Results of December 2012 - October 2013 Sampling Events

Orange County, FL *Naegleria fowleri* Environmental Factors Longitudinal Study

Vincent R. Hill, PhD, PE
Team Lead; Water, Sanitation and Hygiene Laboratory Team
Centers for Disease Control and Prevention
Division of Foodborne, Waterborne, and Environmental Diseases
Waterborne Disease Prevention Branch
1600 Clifton Rd NE
Mailstop D-66
Atlanta, GA 30329
Phone: 404-718-4151
vhill@cdc.gov

Bonnie Mull, MPH
Regional Environmental Epidemiologist
Food & Waterborne Disease Program
Florida Department of Health
224 SE 24th St
Gainesville, FL 32641
(352) 334-8836 (Office)
(850) 210-5200 (Cell)
Bonnie.Mull@flhealth.gov

STUDY SUMMARY

Beginning in December 2012, the Florida Department of Health (DOH) - Orange County and Food and Waterborne Disease Program staff collaborated with scientists in the CDC Waterborne Disease Prevention Branch (WDPB) to conduct environmental testing of samples from lakes in the Orlando metro-area over a period of 11 months. The goal of this investigation was to improve scientific understanding of *Naegleria fowleri* in natural water bodies and to assess physical, chemical and biological factors contributing to its presence and concentration over time. For this study, DOH staff collected 1-L water and sediment samples from 9 lakes in December 2012, June and October, 2013 in Orange County, Florida (Figure 1). Additionally, two lakes were sampled more frequently (n=10) over the study period. At the time of sample collection, the field team measured water temperature, pH, specific conductance, and dissolved oxygen (DO). Each 1-L sample was a composite of 4, 250-mL samples collected from a bathing area at each lake. Additional water samples were also collected for bacterial and chemical testing. All samples were shipped priority overnight for testing at CDC by scientists in the WDPB Environmental Microbiology Laboratory. The lake samples were tested using the 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 polymerase chain reaction (PCR) (to detect and estimate the concentration of *N. fowleri*) and by culture (for *N. fowleri* isolation). The sample pellets were also cultured directly without IMS processing. Positive *N. fowleri* detection was confirmed using a second PCR assay (Qvarnstrom et al, 2006).

The CDC and Florida DOH team is in the process of analyzing these data and plan to report the results in a peer-reviewed journal manuscript. This report was prepared as a summary of the laboratory results for consideration by DOH and has not been subjected to statistical analysis or peer review.

Other water quality parameters tested but data not shown in this report include specific conductance, dissolved oxygen, total suspended solids, turbidity, total organic carbon, calcium and magnesium hardness, total nitrogen, total iron, manganese, and total phosphorus.

Figure 1. Location of Study Lakes in Orange County, FL

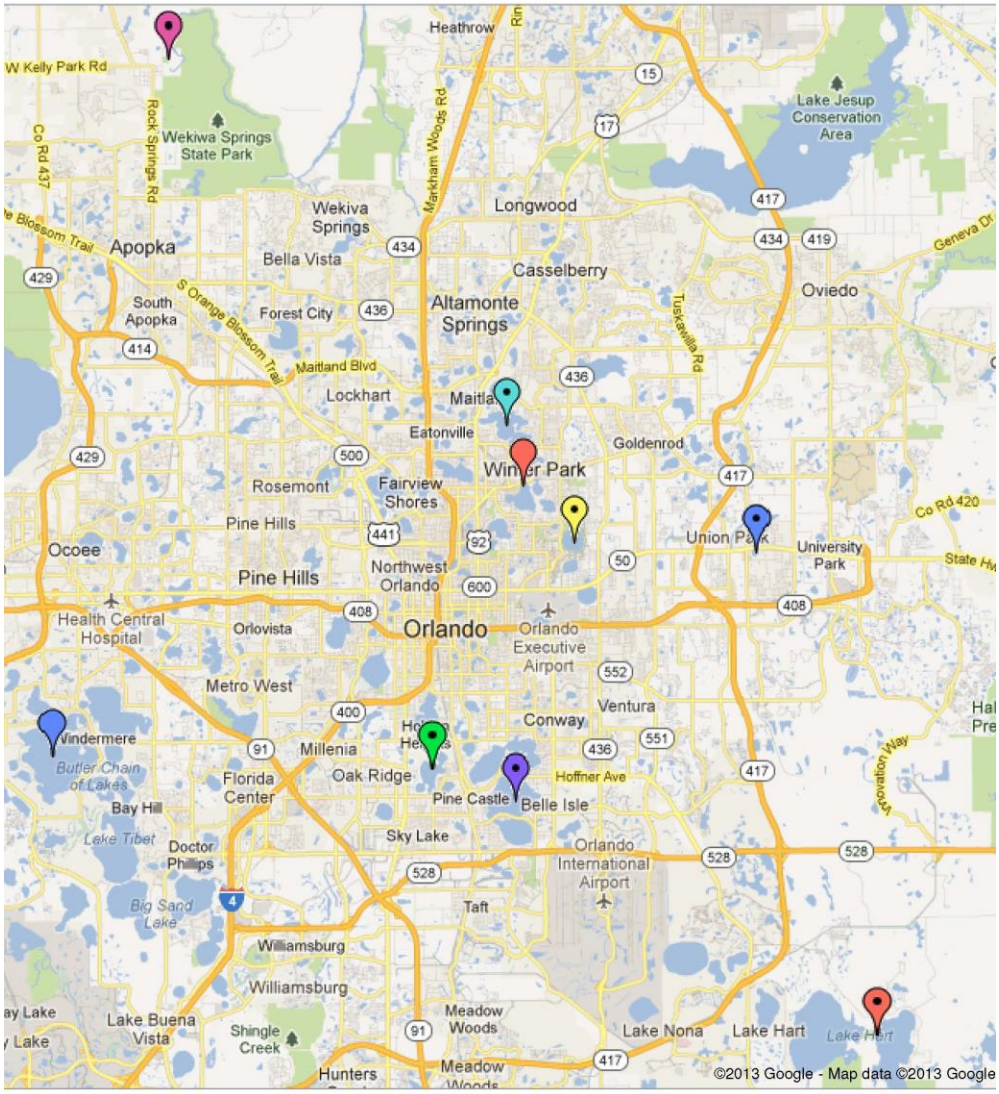


Table 1. Characteristics of lakes sampled for presence of *Naegleria fowleri*, December 2012-October 2013

