Studies to Identify *Naegleria fowleri* Amebae, Causative Agent of Primary Amebic Meningoencephalitis, in Lake Anna.

A Final Report Submitted to The Lake Anna Civic Association.

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I. Executive Summary

Naegleria fowleri* is the causative agent of a rapidly fatal disease of the brain that occurs in previously healthy children and young adults with a history of swimming and diving in freshwater lakes and ponds. In the summer of 2007, 6 cases of fatal amebic encephalitis were reported in the US. This news, along with a letter from Dr. Robert Stroube the State Health Commissioner concerning the possible health risk of *Naegleria* in Lake Anna, prompted the Lake Anna Civic Association (LACA) to commission a study of Lake Anna to determine the presence of *Naegleria fowleri* in Lake Anna. LACA provided $10,000 to Dr. Francine M. Cabral at Virginia Commonwealth University to obtain water samples from Lake Anna to analyze the samples for the presence or absence of *Naegleria fowleri.

What was done:

Water was collected from Lake Anna on three different days during the summer of 2007. A total of 16 locations (9 on the cold side and 7 on the warm side) were sampled during June and September of 2007. **See Figure 1 for Identification of Sites Tested**. Not all locations were sampled on each of 3 sampling visits of 6/29, 9/2, and 9/20.The water was taken to the laboratory the same day as sampling and cultured to determine whether amebae were present. Cultures were examined daily with a light microscope to identify amebae. The water was tested for *N. fowleri* using a Polymerase Chain Reaction (PCR) Assay which is a sensitive and specific assay to detect *Naegleria fowleri* DNA in water. Water temperature, pH, and dissolved oxygen content also were determined. Studies of Lake Anna during the summer of 2007 demonstrated that *Naegleria fowleri* amebae are present in Lake Anna. Eight samples were collected from 4 sites on the warm side of the Lake and 4 sites on the cool side of the Lake June 29, 2007. Of 8 samples collected, 6 samples were positive for *N. fowleri.* At that time, the water temperature at the positive sites ranged from 85º F to 95º F. These temperatures allow for the proliferation of pathogenic amebae and are of concern in terms of human exposure. When water temperatures reach 85º F and higher there is cause for concern as large numbers of amebae may be the source of infection. On September 2, 16 sites on Lake Anna were sampled. Of 16 samples tested for the presence of *N. fowleri,* 7 were positive. At that time, water temperatures ranged from 80º to 96º F with the majority of sites recorded at 80º to 88º F. Sites that recorded temperatures of 81º, 83º, 84ºF were negative for *N. fowleri*. Sites that were positive for *N. fowleri* generally recorded temperatures of 95º to 96º F, indicating that amebae proliferate at high temperatures. For example, Site Nf2 (Aspen Hill) and Nf3 (Pt Dike 1 Canal) which were recorded at 95º and 96º F respectively, were positive for *N. fowleri* by PCR. On September 20, 2007, water and sediment samples were collected since the water level had fallen due to a lack of rain. Five sites were sampled and water and sediment were collected from the five sites. Four of the five samples tested were positive for *N. fowleri* amebae. Samples taken at sites with temperatures recorded at 90º and 92º F were positive for *Naegleria fowleri*. This study indicates that increased temperatures at sites on the lake are associated with the presence of *Naegleria fowleri*. A total of 9 out of 16 sites tested were positive for *N.fowleri* amebae during the summer. The number of amebae present in a site was determined by counting with a hemacytometer. This count will include all amebae present, not all of which are *Naegleria*. Other water and soil amebae will be present in the sample which is why it is important to have a specific test for *Naegleria fowleri*. A DNA test is performed to identify *N. fowleri.* All tests were performed at least two times to verify the results.

Why was this study done?

This study was performed to determine whether *N. fowleri* amebae were present in Lake Anna and to determine whether there was a health risk to humans associated with the presence of the amebae. Dr. Robert Stroube, the State Health Commissioner indicated in a letter to Mr.Robert Burnley, Director of the Department of Environmental Quality in Richmond, Virginia that *Naegleria fowleri* amebae have been isolated from bodies of water that were thermally enriched by power plant effluents in several states including Virginia. He also indicated, that "the organism begins to proliferate at temperatures around 86º F and thrives especially well at temperatures of 95º F to113º F where it can reach concentrations in water and sediments of 10 to 100 organisms per liter or gram." Our studies at Lake Anna support this statement as the majority of sites that were positive for *N. fowleri* recorded temperatures of 86º F and higher. There are no Virginia or U.S. Public Health standards for identifying the risk of Primary Amebic Meningoencephalitis. Sites where *N. fowleri* are present should be monitored beginning early in the summer and continue during the summer months, to determine whether increased water temperatures or

other parameters account for an increase in the number of amebae, in order to prevent primary amebic meningoencephalitis. The presence of *N. fowleri* amebae in water is a potential risk to humans who swim and dive in the water. Reports of 6 deaths in the US in the summer of 2007 indicate that there is an increase in the number of cases of Primary Amebic Meningoencephalitis. This number may be an **under estimate** of this disease as it is not always recognized and autopsies are not always performed to verify the cause of death.

Who Participated in this study and Collection Dates: Samples from 8 sites in Lake Anna were collected June 29, 2007 by Kenneth Remmers, Harry Ruth and Francine Marciano-Cabral. On September 2, 2007 a second sampling was performed at Lake Anna. Kenneth Remmers and Francine M-Cabral collected surface water samples from 16 different sites at Lake Anna. September 20, 2007, Kenneth Remmers, Harry Ruth, and Francine M-Cabral collected water and sediment samples at 5 different locations at Lake Anna. Melissa Jamerson, a doctoral student in the laboratory, performed the PCR assays. **Please refer to Page 6, Table 1, for a summary of the results of these studies performed on surface samples at a depth of 0.3m at specific sites on Lake Anna and to Page 12 for Tables 2 and 3 with temperature and PCR results from the individual days. These Tables contain the separate results of our 3 studies indicating the temperature, the site, and PCR results (+ or -) for the presence of *N. fowleri.***

