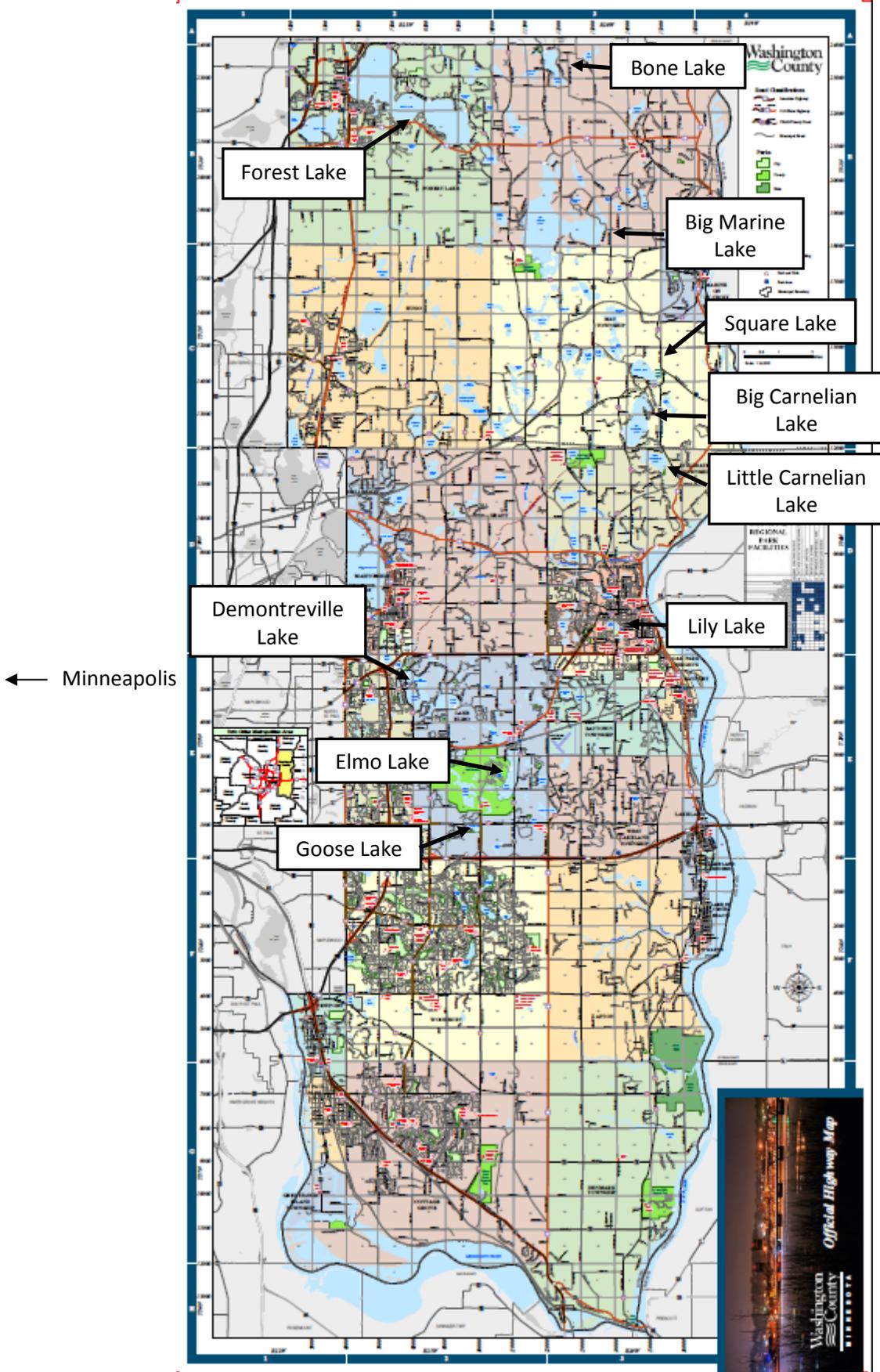


Figure 1. Field sampling locations, Washington County, MN, 2014

WATER QUALITY TESTING RESULTS

At the time of sample collection, water temperatures in the study lakes were 24.5 to 26.1 °C (76.1 to 79.0 °F) (Table 1). On average the lakes were 1.5 °C warmer than at the time of sample collection in 2013. However, average monthly and average maximum monthly air temperatures in the Washington County area appear to have been appreciably lower in 2013 and 2014 versus 2010-2012, the period coinciding with previous detections of *N. fowleri* and the two cases of primary amebic meningoencephalitis (PAM). For example, in 2010, 2011 and 2012 the average maximum air temperatures for July were 82 °F, 84 °F and 85°F, respectively. In 2013 and 2014 the average maximum air temperatures for July were 79 °F and 77 °F, respectively. Similarly, the average overall air temperatures (all hourly temperatures averaged) for July in 2010, 2011 and 2012 were 73 °F, 75 °F and 76°F, respectively. In 2013 and 2014 the average overall air temperatures for July were 70 °F and 69 °F, respectively.

