

An investigation into the use of Class A fire-fighting foams to control the spread of whirling disease by wildland fire-fighting activities

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Abstract

Whirling disease is a serious problem that impacts wild and hatchery salmonid stocks across the globe. Whirling disease is caused by a microscopic parasite, *Myxobolus cerebralis*, with a complex life history involving both salmonid and oligochaete worm hosts. In this study, we investigated the capability of wildland firefighting foams to inactivate *M. cerebralis* by indirect methods. Specifically, we tested the effectiveness of firefighting foams as disinfecting agents against surrogate organisms (*E. coli* and coliphage) in bench-scale laboratory experiments. The USFS firefighting foams tested in this study did not inactivate *E. coli* or coliphage during a 60-min contact time at a 10% mix ratio. We did not examine whether firefighting foams were effective at displacing bacterial and viral organisms when treated as a surfactant. Nevertheless, we conclude that continued research into the use of dilute fire retardant foams to inactivate aquatic invasive microorganisms is not warranted.

Introduction

Myxobolus cerebralis is a freshwater myxozoan parasite that causes whirling disease in salmonid species. *M. cerebralis* has been detected in hatcheries or natural waterways in 25 states since its initial discovery in the United States in 1958 (Figure 1; Hoffman et al. 1962, Elwell et al. 2009). *M. cerebralis* became a widespread problem in fish culture facilities between the 1950s and 1990s, leading to the development of a variety of successful control strategies to produce parasite-free fish (Elwell et al. 2009). Major losses of wild fish were not observed until the mid-1990s, and the parasite continues to spread in wild populations today (Nehring and Walker 1996, Vincent 1996, Elwell et al. 2009).

M. cerebralis has a complex life cycle involving salmonid and oligochaete worm hosts (Wolf and Markiw 1984,

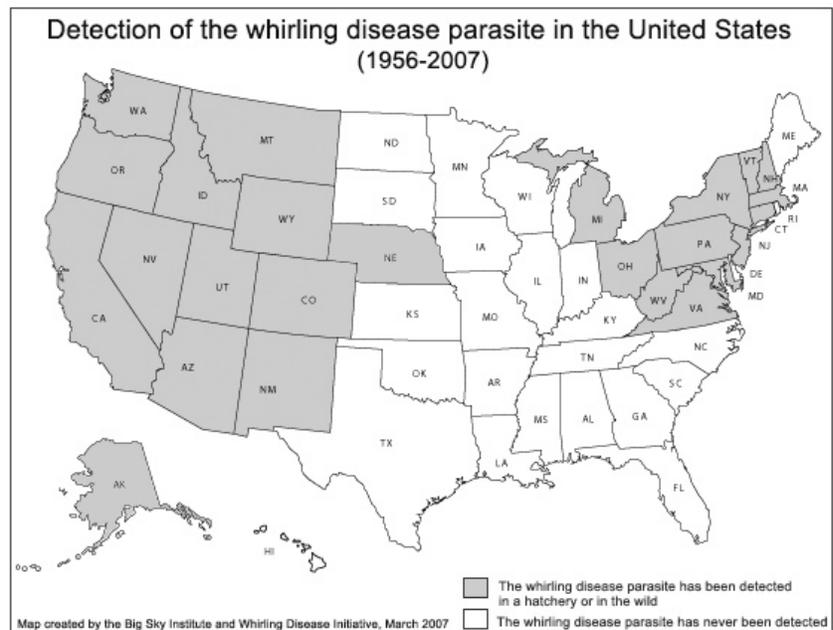


Figure 1. Distribution of *Myxobolus cerebralis* in the United States.

Image from <http://whirlingdisease.montana.edu/about/map.htm>

Hedrick and El-matbouli 2002, Gilbert and Granath 2003; Figure 2). In brief, a salmonid host is infected by ingestion of infected worms or attachment of the Triactinomyxon (TAM) stage to the skin. Once the TAM attaches to the host fish, the sporoplasm is injected and spreads to cartilaginous tissue (El-matbouli et al. 1995). Myxospores develop in the cartilage and are released to the environment in large numbers when the fish dies. Once the myxospores are ingested by *Tubifex tubifex* worms they attach to the gut epithelium. The myxospores then undergo a series of asexual and sexual development stages to eventually produce pansporocysts, each containing eight TAMs (El-Matbouli and Hoffmann 1998). Mature TAMs are released from the worm to the water allowing the infection cycle to repeat.