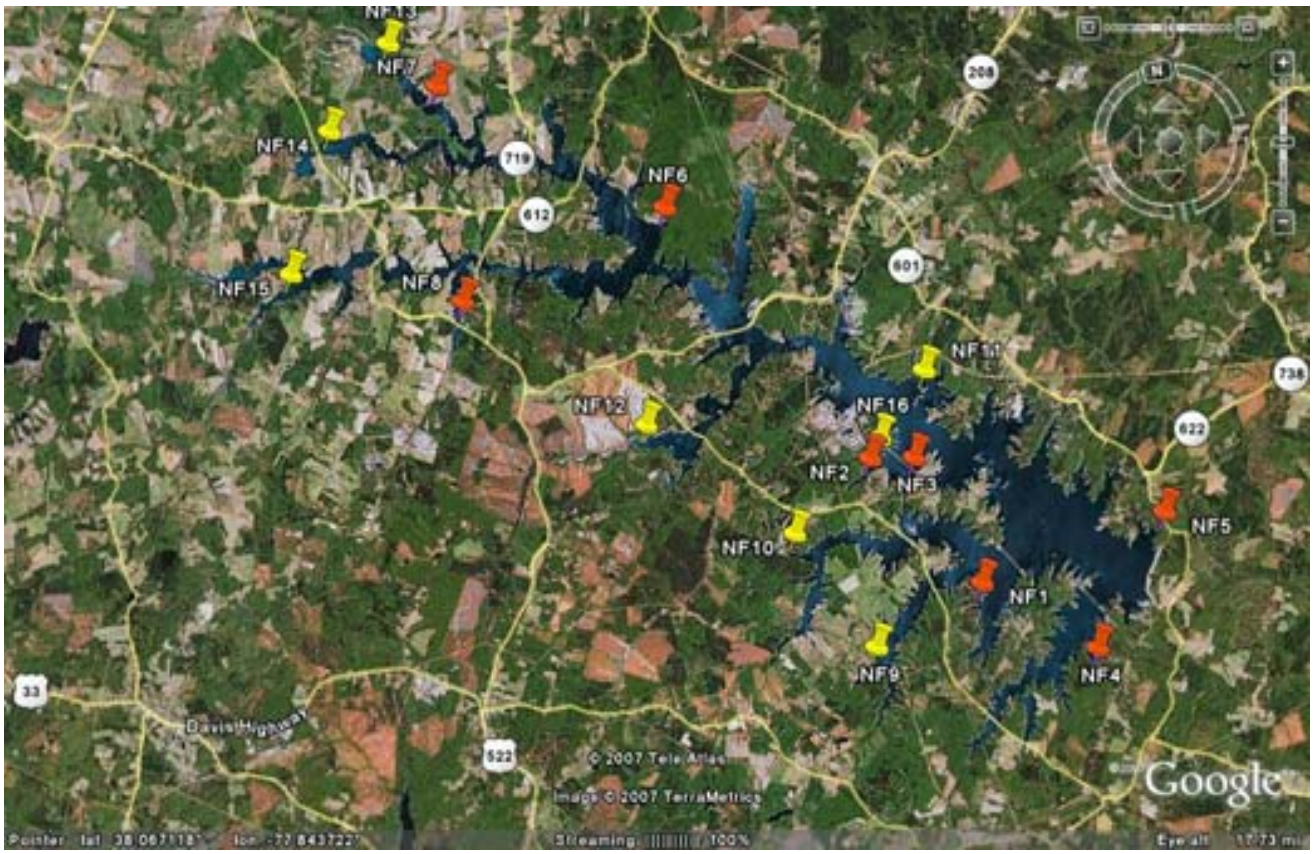
VCU Related Research About NF Amebae. *N. fowleri* have been identified from a variety of habitats including soil, freshwater lakes, ponds, thermal springs, air, and humidifier systems (Marciano-Cabral, 1988). Although *N. fowleri* is generally isolated from water, soil is the preferred habitat and contamination of water occurs via runoff from soil after rain (Singh, 1975). Environmental studies suggest that man-made factors as well as climatic changes contribute to the propagation of *Naegleria fowleri*. ***N. fowleri* is thermophilic and thrives and proliferates at high temperatures.** Variable physical parameters such as temperature and pH are equally tolerated over a wide range with growth reported at water temperatures of 80°F to 111°F and a pH range of 4.0 to 9.5. **Thermal enrichment of water can cause proliferation of amebae especially at temperatures of 86°F to 111°F.** Apparently, localized 'hot spots'of water serve as a propagation source. Furthermore, the propagation of *Naegleria* may be enhanced in these "hot spots" since high water temperatures eliminate non-thermophilic competitors and favor the growth of *N. fowleri*. *Naegleria* also grow vigorously in the presence of Enterobacteriaceae and related bacteria (Kyle and Noblet, 1985; Singh and Dutta, 1984). Thermal pollution from industrial plants and cooling towers facilitates the growth of thermophilic *Naegleria* and of bacteria that serve as a food source for the amebae (Tyndall et al., 1989). In studies of fresh water lakes associated with power plants, *N. fowleri* was routinely isolated. The heated water is a breeding ground for pathogenic amebae (Kasprazak et al 1982). In addition, it has been reported that iron in the environment has a positive effect on the growth of *N. fowleri*, while copper inhibits its growth. Consistent with these observations, concentrations of *N. fowleri* have been shown to be almost 60 times higher in cooling systems that are equipped with stainless steel condensers as compared to those equipped with brass condensers due to the release of copper from brass into the water.

U.S. CENTER FOR DISEASE CONTROL RESEARCH INDICATES: Infection with *N. fowleri* occurs when the ameba enters the body through the nose. Generally this occurs when people are participating in water-related activities such as swimming, diving, or other water sports that result in water going up the nose. Amebae travel to the brain where they destroy brain tissue.

a. **What are the signs and symptoms of *Naegleria* infection?** Infection with *N.fowleri* causes the disease Primary Amebic Meningoencephalitis (PAM), a brain inflammation, which leads to destruction of brain tissue. Initial signs and symptoms of PAM start 1 to 7 days after infection. These symptoms include headache, fever, nausea, vomiting, loss of smell, and stiff neck. As the ameba cause more extensive destruction of brain tissue this leads to confusion, loss of balance, seizures and hallucinations. After onset of symptoms, the disease progresses rapidly and usually results in death within 3 to 14 days.
b. **Is there treatment for infection with NF?** Several drugs are effective against *N.fowleri* in the laboratory. Although a variety of treatments have been used to treat infected persons, their effectiveness is unclear since most infections have been fatal.

<u>* Recommendations to reduce the risk of infection:</u> Since it has been shown that *N. fowleri* is present in Lake Anna, the public should be warned to wear nose plugs while diving, swimming or engaging in water activities in which the head is submerged when temperatures of Lake Anna reach 84F and higher. Additionally, signs should be posted to warn the public of a possible human health risk due to *Naegleria fowleri* present in the water. If possible, the water should be tested early in the summer, during the summer, and late in the summer to determine whether there is an increase in the number of amebae that occurs with increased water temperatures. Lake Anna Civic Association studies indicate that Lake Anna is unique in that 99% of the water between the power plant and the dam is recirculated by the North Anna Power cooling pumps. During the summer months water temperatures are in excess of 100 degrees F at some locations. Amebae that are present in one location today may be at another location tomorrow since the water is recirculated.**

(Figure 1): A map of sites that were sampled during the summer of 2007.