Dissolved oxygen concentrations and turbidity values for the 2014 samples varied between water and sediment, with sediment DO being 2.0 mg/L higher in sediment. Turbidity of the water ranged from 1.29 in Square Lake to 13.3 NTU in Forest Lake.

Grab samples collected for water quality testing from Big Carnelian, Demontreville, Elmo, Little Carnelian, and Lily lakes were analyzed 48 hours after collection instead of 24 hours, due to a shipping delay. Total coliform and *E. coli* concentrations were highest at Goose Lake. Heterotrophic plate count (HPC) bacteria concentrations were highest at Elmo Lake. Overall *E. coli* concentrations were below the USEPA recreational water statistical threshold value of 320 cfu/100 mL, with the exception of Forest Lake and Goose Lake. The Goose Lake *E. coli* result was the highest we have measured for any of the 10 lakes we have studied for the past four years.

Other water quality parameters tested but not shown included pH and specific conductance.

NAEGLERIA FOWLERI TESTING RESULTS

Unlike the test results determined by CDC for these 10 lakes in 2011 and 2012 (see Appendix), *N. fowleri* was not detected in any of the samples. Samples were determined to be negative for *N. fowleri* by real-time PCR analysis directly on the sample concentrate and after culture. Additionally, we did not detect *N. fowleri* in lake water or sediment samples collected in August 2013.

Table 1. Water quality results, 2014

Sample site	Sample type	Field water temp (°C)	Field DO (mg/L)	Turbidity (NTU)	Total Coliforms (cfu/100mL)	<i>E. coli</i> (cfu/100mL)	Heterotrophic plate count (cfu/1mL)
Big Carnelian Lake*	Sediment	25.1	6.45				
	Water	25.1	8.11	2.25	6,700	2.0	24,000
Big Marine Lake	Sediment	25.2	7.3				
	Water	25.2	8.66	2.06	8,820	38.8	35,500
Bone Lake	Sediment	25.6	5.19				
	Water	25.6	8.29	8.06	6,160	1.0	5,450
Demontreville Lake*	Sediment	25.5	7.41				
	Water	25.5	8.15	3.93	11,870	4.1	69,500
Elmo Lake*	Sediment	25.8	6.67				
	Water	25.8	8.65	1.42	3,990	21.3	108,500
Forest Lake	Sediment	24.5	5.8				
	Water	24.5	7.8	13.3	8,600	344.8	64,000
Goose Lake	Sediment	25.9	5.14				
	Water	25.9	7.5	7.07	23,590	2,419.6	11,500
Little Carnelian Lake*	Sediment	25.2	6.31				
	Water	25.2	8.43	1.37	308	1.0	17,000
Lily Lake*	Sediment	26.1	6.79				
	Water	26.1	9.02	11.4	10,170	3.1	65,000
Square Lake	Sediment	25.5	6.15				
	Water	25.5	8.57	1.29	1440	2.0	4750

*Water quality testing conducted 48 hours after sample collection due to a shipment delay

Table 2. *N. fowleri* presence/absence and genotyping test results, 2013

Sample site	Sample type	IMS		w/out IMS	
		Direct	Culture	Direct	Culture
Big Carnelian Lake	Sediment	Negative	Negative	Negative	Negative
	Water	Negative	Negative	Negative	Negative
Big Marine Lake	Sediment	Negative	Negative	Negative	Negative
	Water	Negative	Negative	Negative	Negative
Bone Lake	Sediment	Negative	Negative	Negative	Negative
	Water	Negative	Negative	Negative	Negative
Demontreville Lake	Sediment	Negative	Negative	Negative	Negative
	Water	Negative	Negative	Negative	Negative
Elmo Lake	Sediment	Negative	Negative	Negative	Negative
	Water	Negative	Negative	Negative	Negative
Forest Lake	Sediment	Negative	Negative	Negative	Negative
	Water	Negative	Negative	Negative	Negative
Goose Lake	Sediment	Negative	Negative	Negative	Negative
	Water	Negative	Negative	Negative	Negative
Little Carnelian Lake	Sediment	Negative	Negative	Negative	Negative
	Water	Negative	Negative	Negative	Negative
Lily Lake	Sediment	Negative	Negative	Negative	Negative
	Water	Negative	Negative	Negative	Negative
Square Lake	Sediment	Negative	Negative	Negative	Negative
	Water	Negative	Negative	Negative	Negative