The spread of *M. cerebralis* throughout the United States has occurred primarily by the movement of infected fish through natural or human activities (Hedrick et al. 1998, Bartholomew and Reno 2002). Other vectors of transmission include the movement of infected water or sediments (Elwell et al. 2009); piscivorous predators (Taylor and Lott 1978, El-Matbouli and Hoffmann 1991, Arsan and Bartholomew 2008); and effluent from fish processing plants (Arsan and Bartholomew 2008). The project discussed herein was initiated due to the concern that fire-fighting activities may also transplant *M. cerebralis* from an infected watershed to a pristine watershed.

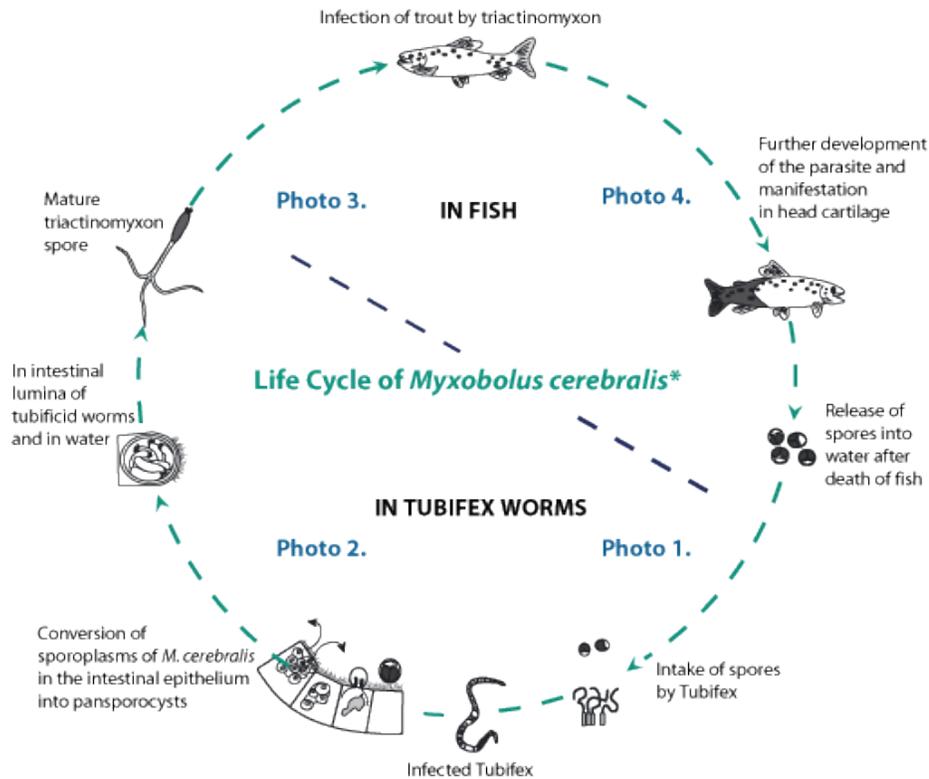


Figure 2. Lifecycle of *Myxobolus cerebralis*.
Image from <http://whirlingdisease.montana.edu/about/lifecycle.htm>

Once *M. cerebralis* has been established in a watershed, the parasite is nearly impossible to eradicate. One of the primary difficulties in controlling *M. cerebralis* is the different physical forms of *M. cerebralis* present at different stages of their life cycle that are highly resistant to chemical and or physical disinfection. Organisms with spore or cyst life-stages are the most difficult (resistant) to inactivate with traditional chemical disinfectants (White 1992). For example, a 15 minute application of 500 mg/l chlorine is required to inactivate the myxospore stage of *M. cerebralis* by 5.07-logs¹ (Hedrick et al. 2008). However, strategies to control the spread and prevent introduction of *M. cerebralis* to fish culture facilities has been relatively successful. For example, the replacement of earthen ponds that provide habitat for the worm host, *T. tubifex*, with concrete raceways (Markiw 1992, Bartholomew et al. 2007), the application of chemicals for disinfection (see Wagner 2002), the use of groundwater instead of surface waters or the treatment of incoming water, regular testing of fish and surface waters for the parasite, and the destruction of infected fish have all proven successful to some degree (Elwell et al. 2009).