Sample site	Surface area (acres)	Mean Depth (feet)	Volume (gallons)	Watershed	Lake Region
Baldwin	196	15	9.87×10^8	Little Econ	Orlando Ridge
Moss	1,135	7	2.55×10^9	Lake Hart	Osceola Slope
Downey	18	16	9.38×10^7	Little Econ	Eastern Flatland
Jessamine	292	16	1.53×10^9	Boggy Creek	Orlando Ridge
Conway	1,773	23	9.48×10^9	Boggy Creek	Orlando Ridge
Kelly	Unk	Unk	Unk	Wekiva River	Unk
Ft Maitland	449	Unk	Unk	Howell Branch	Orlando Ridge
Dinky Dock	225	Unk	Unk	Howell Branch	Orlando Ridge
Keene	1,579	14	7.43×10^9	Cypress Creek	Doctor Phillips

Provided by: Orange County Water Atlas at http://www.orange.wateratlas.usf.edu/waterresourcesearch.aspx

Table 2. *N. fowleri* presence/absence test results for 9-lake dataset

Sample site	Sample type	12/11/12			6/4/13			10/1/13		
		IMS		w/o IMS	IMS		w/o IMS	IMS		w/o IMS
		Direct	Culture	Culture	Direct	Culture	Culture	Direct	Culture	Culture
Baldwin	Water	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Sediment	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Moss	Water	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Sediment	Neg*	Neg*	Neg*	Neg	Neg	Neg	Neg	Neg	Neg
Downey	Water	Neg	Neg	Neg	Neg	Neg	Neg	**Positive**	Neg	Neg
	Sediment	**Positive***	Neg*	**Positive***	Neg	Neg	Neg	Neg	Neg	Neg
Jessamine	Water	Neg	Neg	Neg	**Positive**	Neg	Neg	**Positive**	Neg	Neg
	Sediment	Neg*	Neg*	Neg*	Neg	Neg	**Positive**	Neg	Neg	Neg
Conway	Water	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Sediment	Neg*	Neg*	Neg*	Neg	Neg	Neg	Neg	**Positive**	Neg
Kelly	Water	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Sediment	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Ft Maitland	Water	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Sediment	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Dinky Dock	Water	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Sediment	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Keene	Water	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Sediment	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

*Water was collected from the lake bottom above the sediment; Neg= Negative

On December 11, 2012, one sampling team collected water from the lake bottom above the sediment instead of sediment at Moss, Downey, Jessamine, and Conway lakes.

Table 3. *N. fowleri* direct detection test results

Sample site	Sample type	12/11	1/3	2/12	3/5	4/2	4/23	5/7	6/4	6/18	7/2	7/30	8/18	9/10	10/1
Baldwin	Water	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	**Positive**	**Positive**	**Positive**	Neg
	Sediment	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	**Postive**	**Positive**	**Positive**	Neg
Downey	Water	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	**Positive**	**Positive**	**Positive**
	Sediment	**Positive***	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

*Water was collected from the lake bottom above the sediment; Neg= Negative

Table 4. *N. fowleri* culture detection with IMS test results

Sample site	Sample type	12/11	1/3	2/12	3/5	4/2	4/23	5/7	6/4	6/18	7/2	7/30	8/18	9/10	10/1
Baldwin	Water	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	**Positive**	Neg	Neg	Neg
	Sediment	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Downey	Water	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	**Positive**	Neg	Neg
	Sediment	Neg*	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	**Positive**	**Positive**	Neg	Neg

*Water was collected from the lake bottom above the sediment; Neg= Negative

Table 5. *N. fowleri* culture detection without IMS test results

Sample site	Sample type	12/11	1/3	2/12	3/5	4/2	4/23	5/7	6/4	6/18	7/2	7/30	8/18	9/10	10/1
Baldwin	Water	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	**Positive**	Neg	Neg	Neg
	Sediment	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Downey	Water	Neg	Neg	Neg	**Positive**	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	Sediment	**Positive***	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	**Positive**	Neg	Neg

