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Naegleria fowleri in Spirit Lake, Mount Saint Helens: Supporting Case For The Flagellate-Empty Habitat Hypothesis

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Abstract

Naegleria fowleri is an opportunist parasite that infects humans, and other mammals, causing a very rare, but almost always fatal brain infection, primary amebic meningoencephalitis. The criterion for initiating tests for *N. fowleri* in a water-body is currently based on temperature, leading to most testing occurring in the southern United States. The flaw in this criterion was shown in 1991, Detterline *et al*, discovered the presence of *N. fowleri* in Spirit Lake, Washington. This discovery is noteworthy due to the fact that *N. fowleri* was not thought to be commonly found in northern habitats, outside of man-made artificially heated waters. By exploring the physiological requirements and life-cycle of *N. fowleri*, I was able to indicate a probable blooming date in Spirit Lake and its possible effects on the biological community. The eruption of Mt. St. Helens was the key factor to inducing the proper conditions for the bloom. Using the known physiological requirements for temperature, water acidity, dissolved iron, dissolved O2, prey availability, and competition presence levels, the probable initiation of the bloom was dated at July 1981. The limiting factor for the bloom was in line with J. L. Griffin's (1983) flagellate-empty habitat hypothesis. This approach of viewing the presence or absence of competition as the limiting factor in the growth of *N. fowleri* populations, as opposed to the currently observed factor of temperature, could lead to more efficient detection rates in other possible *N. fowleri* habitats.

Keywords: *Naegleria fowleri,* flagellate-empty habitat hypothesis, Mt. St. Helens

1. Introduction:

In 1991, Detterline *et al.* [3], discovered the presence of the parasitic amoebae *Naegleria fowleri* Carter, 1970 in Spirit Lake, Kootenai County, Washington. *Naegleria* species are commonly found as harmless bacterivores inhabiting soils and fresh water bodies. When amoeba-laden water is introduced to the nasal passages of certain mammals, such as humans, *N. fowleri* follows the olfactory nerve into the brain where it feeds, causing primary amebic meningoencephalitis (PAM). *N. fowleri* infections, though rare, are almost always fatal in humans, due to the damage done by the amoeba and the immune response of the host [1]. Children are thought to be at highest risk because of their tendency to engage in activities in which water is more likely to enter the nasal passages [1]. The discovery of *N. fowleri* in Spirit Lake is noteworthy because public health organizations such as the Center for Disease Control and Prevention (CDC) do not test for the parasite in northern habitats, outside of artificially or geothermically heated waters [1]. The CDC's testing protocol are currently focused on the thermophilic hypothesis, where water temperature is the most important factor for predicting the presence or absence of the parasite [1]. The purpose of this paper is to examine the literature in an effort to determine if the history of Spirit Lake supports the thermophilic hypothesis employed by the CDC or the flagellate empty habitat hypothesis proposed by Griffin in 1983 [5]. Griffin's hypothesis states that the most important limiting factor for *N. fowleri* is a lack of amoeba-flagellate competition. The physical requirements and life-cycle of *N. fowleri* can also indicate the probable blooming date in Spirit Lake.

The importance of recognizing the various limiting factors of *N. fowleri* infestations is mostly focused around the organism's pathogenicity in humans, wildlife and domestic animals. *N. fowleri,* has been shown to infect house mice, guinea pigs, sheep, cotton rats, squirrels, and muskrats; however, some mammals such as opossums, raccoons, and rabbits are thought to be highly resistant to infection [7]. In the laboratory, mice have been shown to contract PAM from swimming in contaminated water, in a similar way to humans [8]. May and John (1983) demonstrated that the parasite could be passed between mice, with secondarily-exposed mice showing symptoms of infection 28 days after the death of the originally infected mice [10], suggesting an increase in mean time to death, when compared to the first host mice [10].

1.1 Description of Spirit Lake:

Spirit Lake sits at an elevation of 974.75 meters above sea level [9]. It is within 8 kilometers of the blast crater of Mt. St. Helens, which caused it to receive the full impact of the enormous lateral blast [9]. Before the eruption, the lake was a typical oligotrophic lake, with dilute solutes, surrounded by a Douglas fir (*Pseudotsuga menziesii*) forest [9,11].