<u>Key:</u>

<u>NF 1 Ruth's Dock</u>
<u>NF 2 Aspen Hill</u>
<u>NF 3 Pt. Dike 1 Canal</u>
<u>NF 4 Noahs Landing</u>
<u>NF 5 Remmers Dock</u>
<u>NF 6 State Park</u>
<u>NF 7 Days Bridge</u>
<u>NF 8 Camp Ground</u>
<u>NF 9 Overtons Fork</u>
<u>NF 10 Waters CA</u>
<u>NF 11 Islands Across PP</u>
<u>NF 12 Contrary Creek</u>
<u>NF 13 Upper Terry's Run</u>
<u>NF 14 Upper Pumunkey</u>
<u>NF 15 Goldmine Creek</u>
<u>NF 16 Power Pt Discharge</u>

Table 1: Lake Anna *Naegleria fowleri* Study 2007

Site (Warm or Cold)	Date	Temp(F)	Spec cond.	DO	pH	PCR	# Amebae[A] including non Nf
Ruth's Dock (Nf1)	6/29/07	90.90	0.063	6.39	6.70	_	1/50ml
(Warm Side)	9/2/07	91.18	0.067	6.49	6.90	_	1/50ml
	9/20/07	83.3	0.066	5.85	7.39	_	no amebae
Aspen Hill (Nf2)	6/29/07	95.86	0.064	6.02	6.87	+	5/50ml
(Warm Side)	9/2/07	95.81	0.067	6.20	7.11	+	10/50ml
	9/20/07	90.2	0.067	5.90	7.21	+	5/50ml
Pt.Dike 1 Canal (Nf3)	6/29/07	95.67	0.063	6.28	6.85	+	5/50ml
(Warm Side)	9/2/07	96.62	0.067	6.15	7.12	+	
	9/20/07	90.73	0.67	6.33	7.10	+	
Noah's Landing (Nf4)	6/29/07	88.47	0.063	6.57	7.01	+	5/50ml
(Warm Side)	9/2/07	88.39	0.067	6.55	7.46	+	
Remmers Dock (Nf5)	6/29/07	85.28	0.064	7.05	7.15	+	10/50ml
(Cold Side)	9/2/07	86.29	0.067	6.25	7.20	+	12/50
State Park (Nf6)	6/29/07	84.13	0.062	7.80	7.55	_	No amebae
(Cold Side)	9/2/07	85.48	0.065	7.72	7.72	_	No amebae
Days Bridge (Nf7)	6/29/07	84.49	0.071	8.91	8.82	+	10/50ml
(Cold Side)	9/2/07	82.00	0.066	7.43	7.82	+	
Chr Run (Nf8)	6/29/07	85.39	0.070	8.86	8.77	+	10/50ml
Camp Ground	9/2/07	84.38	0.067	8.59	8.94	_	no amebae
(Cold Side)							
Overton's Fork (Nf9)	9/2/07	87.26	0.067	6.67	7.41	+	10/50
(Warm Side)	9/20/07	80.94	0.66	6.72	7.50	+	12/50ml
Waters CA (Nf10)	9/2/07	86.90	0.067	6.90	7.55	_	1/50
(Warm Side)							
Islands Across PP(Nf11)	9/2/07	84.78	0.067	6.81	7.15	+	10/50ml
(Cold Side)							
Contrary Creek (Nf12)	9/2/07	84.15	0.072	7.05	7.10	_	no amebae
(Cold Side)							
Upper Terry's Run (Nf13)	9/2/07	82.47	0.068	8.77	8.73	_	amebae not Nf
(Cold Side)							
Upper Pumunkey (Nf14)	9/2/07	83.62	0.089	11.65	9.48	_	2/50ml
(Cold Side)							
Goldmine Creek (Nf15)	9/2/07	84.20	0.071	9.50	8.87	_	no amebae
(Cold Side)							
Power Pit Disch (Nf16)	9/2/07	97.52	0.066	6.04	7.05	_	3/50ml
(Warm Side)	9/20/07	91.74	0.067	5.94	7.06	+	10+/50ml

[A]All measurements were made at 0.3m except for NF1 on June 29 which was taken at 0.1m.

[B]Samples were examined for the presence of amebae by light microscopy and counted using a hemacytometer. #Amebae = number of amebae in 50 ml sample (not all *Naegleria fowleri*).

II. Introduction

Amebae belonging to the genus *Naegleria* are found in soil and freshwater habitats throughout the world. One species, *Naegleria fowleri*, has been associated with human disease (DeJonkheere,2004). *N. fowleri* can cause Primary Amebic Meningoencephalitis (PAM) a rapidly fatal disease of the central nervous system (CNS) that occurs generally in previously healthy children and young adults with a history of exposure to contaminated recreational, domestic, or environmental water sources (Martinez, 1985). Cases of PAM have been reported from several states in the US, including Virginia, Texas, Florida, North Carolina, Georgia, and Arizona (Fiordalisi et al., 1992). The majority of cases have occurred in individuals who had been swimming and diving in freshwater lakes and ponds. Infection occurs when amebae enter the nasal passages of an individual while swimming or diving and make their way to the brain **(Figure 2).** Death can occur in 7 to 10 days post infection. Although PAM is a rare disease, there has been an increase in the number of cases worldwide since 1990, including the United States (Taylor,1996; Okuda et al., 2004; DeNapoli et al.,1996; Taylor et al 1996; Gyori, 2002; Schuster and Visvesvara, 2004; Craun et al., 2005; Marciano-Cabral et al., 2003). In the summer of 2007, six fatal cases of PAM were reported in the US in Florida, Texas, and Arizona. The disease may be more common as not all cases are recognized or reported and autopsies are not always performed to confirm the cause of death. Thus, there is a need to monitor fresh water lakes, ponds, and recreational sites where previously healthy individuals swim, dive, water ski and take part in water activities. The major objective of this study was to determine the presence or absence of *Naegleri*a *fowleri* amebae in Lake Anna during the summer of 2007.