* Water was collected from the lake bottom above the sediment; Neg= Negative

Figure 2. Surface water temperature for the 9-lake dataset

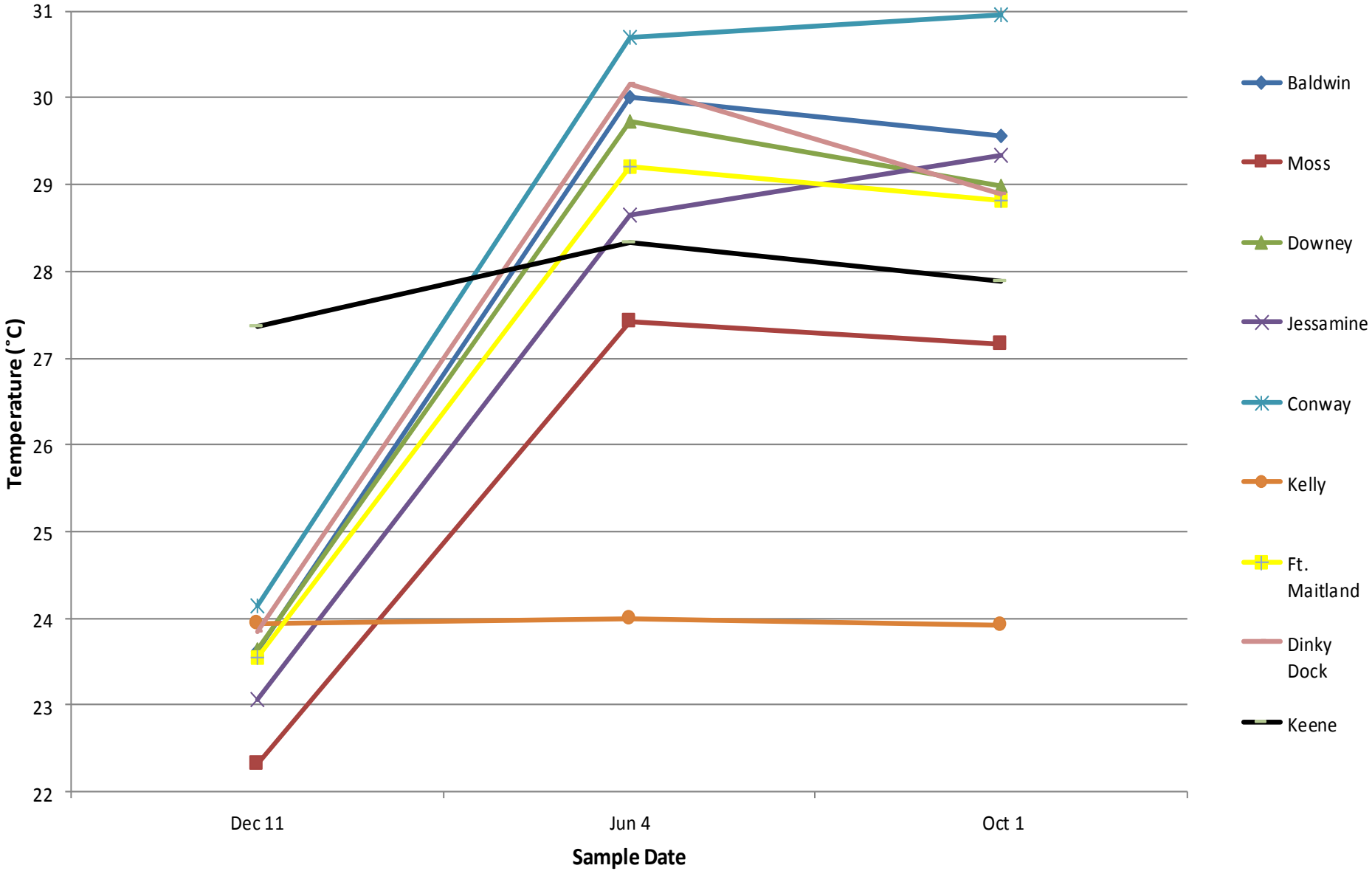
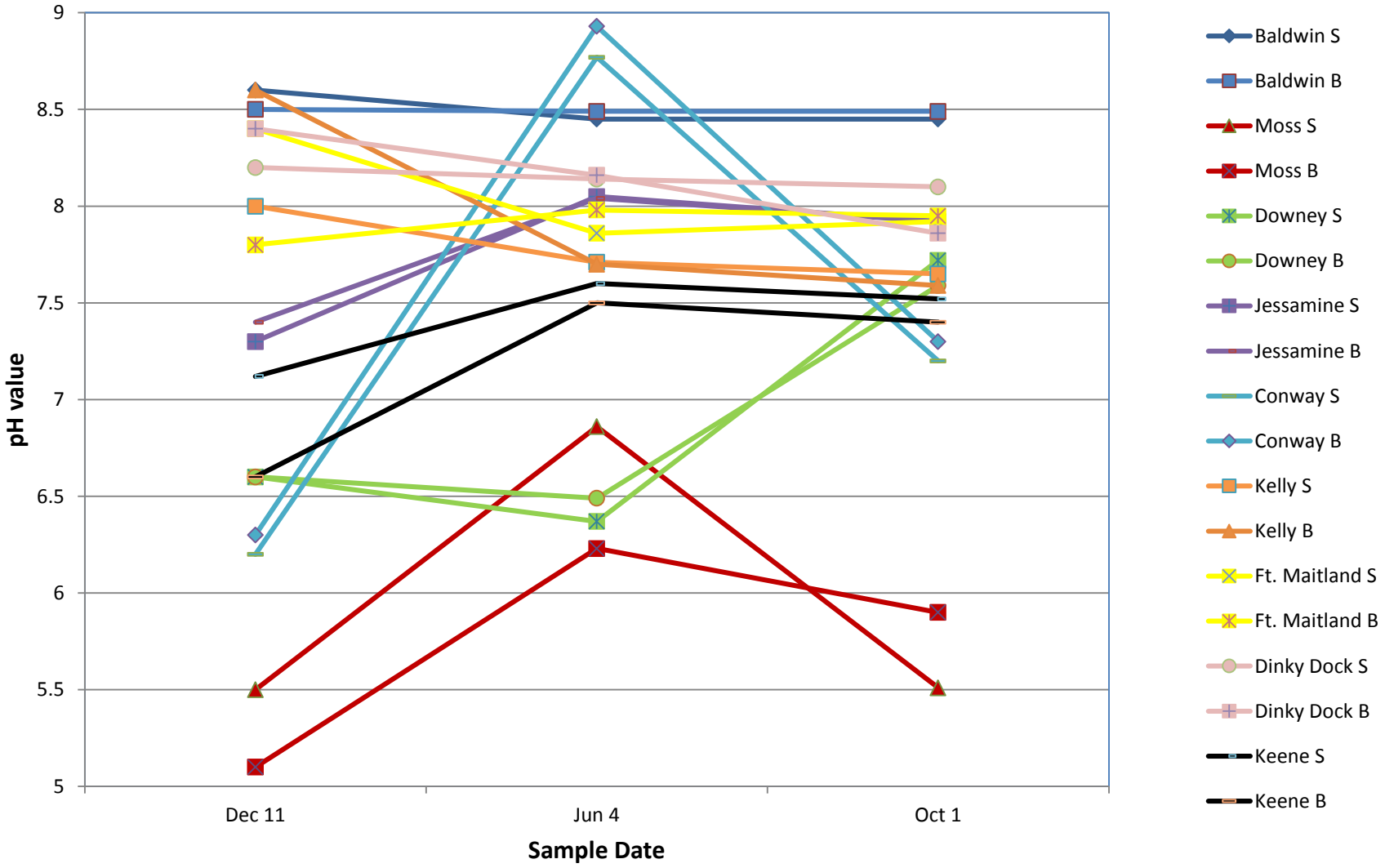
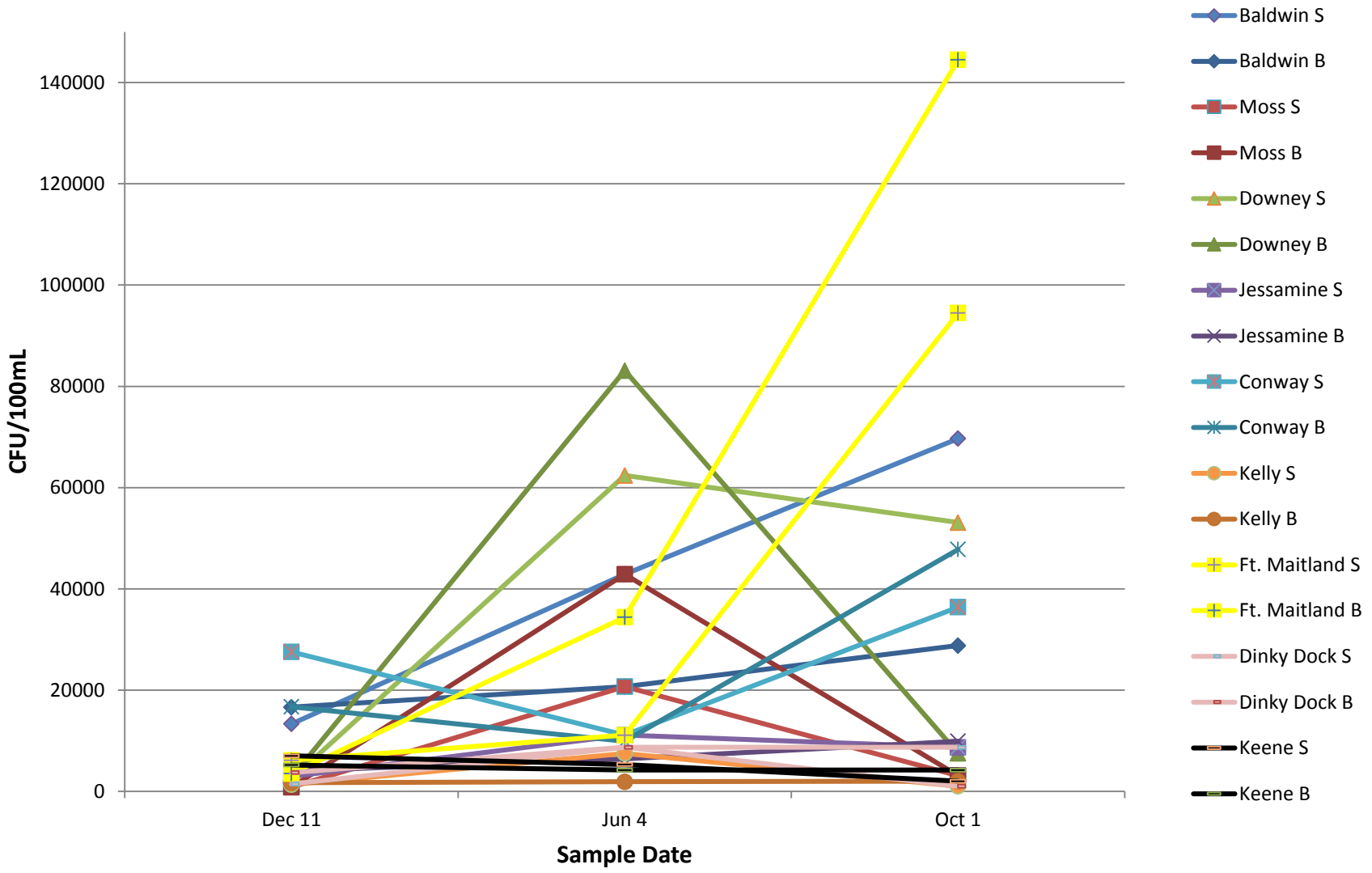