The goal of this project was to investigate the capability of wildland firefighting foams to inactivate *M. cerebralis*. Due to the presence of multiple resistant life-stages of *M. cerebralis*, which are labor intensive and costly to obtain for experimental purposes, surrogate organisms were used to assess the initial efficacy of the firefighting foams as disinfecting agents. The surrogate organisms were selected to represent increasing resistance to traditional chemical disinfectants; from *E. coli* bacteria, to coliphage virus, to spore producing organisms. For each surrogate organism, the performance of the foams was compared to the performance of chlorine, a traditional chemical disinfectant. The inactivation of the surrogate organisms by the foams relative to the inactivation of the surrogate organisms by chlorine can be used to infer the capability of the foams to inactivate the resistant life-stages of *M. cerebralis* at the desired field conditions.

The specific objective of this project was to investigate the capability of four Class A Wildland Fire Foams (foams): Tyco Silv-Ex Plus Class A (Silv-Ex), Phos-Chek WD 881 (WD 881), Phos-Chek First Response (First Response), and Chemguard First Class (Chemguard) to inactivate two surrogate organisms (*E. coli* bacteria and a coliphage virus). The inability of any of the foams to inactivate either *E. coli* bacteria or coliphage virus at the highest desired foam mix ratio and longest desired contact time precluded the need to evaluate the capability of foams to inactivate a more resistant, spore-forming surrogate organism.

¹ A log inactivation refers to the ability of a disinfectant to reduce the number of viable pathogens by one order of magnitude. Therefore, a 5-log inactivation is a 100,000-fold reduction in the number of viable pathogens.

Methods

Experimental Design

The effects of foams and free chlorine on the viability of surrogate organisms were evaluated by bench-scale disinfection experiments. The experiments were conducted with bacteria or virus suspended in dilution buffer (42.5 mg/L KH₂PO₄ and 405.5 mg/L MgCl₂·6H₂O; Method 9050C, APHA et al., 2005) at room temperature and stirred continuously during a 60-minute contact time with the foams or chlorine. After exposing the surrogate organisms to the foams and chlorine, we analyzed their viability and compared that to a Control treatment run under identical conditions. Experiments were performed in triplicate for each combination.

Foams included 10% solutions of Class A wildland firefighting foams Silv-Ex, WD 881, First Response, and Chemguard. The foam treatments were made by stirring 10 ml of select firefighting foam into 90 ml of sterile buffer in a 250 ml glass flask. The firefighting foams were not deactivated or removed prior to enumerating the bacterial or viral surrogate. Pilot experiments indicated the foams did not interfere with either detection assay.

Sodium hypochlorite, at approximately 3.5 mg/L free chlorine, served as a model disinfectant. Chlorine treatments were made by adding a known volume of chlorine stock solution to sterile buffer for a final volume of 100ml. A stock solution of approximately 100 mg/L chlorine was prepared in purified deionized water with 5.65-6% laboratory-grade NaOCl (Fisher Scientific). The stock solution was standardized as per Standard Method 4500-Cl D.4 (APHA et al., 2005) before each experiment. Temperature, pH, and residual chlorine concentration was determined at the beginning and end of each experiment. An appropriate amount of sodium thiosulfate was added to the chlorine treatments for a final concentration of 30 mg/L at the end of the experiment to neutralize any remaining chlorine prior to enumeration of the seeded organism (APHA et al., 2005).

Bacterial surrogate

The historical use of *Escherichia coli* in wastewater disinfection studies, and standardized methods for enumeration, made *E. coli* the optimal surrogate bacteria for our study. *E. coli* No. 13706 was purchased from the American Type Culture Collection (ATCC, Manassas, VA). The bacteria was rehydrated and cultured in nutrient broth (BD Difco, Franklin Lakes, NJ) at 35°C for 20h, and then stock aliquots were preserved in 10% glycerol and stored at -80°C.

A one milliliter aliquot of thawed *E. coli* stock was inoculated in 100 ml of nutrient broth and incubated at 35°C. After 18-24 hours of growth, one milliliter of *E. coli* culture was added to each treatment at time zero, for a final concentration of approximately 10⁶ cfu/ml. At the conclusion of the 60-minute contact time, the seeded *E. coli* was assayed by the multiple-tube fermentation technique as per *Standard Methods* 21th Edition, Method 9221 B (APHA et al., 2005). Briefly, serial decimal dilutions were made by pipetting 10 ml of each treatment into 90 ml dilution buffer. The 100 ml solution was thoroughly mixed and successive 10-fold dilutions were made in an identical manner. Then 1 ml from each select dilution was pipetted into each of five replicate test tubes containing 10 ml lauryl sulfate broth (Fluka analytical, Buchs, Switzerland). For the chlorine treatments (only), ten ml of non-dilute sample was also pipetted into five replicate test tubes of double-strength Lauryl sulfate broth. Lauryl sulfate broth tubes were capped and incubated at 35°C for 24-48 hrs. Bacterial growth was indicated by turbidity in the test tube or trapped gas in the inverted Durham vial. Positive samples were confirmed by transfer with a sterile wooden cotton swab to test tubes filled with 10 ml brilliant green bile lactose (BGBL) broth and an inverted vial. BGBL broth tubes were considered positive for *E. coli* growth if a gas bubble was trapped in the inverted vial after incubation at 35°C for 24-48hrs. The mean density of viable bacteria was then estimated using a most probable number (MPN) technique from *Standard Methods* 21st Edition, Table 9221:IV (APHA et al., 2005).