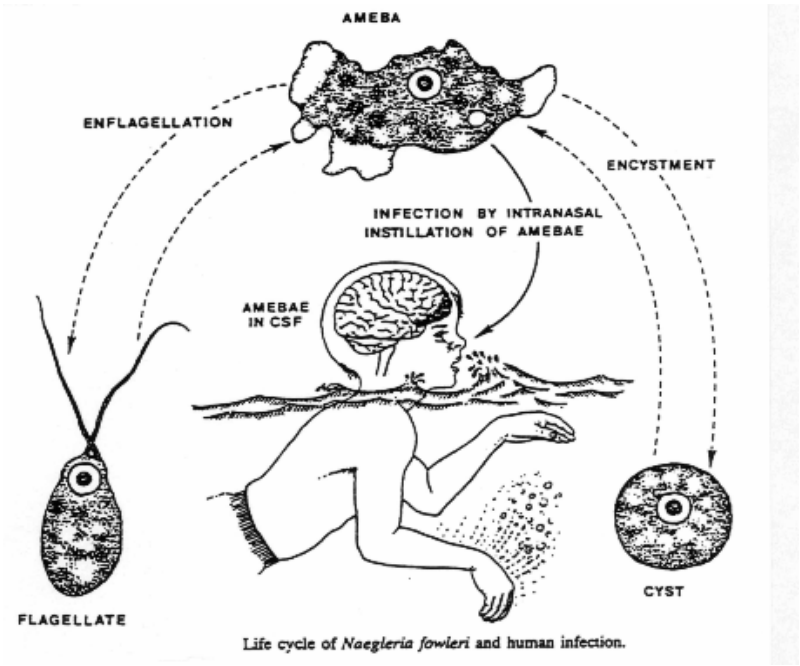


Figure 2 illustrates the three morphological forms of *N. fowleri* that are present in freshwater. The amebae enter the nasal passages and make their way to the brain resulting in Primary Amebic Meningoencephalitis, a rapidly fatal disease of the brain.

III. Methods of Detection

Since *N. fowleri* is considered primarily a waterborne pathogen (Marshall et al. 1997, Schuster and Visvesvara, 2004a), a variety of techniques and rapid methods have been developed for its detection and isolation from aqueous environments. Samples (50 ml) in a sterile centrifuge tube can be obtained from surface water since amebae are found in greater abundance at that location as the flagellates swim to the surface, transform into amebae and feed on bacteria (Preston and King, 2003). Sediment also can serve as a source of amebae and cysts. After collection, samples should be processed as quickly as possible to avoid overgrowth by fungi. Water samples can be concentrated by centrifugation (Pernin et al., 1998). The resultant pellet can be transferred onto 1.5% non-nutrient agar (NNA) that has been seeded with a

lawn of *Escherichia coli* bacteria (ameba food source) and maintained at 108°F or 98° F for 48h to obtain thermotolerant amebae. Molecular techniques have been developed that allow for a more rapid, sensitive, and specific laboratory identification of *N. fowleri*. Polymerase chain reaction (PCR) assays have been developed to identify *N. fowleri* isolated from environmental sources (Behets et al 2003; Reveiller et al. 2002 ; Pelandakis and Pernin, 2002). The PCR assays are based on the DNA of the ameba. A specific DNA or gene sequence is used to amplify the DNA (genetic material) of the ameba that may be present in the water. Our laboratory designed a PCR assay based on a cDNA clone designated Mp2Cl5 derived from *N. fowleri* (Reveiller et al., 2002). This assay was designed with two distinctive sets of primers (DNA sequences) to allow for discrimination of *N. fowleri* from other species of amebae that may be present in environmental samples. PCR amplification can be performed directly on a water sample without prior genomic DNA extraction. The PCR assay can identify as few as 5 amebae from a volume of 50 ml of water. We have used this assay to detect *N. fowleri* in domestic water and in environmental water samples (Marciano-Cabral et al 2003; MacLean et al. 2004).

IV. Lab Analysis

The objective of this study was to determine whether *Naegleria fowleri* were present in Lake Anna. The first **s**amples from Lake Anna were collected **June 29, 2007** by Kenneth Remmers, Harry Ruth, and Francine Marciano-Cabral. Water samples were collected by placing a 50 ml sterile centrifuge tube in the water at the surface. More than one sample was obtained from each area to ensure that a representative sample was obtained. At the time of collection, measurements of temperature, pH, dissolved oxygen and specific conductance of the water were taken and recorded. The samples were numbered Nf1 through Nf8 since collection was at 8 different sites on Lake Anna. Samples were taken back to the laboratory and processed that afternoon as fungi, bacteria or other amebae present in the water may overgrow *Naegleria fowleri* amebae. Prior to collecting samples, Petri plates containing **nonnutrient agar (NNA)** coated with *E. coli* bacteria were prepared. *E. coli* serves as a food source for the amebae. Water samples placed on the agar plates were tested for growth of amebae. Amebae growing on the plates can be visualized by light microscopy.

Water samples were centrifuged upon returning to the laboratory to obtain a pellet which was used for growth studies, counting amebae, and for a polymerase chain reaction assay (PCR). A portion of the pellet was used to plate onto NNA plates coated with *E. coli.* NNA-plates were placed at 107° F and at 98° F to allow amebae to grow. Amebae that grow at 107º F are thermotolerant (heat loving) and may be *N. fowleri*. Amebae that were present grew on the NNA plates and were observed by light microsocopy. The amebae were scraped from the agar plates and tested by a PCR assay (a DNA test specific for *N. fowleri*). Samples were cultured (allowed to grow) after the initial assay and the samples were retested. All samples were tested more than one time. Duplicate samples of water were placed in culture flasks to grow amebae for observation by light microscopy. One tube of water (50ml) from each site was centrifuged and amebae or cysts were counted using a hemacytometer and light microscope.

On **September 2, 2007** a second sampling was performed at Lake Anna. Kenneth Remmers and Francine Marciano-Cabral collected surface water samples from 16 different sites at Lake Anna. The samples were brought back to the laboratory and processed the following morning. Water was centrifuged and pellets were used for PCR and for counting the number of amebae or cysts present.

On **September 20, 2007**, Kenneth Remmers, Harry Ruth, and Francine Marciano-Cabral collected water and sediment samples at 5 different locations at Lake Anna. The same procedures were followed as on June 29 and September 2.