Figure 3. pH values for the 9-lake dataset

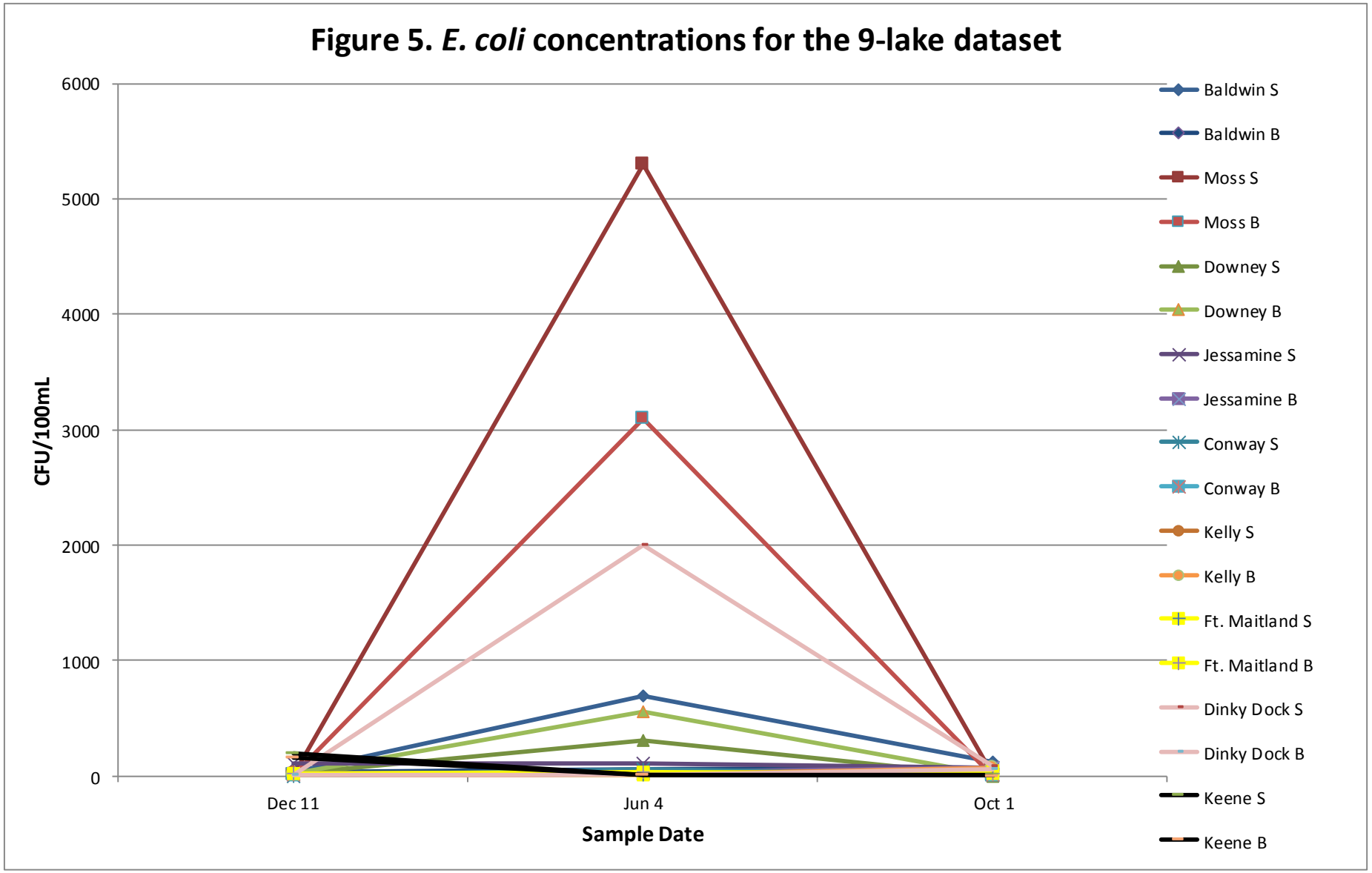


S= Surface; B=Bottom just above sediment

Figure 4. Total coliform concentrations for the 9-lake dataset

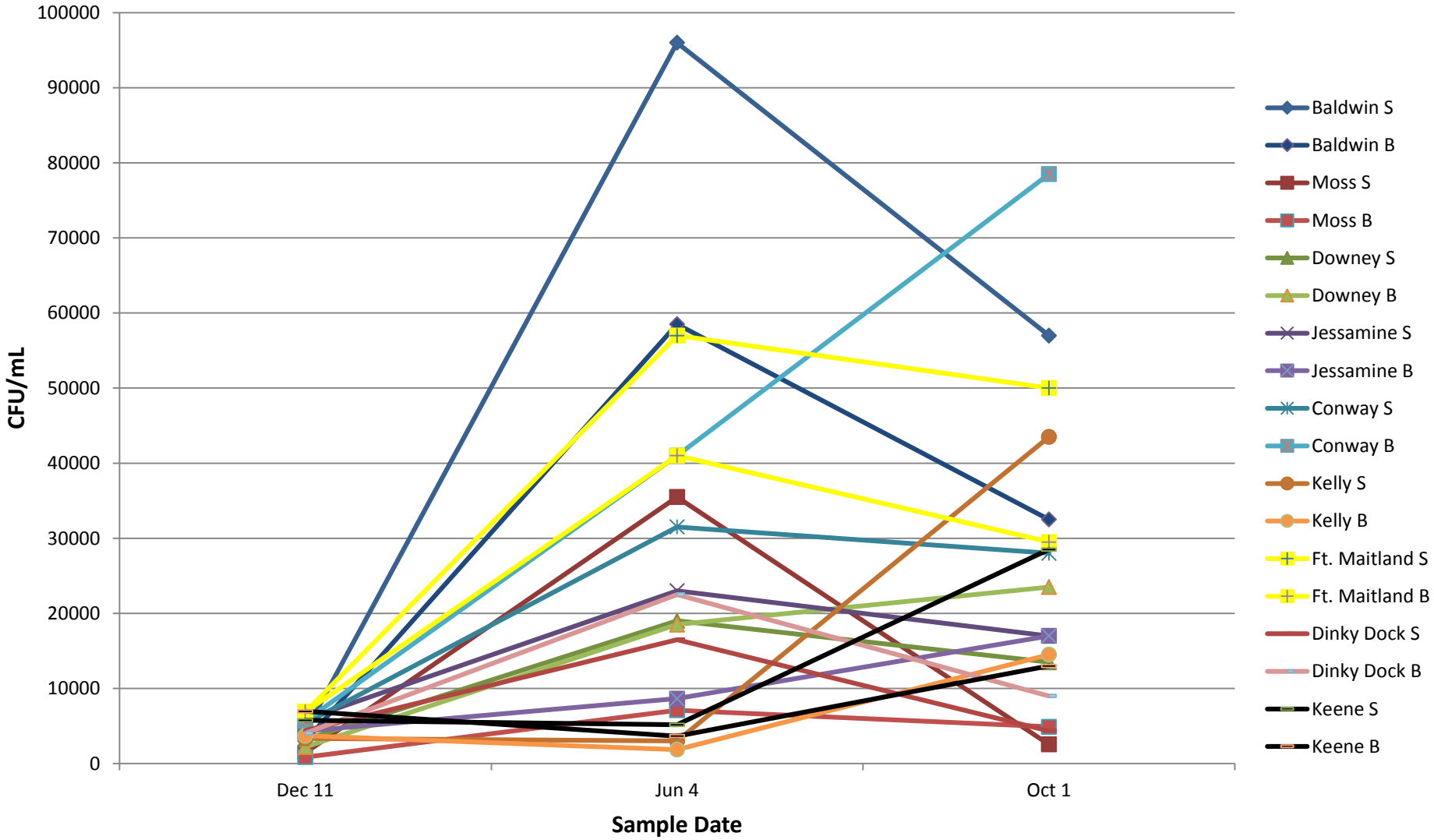


S= Surface; B=Bottom just above sediment