Viral surrogate

Coliphage ϕ X174 was chosen as the optimal surrogate virus because of its wide use as an alternative to human virus assays in wastewater treatment protocols. A virus, or phage, is a small infectious agent that requires a living host cell to replicate; a coliphage is a bacterial virus that infects and replicates in coliform bacteria. Coliphage ϕ X174 was purchased as a frozen concentrate from ATCC (No. 13706-B1; Manassas, VA). A stock solution was created by serially diluting the thawed frozen virus in tryptone broth to approximately 3,000 plaque forming units (pfu) per milliliter. The coliphage stock was stored at 4°C throughout the experimental period in the absence of the bacterial host, during which no

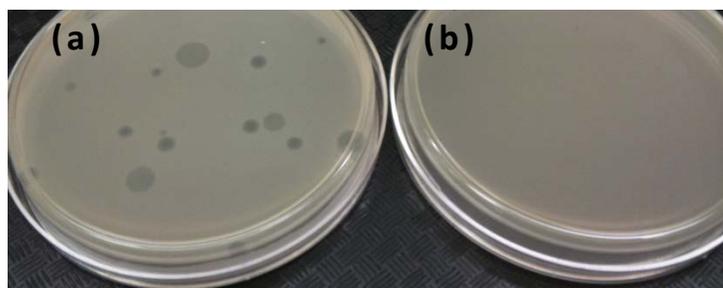


Figure 3. Example of coliphage plaques visualized by the double agar overlay method; (a) depicts circular plaques formed by coliphage in a monolayer of *E. coli* cells, and (b) depicts an intact monolayer of *E. coli* cultured cells without viral plaques.

replication or death of the virus was observed. One milliliter of coliphage stock was used to inoculate each treatment for a final concentration of approximately 20-30 pfu/ml. The bacterial host was not supplied during the experiment, so replication of the virus was not expected. At the end of the 60 minute disinfection period, seeded coliphage was enumerated using a plaque assay based on the double-agar-layer method as per *Standard Methods* 21th Edition, Method 9224 B (APHA et al., 2005; Figure 3). Briefly, one milliliter of sample was inoculated into 3 ml of warm tryptone top agar plus 100 μ l of actively growing *E. coli* host. Three replicate plates were also made with one milliliter of sterile tryptone broth to check for coliphage contamination in the agar. The top agar was gently mixed and poured on top of tryptone bottom agar plates, dried and incubated overnight. Plaques are created when a coliphage particle infects a cell within a monolayer of *E. coli* cells in the top agar. The virus-infected cell replicates the virus and bursts, which spreads the virus infection to the adjacent host cells. This process repeats until the area of infection can be visualized as a clearing of the bacterial host. It is assumed that each plaque is formed by one coliphage. The coliphage was enumerated by counting plaques on 7-15 replicate plates to obtain the average pfu per treatment and 95% confidence intervals, assuming a Poisson distribution.

Results

Bacterial surrogate

The firefighting foams did not demonstrate any inactivation of *E. coli* at 10% concentration within the recommended disinfection time of 60 minutes. The MPN of *E. coli* in each foam treatment was not significantly different than the Control treatment in any of the trials (Figure 4).