V. Results

On June 29, 2007, 5 NNA plates were used for each sample. The plates (labeled Nf 1 to Nf 8) were incubated at 107° F and 98° F for 1 week and observed daily by light microscopy. Amebae and/or cysts were observed on all plates after 1 week of culture. The plates were scraped and used for PCR to determine whether *Naegleria fowleri* were present in the water. After the PCR assay is performed a gel is run to visualize the PCR product (amplification of ameba DNA). The gel should have one band of a predicted size. A positive control consists of *N. fowleri* amebae and a negative control consists of all reagents in the absence of *N.fowleri*. These controls are used to determine whether the assay is working

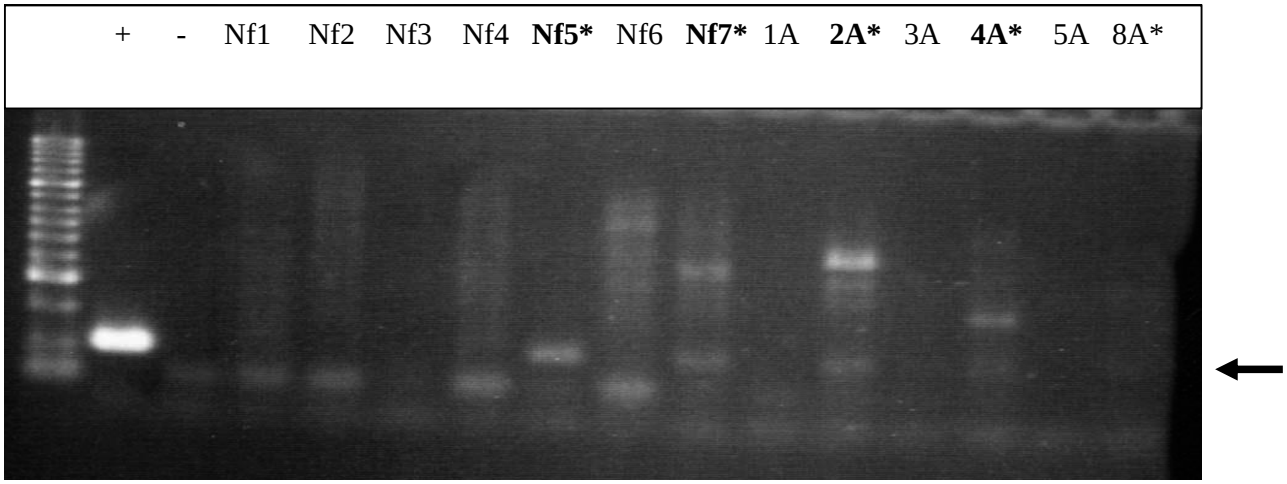
and whether there is contamination in the negative samples. After 1 week of culture, samples were positive for *Naegleria fowleri* by PCR **(Figures 3, 4).**

Figure 3. Agarose gel depicting the results of a PCR assay to determine the positive and negative samples from Lake Anna. (+ Positive Control, - Negative Control, Nf1, Nf2, Nf3, Nf4, Nf5, Nf6, Nf7). Samples labeled 1A, 2A, 3A, 4A, 5A and 8A are duplicate samples i.e., Sample 2 is one collection tube and 2A is a second collection sample from the same site. Four different gels are shown from 4 different PCR assays performed for the samples collected on June 29, 2007 to confirm PCR results. An arrow (←) indicates the PCR product for positive samples, * indicates positive sample.

Samples NF5, Nf7, Nf2A are positive for *N. fowleri*. Sample 4A and 8A are weakly positive.

Weakly positive samples may indicate that there are fewer amebae in the sample or that inhibitors are present in the sample. Environmental samples contain many inhibitors of PCR amplification. Higher bands on the gel indicate that other DNA is being amplified. The DNA in the higher bands was sequenced to determine what was being amplified in addition to *N. fowleri*. Several bacterial DNA sequences (*Pseudomonas, Haemophilus and Anaeromyxobacter*) were obtained for the higher bands.

Figure 3. Agarose gel of the PCR products to demonstrate the presence of *N.fowleri.*



Additional Samples.

NF5 +

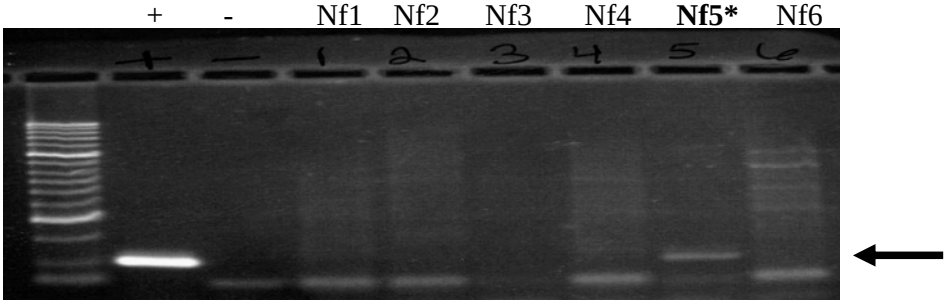
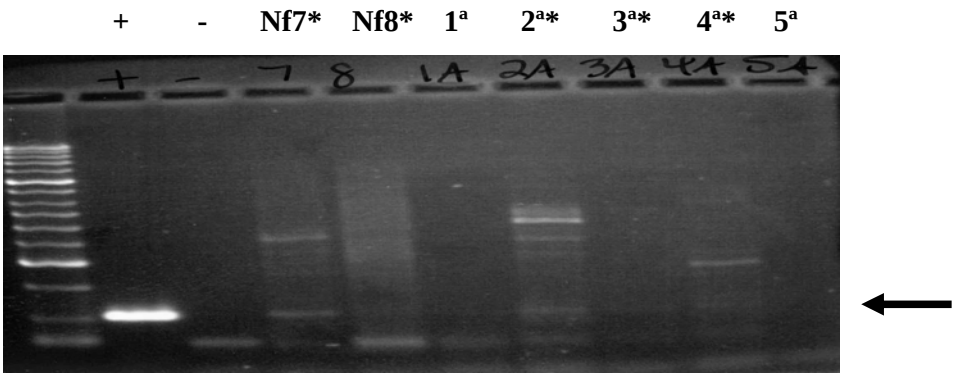


Figure 4. Agarose gel of the PCR products of additional samples.

Samples that have light bands are considered weakly positive suggesting that fewer amebae may be present at that collection site or that PCR inhibitors are present in the water sample.

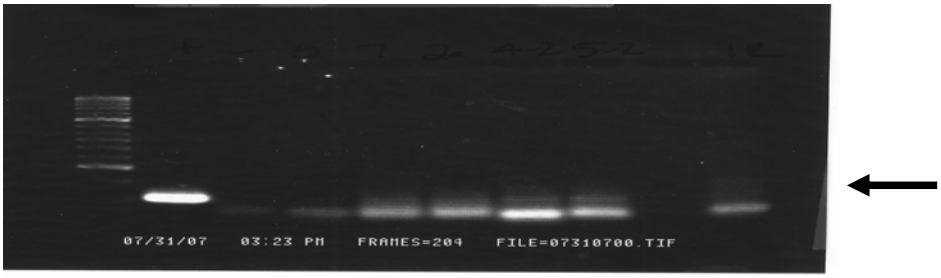
Fig. 5A & B. Agarose gels depicting the results of the PCR assay to determine the presence of *N.fowleri* (June 29,2008).