S= Surface; B=Bottom just above sediment

Figure 6. Heterotrophic plate count concentrations for the 9-lake dataset



S= Surface; B=Bottom just above sediment

Figure 7. Water temperatures for 2-lake dateset

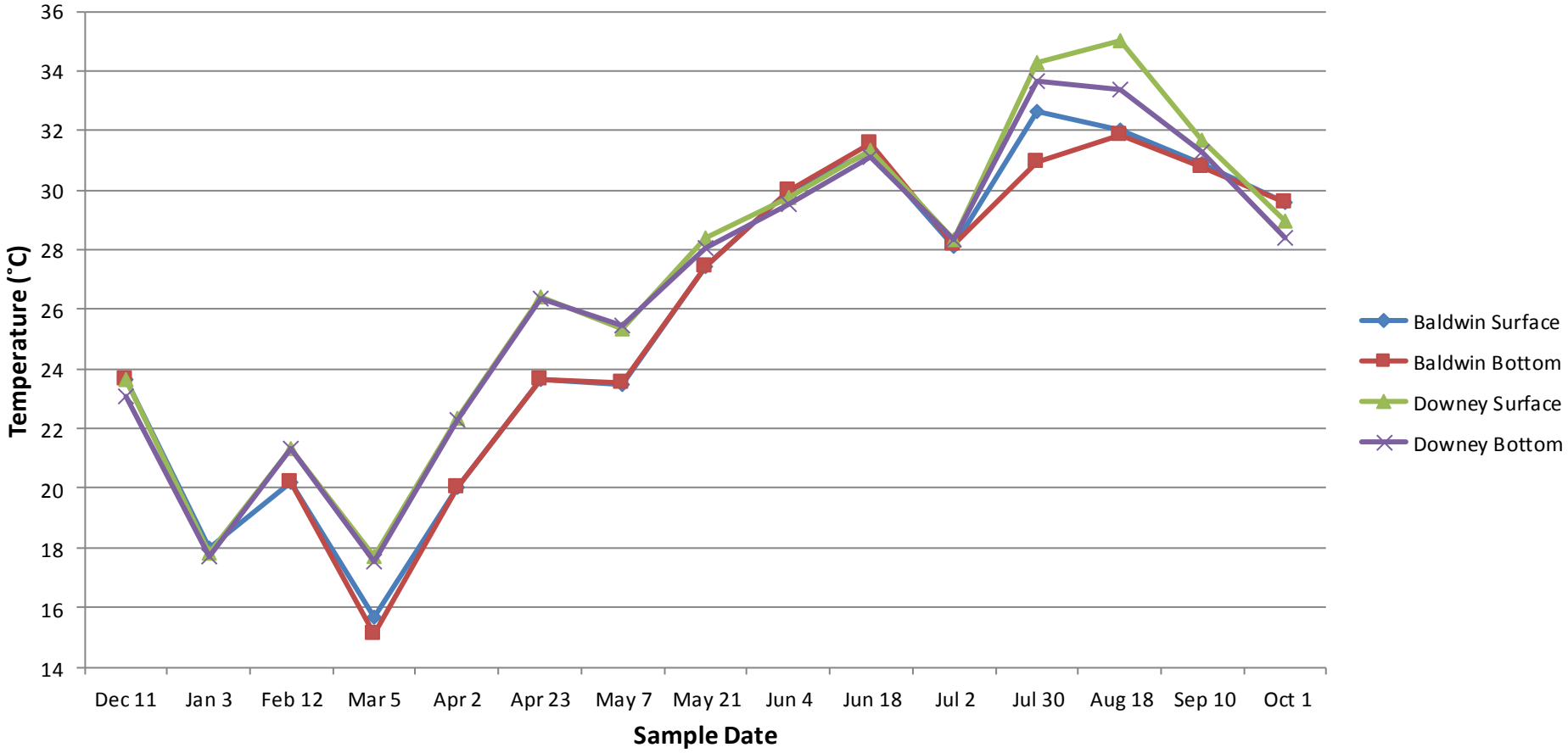