DISCUSSION

In August 2014, samples collected from 10 lakes in Washington County, MN were tested at CDC and all were found to be negative for *N. fowleri*. No *N. fowleri* was detected in the lakes in August 2013, but in July 2012 one sample (Lily Lake sediment) was found to contain *N. fowleri*. The 2011 sampling yielded positive detections of *N. fowleri* in water and/or sediment samples from 6 of the 10 lakes, including Lily Lake. It is not clear why *N. fowleri* was detected in more lakes in 2011 than 2012, but it appears that maximum and sustained elevated air temperatures during the summers of 2011 and 2012 may have been associated with detection of *N. fowleri*. The one lake to yield detections of *N. fowleri* in both 2011 and 2012 was Lily Lake. *N. fowleri* was also detected in water and sediment samples collected from Lily Lake in 2010 during the investigation of a PAM infection related to swimming in Lily Lake. The *N. fowleri* isolates from two separate PAM case-patient clinical samples collected in 2010 and 2012 were both genotype 3. *N. fowleri* genotype 3 was also detected in Lily Lake samples in 2010 and 2012, but not in 2011 (only genotype 1 was detected). The lack of a large, robust database of isolates for comparison purposes combined with the current genotyping method used by CDC do not

have sufficient discriminatory power to confidently link cases with exposure sources. CDC is working on developing new genotyping tools and expanding the comparison database for *N. fowleri*.

The 2011 to 2014 lake sampling data from Washington County have helped CDC evaluate the effectiveness of new sample processing and analytical methods for *N. fowleri* in water and sediment samples. CDC hopes to continue to conduct studies such as these to develop an evidence base that can help CDC better understand the relationship between environmental factors and the presence and concentration of *N. fowleri* in lake environments. Cases of PAM are known to correlate with warm weather (and, by association, warm water temperatures). However, within regions and states, it is not known why cases of PAM are associated with certain water bodies but not others. CDC hopes to continue to conduct ecological studies to investigate various factors that may be associated with the distribution of PAM case exposures in the US. It is also difficult to interpret the negative testing results for the 2014 study since the results are from relatively few samples and there are limited data on *Naegleria* sampling to demonstrate how sampling results change by lake location, time of day or season, location in the water column, etc. Sample results may have been different under other conditions or if more intensive sampling had occurred.

The environmental *N. fowleri* detection data summarized in this report will be useful to CDC in conjunction with future ecological studies of *N. fowleri*. We also hope the data are useful to Washington County, MN public health officials. The growing dataset from Washington County, MN between 2010 and 2014 suggests that maximum and sustained air temperatures (and associated water temperatures) may be a primary factor contributing to the likelihood of detecting *N. fowleri* in some lakes in Washington County. It is still not clear why *N. fowleri* has been detected in some Washington County lakes, but not others. CDC would like to statistically analyze the water, sediment and climatological data associated with the monitoring studies conducted at the 10 lakes in 2011-2014, with the intent to publish findings from the analysis. CDC will engage with Washington County staff on the analyses and plans to report the results in a peer-reviewed journal manuscript.

APPENDIX

Water Quality Results, 2012

Sample site	Sample type	Field water temp (°C)	Field DO (mg/L)	Turbidity (NTU)	Total Coliforms (cfu/100mL)	<i>E. coli</i> (cfu/100mL)	Heterotrophic plate count (cfu/1mL)
Big Carnelian Lake	Sediment	28.8	6.23	4.66	6770	5	4200
	Water	28.8	7.64	3.84	8260	21	10200
Big Marine Lake	Sediment	28.0	5.30	2.65	2420	8	101
	Water	27.8	7.50	4.5	3130	5	122
Bone Lake	Sediment	28.7	6.90	11.3	8360	9	2100
	Water	28.7	7.35	10.7	882	12	2300
Demontreville Lake	Sediment	28.7	6.14	2.4	17930	1	1800
	Water	28.4	6.60	6.1	1350	1	2600
Elmo Lake	Sediment	28.7	missing	1.4	2460	<1	720
	Water	28.8	6.57	4.1	4280	1	3000
Forest Lake	Sediment	29.4	5.3	6.2	3590	75	8100
	Water	29.3	6.3	6.2	4350	40	9500
Goose Lake	Sediment	29.2	5.3	9.0	14390	70	4000
	Water	29.4	7.15	7.4	8780	99	1420
Little Carnelian Lake	Sediment	28.8	6.5	1.6	2980	2	1300
	Water	28.7	7.3	1.3	2310	2	560
Lily Lake	Sediment	29.8	5.36	9.9	18720	921	3200
	Water	29.9	7.5	16.8	29000	1553	1900
Square Lake	Sediment	28.1	4.55	1.7	866	<1	79
	Water	28.1	6.96	1.6	1120	<1	83