A.



Nf7+ Nf8+ Nf2A+ Nf3A+ Nf4A+

B.



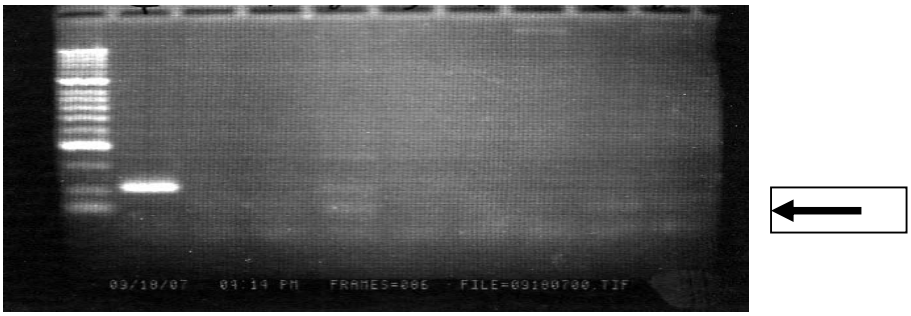
Additional Samples from June 29, 2007

PCR Positive samples Nf2, Nf3, Nf4, Nf5

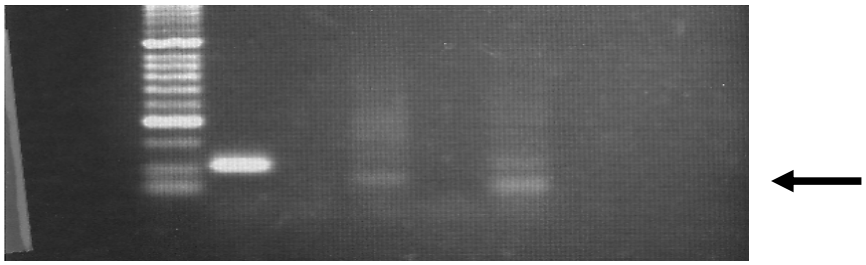
On **June 29, 2007,** surface water samples from Lake Anna were tested by PCR for *Naegleria fowleri* amebae. Of 8 samples collected, **6 samples were PCR positive** for *Naegleria fowleri* including: **Nf2,** Nf3, Nf4, Nf5, Nf7 and Nf8.

On **Sept 2, 2007**, a second sampling was performed. Sixteen surface water samples (Nf1-Nf16) were collected from Lake Anna. The samples were cultured on NNA-*E. coli* plates and PCR was performed on each sample to determine whether *Naegleria fowleri* was present. The samples were examined by light and electron microscopy to visualize what organisms were present. PCR was performed one week after collection. (Nf1-8) were run on this gel. * Indicates positive samples. Nf 2, 3, 4, 5, 7, 9 and 11 were positive for *N. fowleri* by PCR. Arrow indicates area of positive PCR band on gel. **Seven of sixteen samples were positive for *N. fowleri* by PCR (Figures 6, 7, 8).**

+ - Nf1 Nf2* Nf3* Nf4* Nf5*Nf6 Nf8 **Fig. 6.**



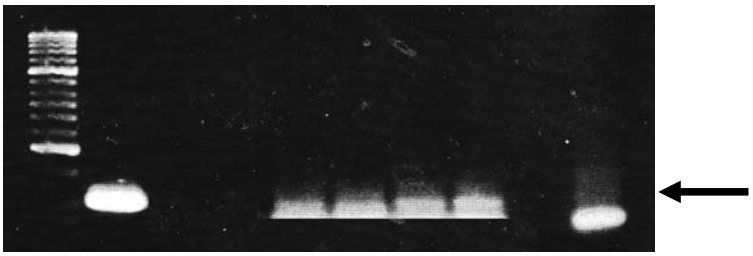
+ - Nf9* Nf10 Nf11* Nf12 Nf13 **Fig. 7.**



Samples **Nf9** and **Nf11** were positive for *N. fowleri* by PCR. All other samples collected at that time were negative(Nf1, Nf6, Nf8, Nf10, Nf12, Nf13, Nf14, Nf15, Nf16).

Samples were cultured continuously for 1 month and repeat PCR assays were performed. At that time Nf 2, Nf4, Nf5, Nf7, and Nf3 were positive for *N.fowleri* by PCR.

+ - Nf1 Nf2* Nf4* Nf5*Nf7* Nf6 Nf3 **Fig. 8**



September 20, 2007. On Sept. 20, both surface water and sediment samples were collected. The purpose of this collection was to determine whether the amebae had encysted and settled to the bottom of the lake rather than swimming to the surface. Five surface water and sediment samples were collected from the same sites. Sediment samples consisted of 1 to 3 inches deep of sediment at the water/sediment interface. Both water and sediment samples were plated on NNA-*E.coli* plates for culture and for daily observation of the cultures by light microscopy. PCR was performed. **(Fig. 9).**

- + Nf1 1A **Nf2* 2A* Nf3* 3A**** + **-** **Nf9* Nf16***

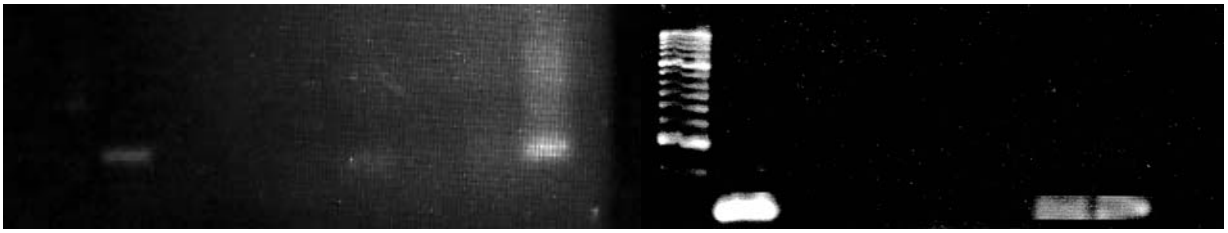


Figure 9. Samples Nf2 (water), 2A(sediment), Nf3(water), Nf3A (sediment), Nf9(water) and Nf16 (water) were positive for *N.fowleri*. Nf1 (water) and 1A (sediment) were negative for *N.fowleri* by PCR.