Figure 8. Total coliform concentrations for 2-lake dataset

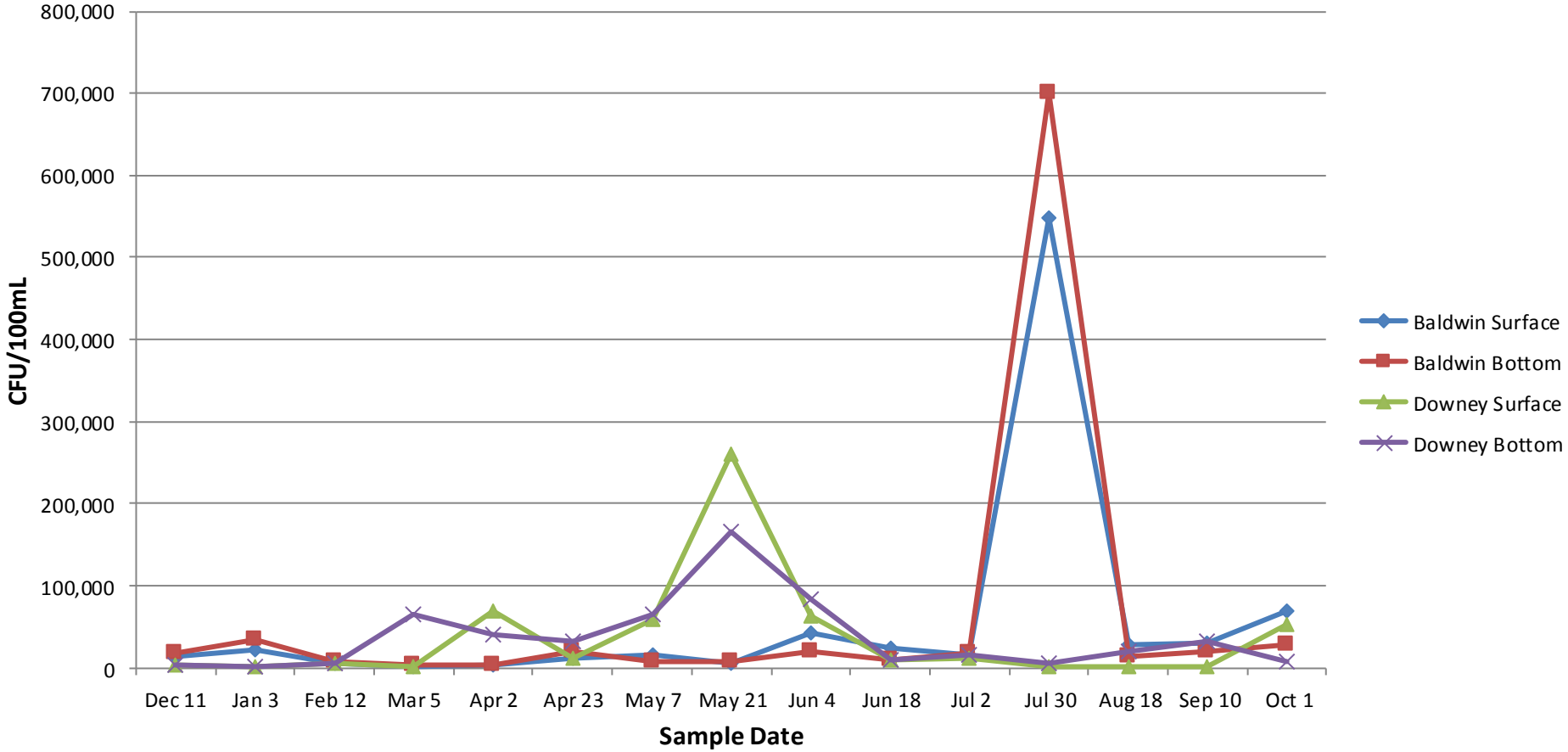


Figure 9. *E. coli* concentrations for 2-lake dataset

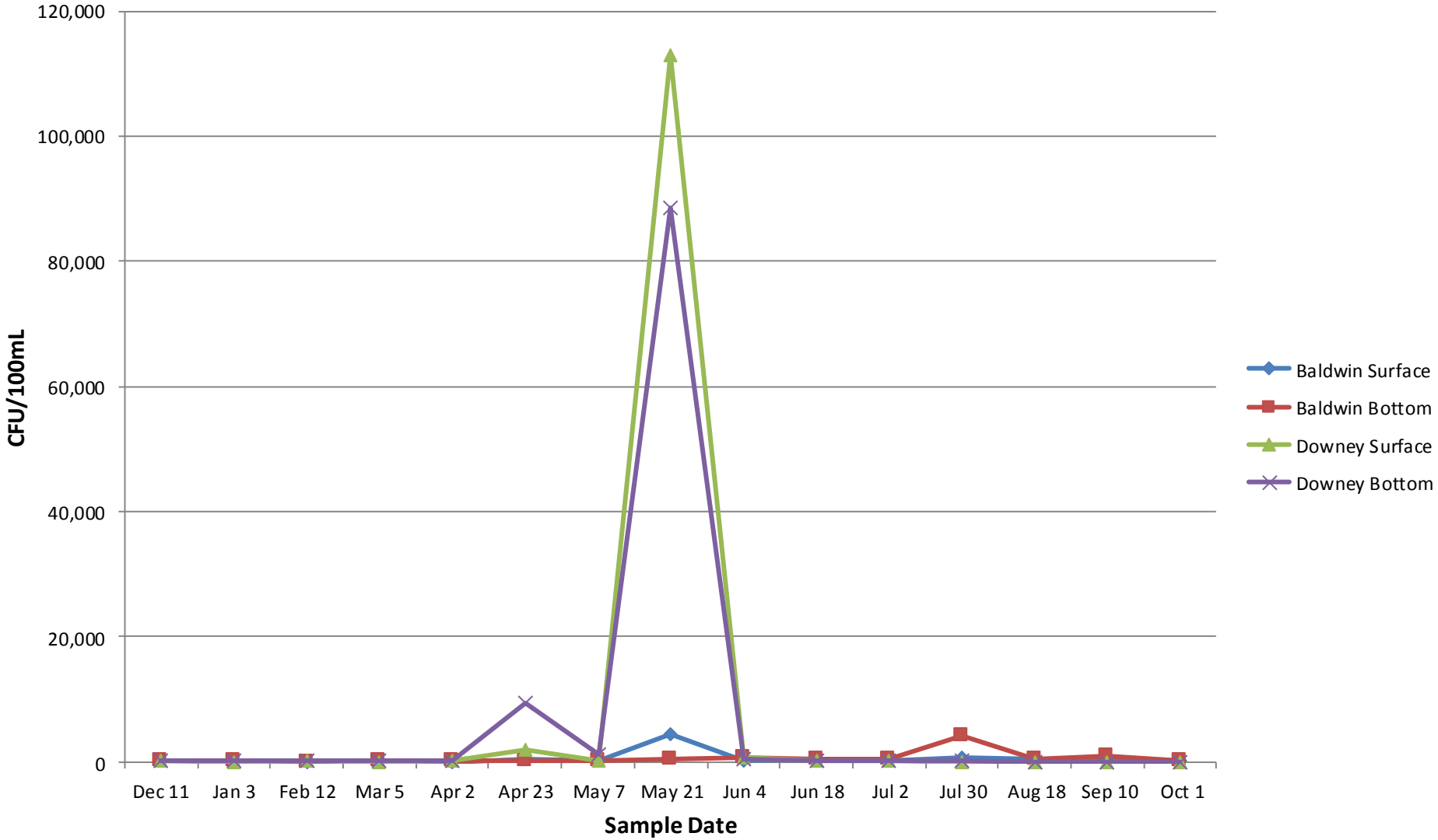
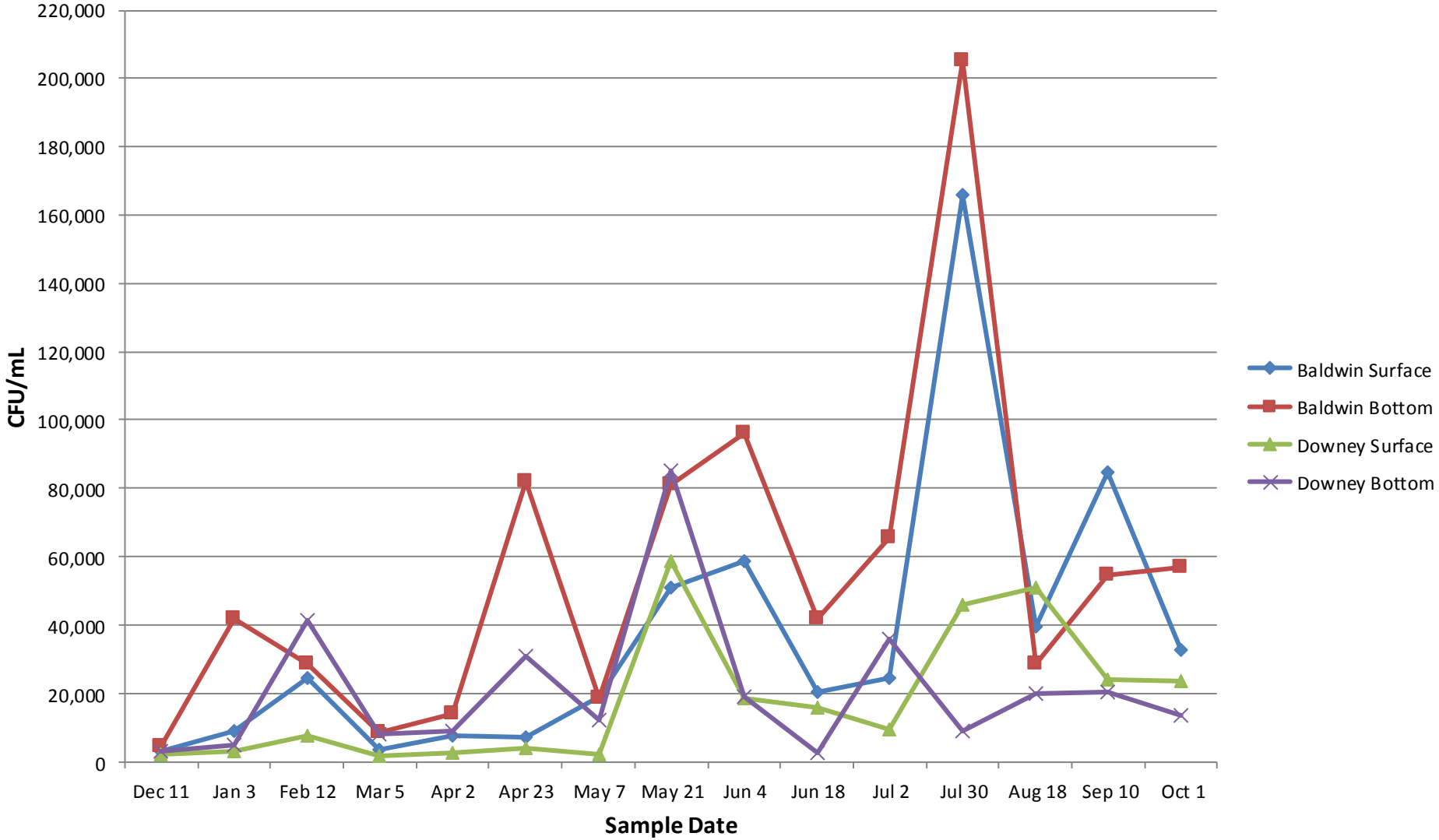