Water Quality Results, 2011

Sample site	Water Temp (°C)	Air Temp (°F)	DO @ surface (mg/L)	DO @ sediment (mg/L)	Turbidity (NTU)	Total Coliforms (cfu/100mL)	<i>E. coli</i> (cfu/100mL)	HPC (cfu/1mL)
Big Carnelian Lake	25.2	65	8.27	7.77	1.39	866.4	0	1040
Big Marine Lake	25.6	72	7.65	6.97	3.03	980.4	1	1080
Bone Lake	25.4	75	8.27	8.00	10.6	816.4	1	630
Demontreville Lake	26.1	ND	8.33	7.71	3.38	613.1	1	150
Elmo Lake	26.9	69	7.40	6.60	1.24	1986.3	2	2060
Forest Lake	23.1	68	8.27	7.73	9.1	1413.6	35.5	930
Goose Lake	25.1	74	5.43	5.15	10.7	2419.6	5.2	1780
Little Carnelian Lake	25.9	65	8.54	8.20	0.75	2419.6	1	800
Lily Lake	26.3	70	9.55	6.01	8.36	1413.6	17.3	490
Square Lake	25.8	68	7.23	6.60	2.8	727	1	2110

***N. fowleri* presence/absence and genotyping test results**

Sample site	Sample type	IMS		w/out IMS	Genotype
		Direct	Culture	Culture	
2012					
Big Carnelian Lake	Sediment		Negative	Negative	
	Water	—	Negative	Negative	
Big Marine Lake	Sediment		Negative	Negative	
	Water	Negative	Negative	Negative	
Bone Lake	Sediment	Negative	Negative	Negative	
	Water	Negative	Negative	Negative	
Demontreville Lake	Sediment	Negative	Negative	Negative	
	Water	Negative	Negative	Negative	
Elmo Lake	Sediment	Negative	Negative	Negative	
	Water	Negative	Negative	Negative	
Forest Lake	Sediment	Negative	Negative	Negative	
	Water	Negative	Negative	Negative	
Goose Lake	Sediment	Negative	Negative	Negative	
	Water	Negative	Negative	Negative	
Little Carnelian Lake	Sediment	Negative	Negative	Negative	
	Water	Negative	Negative	Negative	
Lily Lake	Sediment	Positive	Positive	Negative	3
	Water	Negative	Negative	Negative	
Square Lake	Sediment	Negative	Negative	Negative	
	Water	Negative	Negative	Negative	
2011					
Big Carnelian Lake	Sediment	Negative	Positive	Negative	indeterminate
	Water	Negative	Negative	Positive	indeterminate
Big Marine Lake	Sediment	Negative	Negative	Negative	
	Water	Negative	Negative	Negative ¹	indeterminate
Bone Lake	Sediment	Negative	Negative	Negative	
	Water	Negative	Negative	Negative	
Demontreville Lake	Sediment	Negative	Positive	Negative	1
	Water	Negative	Negative	Negative	
Elmo Lake	Sediment	Negative	Negative	Negative	
	Water	Negative	Negative	Positive	indeterminate
Forest Lake	Sediment	Negative	Negative	Negative	
	Water	Negative	Negative	Negative	
Goose Lake	Sediment	Negative	Negative	Negative	
	Water	Negative	Negative	Negative	
Little Carnelian Lake	Sediment	Negative	Positive	Negative	indeterminate
	Water	Negative	Negative	Negative	
Lily Lake	Sediment	Positive	Positive	Negative	1
	Water	Negative	Negative	Negative	
Square Lake	Sediment	Negative	Negative	Negative	
	Water	Negative	Negative	Negative	

¹ After additional effort by a free living amoeba expert (Dr. Govinda Visvesvara), *Naegleria fowleri* was detected in this sample.

N. fowleri concentration estimates for lake samples and controls

Sample site	Sample type	Average Cycle threshold (Ct) value	Estimated Concentration (Amebas/L)
2012			
Lily Lake	Sediment	38.71	6
Control (229 amebas)	WB Saline	31.87	220
Control (88 amebas)	WB Saline	34.95	64
2011			
Lily Lake	Sediment	38.34	12
Control (48,500 amebas)	DI water	31.76	806
Control (37,200 amebas)	DI water	31.37	1034
Control (37,200 amebas)	WB Saline	27.82	10005
Control (9,750 amebas)	WB Saline	28.36	7106