On the 18th of May 1980, Mt. St. Helens experienced a violent eruption. Spirit Lake was in the direct path of the debris avalanche caused by the massive lateral blast. The debris displaced the water of the lake, creating a wave 260 m above lake level, the wave moved up the mountain sides around the northern portion of the lake [9]. This wash over the slopes brought pyrolized trees, plants, soil, and ash down into the lake bed, contributing to the rise of the lake surface by approximately 60 meters [9]. In the aftermath of the eruption temperature, acidity, dissolved iron, dissolved O_2, prey availability, and lack of competition developed into a suitable habitat for *N. fowleri*.

The waters, once known for clarity, now had little transparency due to the deposits of pyrolized organics and partially degraded lignin molecules from the forest. This created a significant change in the chemistry of the lake waters [9]. These chemical changes, combined with the mechanical force of the lateral blast, would certainly have caused the death of any macrolife-forms in Spirit Lake [9]. In doing so, the trophic structures changed to chemoorganotrophic and chemolithotrophic structures [15]. Dilution of the dissolved organic material could not occur for four years as the natural outlets of the lake became dammed by debris. Little water flowed into the lake from the volcano. The result was an effectively closed system [9,11]. In 1984- 85, the construction of a tunnel opened the system to the North Fork Toutle River [9]. Snow melt-water, together with rain, returned the cycle of annual dilution beginning the spring of 1981 [11].

In 1991, while testing public waters all over the country, Detterline *et al.*, showed a significant population density of *N. fowleri* in Spirit Lake [3]. It is not known if *N. fowleri* existed in Spirit Lake before the eruption. How *N. fowleri* came to be a cold water lake such as Spirit Lake is worth exploring; especially in relation to the post-eruption devastation and slow recovery of populations of other species. Other species required many years, or the intervention of man, to regain their previous densities.

1.2 Description of *Naegleria fowleri*:

Naegleria fowleri is a thermotolerant micropredator, limax-type amoebae, which lives in soil and warm freshwater [3]. Other related thermotolerant amoeboflagellates are amoeboflagellates *Naegleria gruberi*, *N. lovaniensis*, and *N. australiensis*, which is pathogenic to mice. *N. fowleri* is an opportunistic parasite, causing a very rare, but almost always fatal, brain infection when forced (in the flagellated or trophozoite form) up the nasal cavity of humans and some other mammals [1,8]. The amoebae follows the olfactory nerve into the brain where it feeds, causing primary amebic meningoencephalitis (PAM) [1,8]. There is no cure for PAM, although *N. fowleri* shows sensitivity to hyperbaric pressure, and hyperbaric treatments have been suggested, though not yet tested [3].

One of the most noteworthy cases of PAM that illustrates the hardiness of *N. fowleri*, is the epidemic of 16 deaths between 1962 and 1965 in Czechoslovakia [12]. After the deaths of seven people traced back to a swimming pool, the pool closed and underwent cleaning. Within days of the reopening, five more people became ill with PAM and died [12].

N. fowleri has multiple life stages, each stage helps the amoebae to survive under different conditions. The encystment stage helps *N. fowleri* survive in harsh environments and gives them the ability to overwinter in freshwater or sediment[3]. Death of the cyst can occur in situations such as, overcrowding, the environmental temperatures below 5°C or above 45°C, prolonged starvation, desiccation, lack of oxygen, etc [3,6,13]. The flagellated stage increases the mobility of *N. fowleri* and increase its ability to infect host organisms [8].

In 1972, Griffin greatly expanded our knowledge of *N. fowleri* behavior [4,5]. He proposed a hypothesis which has been supported in the research of other. This hypothesis referred is to as the flagellate-empty habitat hypothesis [5]. It states that in a non-devastated, species-rich environment, the occurrence of *N. fowleri* should represent one for every twenty thermotolerant amoebae; and in turn, the presence of thermotolerant amoebas should represent one for every 10,000 non-thermotolerant amoebae [4]. The competition of the other thermotolerant amoebae dramatically limits *N. fowleri* numbers [5]. Conversely, when the environment is devoid of competition, as in the case of a volcanic eruption into a lake, or a chlorinated swimming pool, the population increases exponentially [3]. This competitor and predator-free environment is now considered to be the most important contributing factor to the presence or absence of *N. fowleri* when compared to other important factors such as, temperature, dissolved iron content, and species diversity, in suitable waterways [3 5].