Figure 10. Heterotrophic plate count concentrations for 2-lake dataset



DISCUSSION

The December 2012 to October 2013 lake sampling data from Orange County have helped CDC evaluate the effectiveness of new sample processing and analytical methods for detecting *N. fowleri* in water and sediment samples. The environmental *N. fowleri* detection data summarized in this report will be useful to CDC in conjunction with future ecological studies of *N. fowleri*. The sampling methods used in this study and associated analytical results should be useful to Florida DOH public health officials for future *Naegleria* investigations.

Seventeen of the 106 samples (16%) were positive for *N. fowleri* by at least one test method. The frequency of positive detection was similar for both sample types; eight were sediment samples and nine were water samples. Downey Lake had the highest frequency (23%) of positive samples followed closely by Baldwin Lake (20%). Twelve of the samples were positive for *N. fowleri* when the PCR test was conducted directly on the sample without culture. These positive direct detection test results indicate the presence of *N. fowleri* DNA in the sample, however it cannot differentiate between viable or dead *N. fowleri* cells. Three of those twelve samples were also positive for culturable thermophilic amebas, confirmed to be *N. fowleri* by PCR. This could be due to overgrowth of more rapidly growing but nonpathogenic free-living ameba species or that the *N. fowleri* cells were no longer viable. Five additional samples were determined to be positive for *N. fowleri* after culture that were negative by direct detection testing. It is not clear whether this is due to the sample volume tested (which was lower for PCR than for culture), PCR inhibition, or other factors. *N. fowleri* was detected when the average water temperature was 29.9 ° C (±4.1° C) compared to the average water temperature of 25.9° C (±4.1° C) when *N. fowleri* was not detected. On average, total coliform concentrations were slightly higher in *N. fowleri* positive samples (8.8×10^4 CFU/100mL) compared to samples in which *N. fowleri* was not detected (2.5×10^4 CFU/100mL), but these data have not been statistically analyzed. The data for this project suggest that water temperatures and bacterial concentrations may be factors contributing to detection of *N. fowleri* in some lakes in Orange County. It is still not clear why *N. fowleri* was detected in some Orange County lakes, but not others. It is also difficult to interpret the negative test results since the results are from relatively few samples; limited data on *Naegleria* sampling methods exists to understand how sample test results are affected by lake location, time of day, season, or location in the water column. Test results may have been different under other conditions or if more intensive sampling had occurred.

When considering the data in this report, it is important to note that *Naegleria fowleri* is normally found in the natural environment and is well adapted to surviving in various habitats, particularly warm-water environments. There is no established relationship between detection or concentration of *Naegleria fowleri* and risk of infection.Therefore the data reported in this document are not useful for public health risk estimation. Environmental investigations, such as the investigation in Florida discussed in the present report, are useful for building an evidence base that may assist scientists to better understand the environmental dynamics of *N. fowleri*, factors influencing the ameba's presence and growth, and its geographical distributions in new environments.

Additional research such as the present study are needed to develop a more extensive evidence base that can help scientists better understand the relationship between environmental factors and

the presence and concentration of *N. fowleri* in lake environments. Cases of primary amebic meningoencephalitis (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 that also have *Naegleria* present. We hope to continue to conduct ecological studies to investigate various environmental factors that may be associated with the distribution of PAM case exposures in the United States. Work is planned to perform statistical analysis of the water, sediment and climatological data collected for this study, with the intent to publish findings from the analysis. CDC will engage with Florida DOH public health officials on the analyses and plans to report the results in a peer-reviewed journal manuscript.

REFERENCES

Mull BJ, Jothikumar N and Hill VR. (2013) Improved method for the detection and quantification of *Naegleria fowleri* in water and sediment using immunomagnetic separation and real-time PCR. *J Parasitol Res*, Article ID 608367, doi: 10.1155/2013/608367.

Qvarnstrom Y, Visvesvara GS, Sriram R, and da Dilva AJ. (2006) Multiplex real-time PCR assay for simultaneous detection of *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Naegleria fowleri*. *J Clin Microbiol*, 44(10):3589-3595.