Mean MPN of three replicates of Chemguard First Class was 2.21×10^6 cfu/100ml compared to 1.10×10^6 cfu/100ml for the

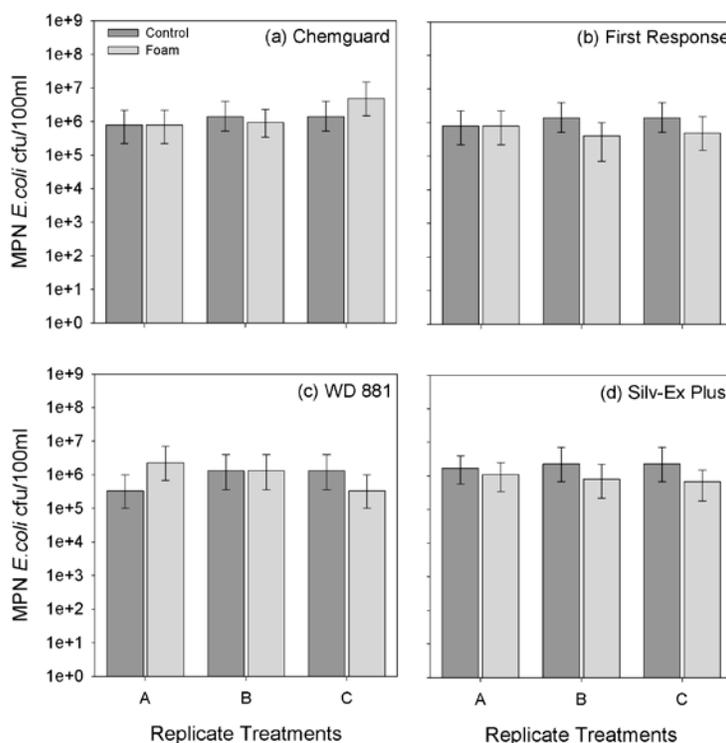


Figure 4. Inactivation experiments of *E. coli* with wildland firefighting foams compared to control treatments. Error bars denote 95% confidence intervals. No significant differences were detected between foam and control treatments.

control. Mean MPN of three replicates of First Response was 5.60×10^5 cfu/100ml compared to 1.10×10^6 cfu/100ml for the control. Mean MPN of three replicates of PHOS-CHEK WD 881 was 1.31×10^6 cfu/100ml compared to 8.15×10^5 cfu/100ml for the control. Mean MPN of three replicates of Silv-Ex Plus was 8.57×10^5 cfu/100ml compared to 2.00×10^6 cfu/100ml for the control.

The Chlorine treatment of 3.33 mg/L initial free chlorine resulted in a mean *E. coli* reduction of 6.14-logs relative to the Control treatment (Figure 5). The mean MPN of three replicates of the chlorine treatment was 3.10×10^6 cfu/100ml, and 2.23 cfu/100ml for the Control treatment. No residual chlorine was detected at the end of the experiments. The temperature rose from 14.1°C to 18.1°C over the course of the 60 min. contact time, with a nearly neutral pH of 6.61 and 6.74 at the beginning and end, respectively. The ability to inactivate *E. coli* with free chlorine using an identical experimental design as the fire retardant foams provides confidence in the results obtained during the foam exposures.

Coliphage surrogate

The firefighting foams did not demonstrate any inactivation of Coliphage ϕ X174 at 10% concentration within the recommended disinfection time of 60 minutes. The mean concentration of coliphage after each foam treatment was not significantly different than the Control treatment in most of the replicate trials (Figure 6). Mean coliphage concentration of all three replicates of the Chemguard First Class treatment was 23.2 pfu/ml compared to 18.0 pfu/ml for the control treatment. Mean coliphage concentration of all three replicates of the First Response treatment was 23.5 pfu/ml compared to 24.1 pfu/ml for the control treatment. Mean coliphage concentration of all three replicates of the PHOS-CHEK WD 881 treatment was 22.1 pfu/ml compared to 22.2 pfu/ml for the control treatment. Mean coliphage concentration of all three replicates of the Silv-Ex Plus treatment was 20.1 pfu/ml compared to 21.2 pfu/ml for the control treatment. A single replicate from the WD881 and Chemguard treatments showed significantly higher concentrations of coliphage than the control, suggesting that the differences

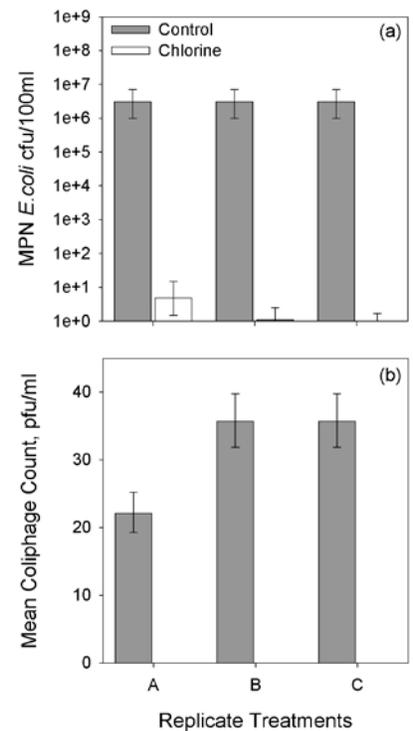


Figure 5. Inactivation experiments of (a) *E. coli* and (b) coliphage ϕ X174 by chlorine. Error bars denote 95% confidence intervals. Chlorine dose for (a) was 3.33 mg/L for all replicates. Chlorine dose for (b) was 3.87 mg/L for replicate A and 3.35 mg/L for replicates B and C. No coliphage was detected in any replicate after chlorine exposure (b).