Table 2. A Summary of PCR Results and Temperature at the Time of Collection at Lake Anna.

Sample	Date 6/29		9/2		
	PCR[a]	Temperature(°F)	PCR	Temperature (°F)	W/C
Nf1 Ruth's Dock	-	90	-	91	Warm
Nf2 Aspen Hill	+	**95**	+	**95**	Warm
Nf3 Dike 1Canal	+	**95**	+	**96**	Warm
Nf4 Noah's Landing	+	**88**	+/-	**88**	Warm
Nf5 Remmers Dock	+	**85**	+	**86**	Cold
Nf6 State Park	-	83	-	84	Cold
Nf7 Days Bridge	+	**84**	+	81	Cold
Nf8 Camp Ground	+/-	**84**	-	83	Cold
Nf9 Overton's Fork			+	**87**	Warm
Nf10 Waters CA			-	87	Warm
Nf11 Islands Across PP			+	**84**	Cold
Nf12 Contrary Creek			-	82	Cold
Nf13 Upper Terry's Run			-	80	Cold
Nf14 Upper Pumunkey			-	83	Cold
Nf15 Goldmine Creek			-	83	Cold
Nf16 Power P Discharge			-	97	Warm

[a] Water samples collected on 6/29 and 9/2, 2007 were surface samples. No sediment samples were collected. + indicates positive PCR for *N. fowleri*, – negative for *N. fowleri*. +/- result not definite.

Table 3. A Summary of PCR Results and Temperature at the Time of Collection at Lake Anna

9/20/2007	Sample	PCR[a]	Temperature(°F)	Warm/Cold Side
Ruth's dock	Nf1	-	83	Warm
	Nf 1A	-		
Aspen Hill	**Nf2**	+	**90**	Warm
	Nf 2A	+		
Pt. Dike 1 Canal	**Nf3**	+	**90**	Warm
	Nf3A	+		
Overton's Fork	**Nf9**	+	**80**	Warm
Power P Discharge	**Nf16**	+	**92**	Warm

Samples numbered 1, 2, 3, 9, and 16 were taken from the water surface. Samples numbered 1A, 2A, 3A were sediment samples. Water and sediment samples taken from the same sites were positive for *N. fowleri* by PCR.

In Conclusion

***N. fowleri* is the causative agent of a rapidly fatal disease of the brain that occurs in previously healthy children and young adults with a history of swimming and diving in thermally-enriched freshwater lakes and ponds. In the summer of 2007, 6 cases of fatal amebic encephalitis were reported in the US but more cases may have occurred but were unrecognized or unreported. Samples were collected three times at different sites on the Lake by Ken Remmers, Harry Ruth, and Francine Marciano-Cabral. In June 2007, of eight samples collected from various sites on the Lake, 6 samples were positive for *N. fowleri* by a PCR assay which is a sensitive and specific assay to detect *Naegleria fowleri* DNA in water. On September 2, of 16 sites on Lake Anna sampled, 7**

were positive. On September 20, 2007, of five water and sediment samples collected, four samples tested were positive for *N. fowleri* amebae. Lake Anna Civic Association studies indicate that Lake Anna is unique in that 99% of the water between the power plant and the dam is recirculated by the North Anna power cooling pumps. During the summer months water temperatures are in excess of 100 degrees F at some locations. Thus, recirculation of the water could account for sites being positive on one sampling date and negative at another sampling date. This study indicates that increased temperatures at sites on the lake are associated with the presence of *Naegleria fowleri*. These sites should be monitored during the summer months when there is increased water activities to determine the abundance of amebae, in order to prevent primary amebic meningoencephalitis. There is a large body of literature that demonstrates that as water temperatures rise, the amebae proliferate. This increased proliferation is consistent with a possible increased risk of human infection. Education is important to inform the public that swimming, diving or other water sports should be undertaken with care at times when the water temperature is above 86F. Nose plugs should be used at times when the water temperature is 86F and higher.

In summary, 9 of 16 Lake Anna sites tested positive for *Naegleria fowleri* amebae. Identifying the risk of contracting Primary Amebic Meningoencephalitis infection when *N. fowleri* amebae are present in the water is a very complex issue and there are no U.S. Standards. When concentrations of amebae are high there is a greater chance of becoming infected, but we do not know what all of the risk factors are and what the actual risk of infection is.

Table 4 : Data from Lake Anna Studies.