2. Temperature:

Temperature tolerance for *N. fowleri* in the laboratory is between 5° and 45°C, and *N. fowleri* has been recorded in the field in water as warm as 41°C [3 12]. Although there is no record of the water temperature on the day of the eruption of Mt St. Helens, it is likely that the water exceeded the tolerance of *N. fowleri* in Spirit Lake. Cysts in the soil may be the source of the colonizing *N. fowleri*, as a considerable amount of soil washed into the lake when the wave reentered the basin. The day after the eruption, the temperature of the surface water was 32.7°C, well-within the tolerance of *N. fowleri* [9]. In October of 1980 (table 1) the temperature was 12.2°C, considered to be back to the lakes pre-eruption averages, also well within tolerance [9].

3. pH levels:

Laboratory culture of *N. fowleri* is optimal when pH is 5.5 to 6.5 for unagitated cultures. Weik and John hypothesized this low pH preference is related to *N. fowleri*'s preferred substrate of amino acids [13]. In Spirit Lake, 80-90% of the organic material was dissolved ionized fulvic acids and hydrophilic acids [16]. Soon after the eruption, on the 30th of June, the pH was 6.21 (table 1), which was a substantial decrease from the measurement of 7.35 from April of that same year. The lake remained within or near *N. fowleri's* preferred pH zone from June 6th to July 28th, staying between 6.0 and 6.8 for the duration. By 1981, the pH had gone back up to the pre-eruption average of 7.35, which is still within the known range of tolerance (if not the preference) [9].

4. Dissolved iron concentration:

Although iron concentration is thought to be one of the more significant factors to the growth of *N. fowleri*, no information could be found on laboratory requirements. Wild *N. fowleri* has been found living in water with iron concentrations as high as 1,210 ug/L, with a mean of 480.ug/L in Yosemite Creek [3]. In early June of 1980, the dissolved iron concentration was measured to be from 1,300 to 3,600 ug/L (table 1), which may or may not be within the livable range of *N. fowleri* [9].

5. Oxygen (O2) levels:

N. fowleri has many mitochondria, unlike some other parasitic amoeba, which have none [14]. It is suspected *N. fowleri's* require high O_2 concentrations because of this high mitochondria content [13]. In the laboratory, 78% saturation of the substrate resulted in O_2 depletion, which initiated the stationary growth phase [13]. When Weik and John designed the procedure of growth for *N. fowleri*, they used a medium with a low pH (as discussed above) which, they speculated, interfered with ATP synthesis, therefore, the O_2 consumption declined as the pH rose [14].

O_2 levels were the most likely limiting factor in Spirit Lake as well as in the laboratory. After the eruption, the methane oxidation was a significant source of O_2 depletion, along with the microbial life in the lake. In June 1980, the total bacterial population was calculated to be 5x10^6 mL^{-1}. There was a minimal number of oxygen releasing organisms, such as those with chlorophyll, by comparison. The lake's chlorophyll *a* count was measured at 0.3 μg/L [15]. The primary source of O_2 was wind-driven on the surface of the lake. The surface area of the lake was 80% larger than pre-eruption surface area, due to lake bottom rise, contributing to more rapid O_2 diffusion [15 9]. In July of 1981, *N. fowleri* could have survived in the epilimnion of the lake with the O_2 concentration up to 5.4mg/L (table 1), at the time [9]. This is the probable time of the beginning of the *N. fowleri* bloom (table 1). At this time the lake met or exceeded all of the life cycle requirements. The population most likely increased as a function of increasing the limiting factor (O_2).

6. Prey source:

As already discussed, large quantities of bacteria inhabited Spirit Lake after the eruption. The literature does not discuss which species are preyed upon in the wild. In labs, *N. fowleri* is grown on Panmede liver digest and calf serum in Page's amoeba saline [13], *Escherichia coli* [4], and cyanobacteria [17]. This is a wide range of diets, which may indicate that *N. fowleri* is unlike other phagotrophic single celled eukaryotes, which are considered to be very selective consumers [17]. This would be logical considering the wide range of locations *N. fowleri* inhabits; it is found where there are low species diversity indices [3]. Cyanobacteria is a prey item used in the lab and was shown be in Spirit Lake before the proposed time of *N. fowleri* bloom. In August of 1980, there were cyanobacteria populations at 2.6 x 105 cells/L (table 1) in Spirit Lake[9].

7. Competition:

The presence or absence of competition is a primary factor in the growth of *N. fowleri* populations. *N. fowleri* has many advantages compared to competitors, in regards to primary succession. In the aftermath of the eruption of Mt. St. Helens, virtually all living inhabitants of Spirit Lake were destroyed [15]. These advantages suit *N. fowleri* best in events such as the eruption. The lack of competition induces *N. fowleri* to transform from the trophozoite to the motile flagellate, which increases its ability to colonize further, leading to a bloom [3]. In August of 1982, there was an extensive study of the organisms in Spirit Lake, flagellates are mentioned, but with no specific species identified [9]. At the time of the discovery of *N. fowleri* by Detterline *et al.*, only two competing species also inhabited in Spirit Lake, *N. lovaniensis* and *Acanthamoeba sp* [3]. These competitors may be the cause of the end of the bloom, but with no indication of when the first sighting occurred there is no way to be certain.

Table 1. Spirit Lake, *N. fowleri* probable bloom time-line. Dates are as specific as possible. Information for this table was taken from various sources given in the body of the paper. The source or sources of the information is given in the citation column.

Requirement/Event	**Date met or exceeded**	**Citation**
Temperature	19 May 1980	Detterline, J. L. and Wilhelm, W. E. 1991; Lee, D. B. 1996
pH	30 June 1980	Weik, R. R. and John, D. T. 1977; Lee, D. B. 1996
Dissolved Iron Concentration	June 1980	Lee, D. B. 1996
Dissolved O2	July 1981	Weik, R. R. and John, D. T. 1977; Lee, D. B. 1996
Prey	August 1980	Xinyao, L. *et al.* 2006; Lee, D. B. 1996
Competition	18 May 1980	Griffin, L. J., 1983; Wissmar, R. C. *et al*. 1982
Pathogen	August 1983	Declerck *et al.; Lee, D. B. 1996*
Probable Bloom begging point	July 1981	
Discovered	1991	Detterline, J. L. and Wilhelm, W. E. 1991

8. Discussion:

N. fowleri appears to have parasites of its own. A possible contributing factor to the lower-than-bloom-level population of *N. fowleri* is the presence of *Legionella pneumophila* in the Spirit Lake waters, found in 1983 [9] (table 1). Declerck *et al.* [2] showed the bacteria, which is also virulent in humans, uses both *N. fowleri* and *N. lovaniensis* as a host.

N. fowleri presence in Spirit Lake does not only have implications for human visitors, it can also affect the local wildlife. This observation has interesting implications for the Spirit Lake habitat, specifically the rodent population. In further research, both the *N. fowleri* population in the water bodies and the rodent population on the shore could be monitored, especially in the event of another reduction in *N. fowleri* competitors. This would give disease ecologists a chance to see if the laboratory results are consistent with *in situ* results.

In the broader scope, the presence of *N. fowleri* in Spirit Lake is a prime example of the flaw in the thermotolerant hypothesis used by public health organization. This warm water focus of the current testing perimeters would have ruled out

Spirit Lake as a testing site [3]. According to this hypothesis *N. fowleri* should not have been present in waters only 12.2°C. Furthermore, its presence in an area lacking competition as a result of the eruption of Mt. St. Helens appears to support the flagellate empty habitat hypothesis. Recognizing the flagellate empty habitat hypothesis, and other limiting factors for *N. fowleri* such as O_2 concentration, will give public health authorities better surface water testing protocols. More efficient testing and warning systems for local human population could decrease incidences of PAM.

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