Site ID	Location	Lattitude	Longitude	Date	Time	Depth(m)	Temp(C)	Temp(F)	Spec cond	Diss Oxg	pH
NF1	Ruth's Dock	38.01420	77.7628	6/29/2007	0921am	0.1	32.72	90.90	0.063	6.39	6.70
NF1	Ruth's Dock	38.01420	77.7628	9/2/2007	0932am	0.3	32.88	91.18	0.067	6.49	6.90
NF1	Ruth's Dock	38.01420	77.7628	9/2/2007	0932am	0.5	32.91	91.24	0.067	6.55	6.96
NF2	Aspen Hill CA	38.04670	77.7878	6/29/2007	1005am	0.3	35.48	95.86	0.064	6.02	6.87
NF2	Aspen Hill CA	38.04670	77.7878	6/29/2007	1005am	1	35.48	95.86	0.063	6.16	6.85
NF2	Aspen Hill CA	38.04670	77.7878	6/29/2007	1005am	1.5	35.45	95.81	0.063	6.25	6.82
NF2	Aspen Hill CA	38.04670	77.7878	9/2/2007	0955am	0.3	35.45	95.81	0.067	6.20	7.11
NF2	Aspen Hill CA	38.04670	77.7878	9/2/2007	0955am	0.5	35.51	95.92	0.067	6.13	7.12
NF2	Aspen Hill CA	38.04670	77.7878	9/2/2007	0955am	1	35.56	96.01	0.067	6.17	7.11
NF3	Pt Dike1 Canal	38.04500	77.7753	6/29/2007	1015am	0.3	35.37	95.67	0.063	6.28	6.85
NF3	Pt Dike1 Canal	38.04500	77.7753	6/29/2007	1015am	1	35.37	95.67	0.063	6.25	6.85
NF3	Pt Dike1 Canal	38.04500	77.7753	9/2/2007	1005am	0.3	35.90	96.62	0.067	6.15	7.12
NF3	Pt Dike1 Canal	38.04500	77.7753	9/2/2007	1005am	1	35.97	96.75	0.067	6.08	7.12
NF3	Pt Dike1 Canal	38.04500	77.7753	9/2/2007	1005am	2	36.00	96.80	0.067	6.13	7.10
NF4	Noahs Landing	37.99470	77.7336	6/29/2007	1050am	0.3	31.37	88.47	0.063	6.57	7.01
NF4	Noahs Landing	37.99470	77.7336	6/29/2007	1050am	1	31.36	88.45	0.063	6.68	7.00
NF4	Noahs Landing	37.99470	77.7336	9/2/2007	1120am	0.3	31.33	88.39	0.067	6.55	7.46
NF4	Noahs Landing	37.99470	77.7336	9/2/2007	1120am	1	31.19	88.14	0.067	6.74	7.46
NF9	Overton Fork CA	38.00433	77.79611	9/2/2007	1050am	0.3	30.70	87.26	0.067	6.67	7.41
NF9	Overton Fork CA	38.00433	77.79611	9/2/2007	1050am	1	30.66	87.19	0.066	6.79	7.43
NF9	Overton Fork CA	38.00433	77.79611	9/2/2007	1050am	2	30.56	87.01	0.066	6.75	7.43
NF10	Waters CA	38.03310	77.81317	9/2/2007	1020am	0.3	30.50	86.90	0.067	6.90	7.55
NF10	Waters CA	38.03310	77.81317	9/2/2007	1020am	1	30.32	86.58	0.067	6.95	7.61
NF16	Power Plt Disch	38.05092	77.78393	9/2/2007	0950am	0.3	36.40	97.52	0.066	6.04	7.05
NF16	Power Plt Disch	38.05092	77.78393	9/2/2007	0950am	1	36.41	97.54	0.066	6.14	7.03
NF16	Power Plt Disch	38.05092	77.78393	9/2/2007	0950am	2	36.41	97.54	0.067	6.11	7.01
NF16	Power Plt Disch	38.05092	77.78393	9/2/2007	0950am	3	36.38	97.48	0.066	6.13	7.00
NF5	Remmers Dock	38.00756	77.73087	6/29/2007	1244pm	0.3	29.60	85.28	0.064	7.05	7.15
NF5	Remmers Dock	38.00756	77.73087	6/29/2007	1244pm	1	29.47	85.05	0.063	7.10	7.06
NF5	Remmers Dock	38.00756	77.73087	6/29/2007	1244pm	1.5	29.35	84.83	0.064	7.00	7.05
NF5	Remmers Dock	38.00756	77.73087	9/2/2007	1240pm	0.3	30.16	86.29	0.067	6.25	7.20
NF5	Remmers Dock	38.00756	77.73087	9/2/2007	1240pm	1	30.06	86.11	0.067	7.05	7.18
NF6	State Park	38.11133	77.83241	6/29/2007	1314pm	0.3	28.96	84.13	0.062	7.80	7.55
NF6	State Park	38.11133	77.83241	6/29/2007	1314pm	1	28.90	84.02	0.062	7.90	7.55
NF6	State Park	38.11133	77.83241	6/29/2007	1314pm	2	28.66	83.59	0.062	7.86	7.50
NF6	State Park	38.11133	77.83241	6/29/2007	1314pm	2.5	28.58	83.44	0.062	7.82	7.43
NF6	State Park	38.11133	77.83241	9/2/2007	1355pm	0.3	29.71	85.48	0.065	7.72	7.72
NF6	State Park	38.11133	77.83241	9/2/2007	1355pm	1	28.90	84.02	0.066	7.79	7.80
NF6	State Park	38.11133	77.83241	9/2/2007	1355pm	2	28.55	83.39	0.065	7.89	7.88
NF7	Days Bridge	38.14806	77.89047	6/29/2007	1347pm	0.3	29.16	84.49	0.071	8.91	8.82
NF7	Days Bridge	38.14806	77.89047	6/29/2007	1347pm	1	29.17	84.51	0.071	9.11	8.88
NF7	Days Bridge	38.14806	77.89047	6/29/2007	1347pm	2	28.64	83.55	0.069	9.10	8.80
NF7	Days Bridge	38.14806	77.89047	6/29/2007	1347pm	2.5	28.52	83.34	0.068	8.50	8.58
NF7	Days Bridge	38.14806	77.89047	9/2/2007	1445pm	0.3	27.78	82.00	0.066	7.43	7.82
NF7	Days Bridge	38.14806	77.89047	9/2/2007	1445pm	1	27.77	81.99	0.066	7.44	7.84
NF7	Days Bridge	38.14806	77.89047	9/2/2007	1445pm	2	27.54	81.57	0.066	7.35	7.80
NF7	Days Bridge	38.14806	77.89047	9/2/2007	1445pm	3	27.29	81.12	0.066	6.28	7.60
NF8	Chr Run Camp Gr	39.09890	77.8947	6/29/2007	1420pm	0.3	29.66	85.39	0.070	8.86	8.77
NF8	Chr Run Camp Gr	39.09890	77.8947	6/29/2007	1420pm	1	29.13	84.43	0.069	8.79	8.65
NF8	Chr Run Camp Gr	39.09890	77.8947	9/2/2007	1536pm	0.3	29.10	84.38	0.067	8.59	8.94
NF8	Chr Run Camp Gr	39.09890	77.8947	9/2/2007	1536pm	0.5	28.29	82.92	0.066	9.20	8.95
NF11	Islands across PP	38.06413	77.76772	9/2/2007	1300pm	0.3	29.32	84.78	0.067	6.81	7.15
NF11	Islands across PP	38.06413	77.76772	9/2/2007	1300pm	1	29.36	84.85	0.067	6.77	7.16
NF12	Contrary Creek	38.06363	77.84944	9/2/2007	1325pm	0.3	28.97	84.15	0.072	7.05	7.10
NF12	Contrary Creek	38.06363	77.84944	9/2/2007	1325pm	1	27.72	81.90	0.079	6.87	6.99
NF13	Upper Terrys Run	38.15964	77.90204	9/2/2007	1500pm	0.3	28.04	82.47	0.068	8.77	8.73
NF13	Upper Terrys Run	38.15964	77.90204	9/2/2007	1500pm	0.5	26.18	79.12	0.067	7.09	8.00

NF14	Upper Pamunkey	38.14249	77.92338	9/2/2007	1410pm	0.3	28.68	83.62	0.089	11.65	9.48
NF14	Upper Pamunkey	38.14249	77.92338	9/2/2007	1410pm	0.5	28.86	83.95	0.089	11.38	9.46
NF15	Goldmine Creek	38.11220	77.94198	9/2/2007	1602pm	0.3	29.00	84.20	0.071	9.50	8.87
NF15	Goldmine Creek	38.11220	77.94198	9/2/2007	1602pm	1	28.30	82.94	0.069	8.71	8.76

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