were not due to disinfection properties of the foam. Since disinfection studies are concerned with log-removal of pathogenic organisms, the small differences observed (less than 1 log) are not likely significant to the question of a foam’s disinfection efficacy.

The Chlorine treatment resulted in a mean coliphage reduction of 2.5-logs relative to the Control treatment, which was the limit of quantification (LOQ) for all three replicates (Figure 5). Mean initial chlorine concentration (\pm SD) was 3.54 ± 0.26 mg/L, ranging from 3.35 mg/L to 3.87 mg/L free chlorine across replicates. No residual chlorine or coliphage was detected at the end of the experiments. The temperature ranged from 19.5°C to 21.3°C, with a near-neutral pH (6.82 and 7.08).

Discussion

The USFS firefighting foams tested in this study did not inactivate *E. coli* or coliphage during a 60-min contact time at a 10% mix ratio. Most bacteria and virus are readily inactivated by traditional chemical disinfectants. We observed this in our side-by-side comparisons with chlorine and *E. coli* or coliphage, in which a 6-log removal of *E.coli* and complete inactivation of coliphage was achieved with ≤ 3.9 mg/l. In contrast, spore-producing organisms, like *M. cerebrialis*, require greater chemical doses (concentration multiplied by the contact time) compared to bacterial and viral species. For example, a

surrogate protozoan parasite for *M. cerebrialis*, *Cryptosporidium parvum*, required 80 mg/l chlorine for 90 minutes for an approximate 2-log reduction (Korich et al. 1990). Given that this chlorine dose was more than 20 times the chlorine concentration and 1.5 times the contact time of our exposure

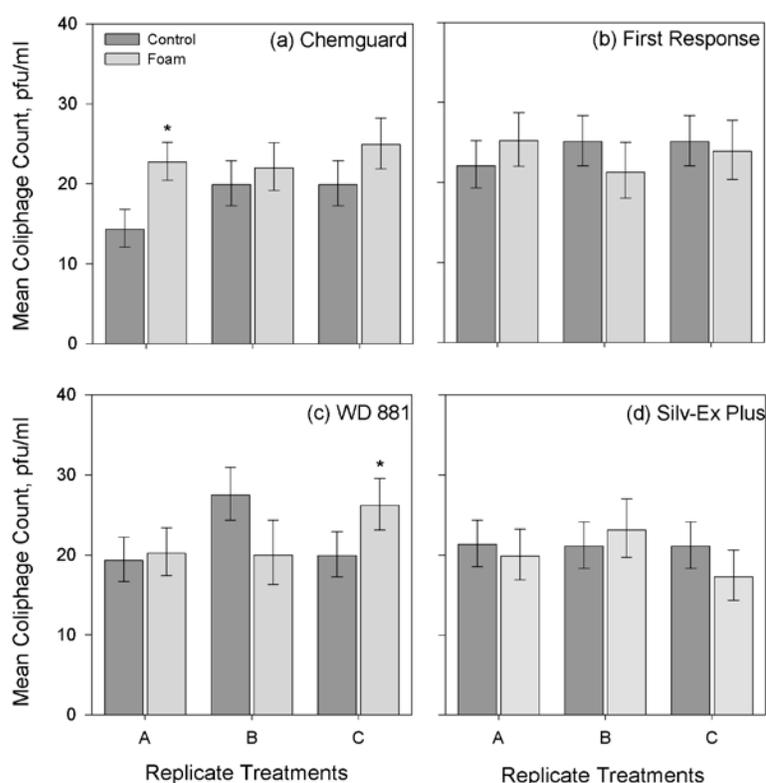


Figure 6. Inactivation experiments of coliphage ϕ X174 by wildland firefighting foams. Error bars denote 95% confidence intervals as determined by Poisson analysis. Asterisks denote replicate foam treatments that were significantly different than the control.

conditions, in which the foams had no inactivation capability; there was no utility in attempting inactivation experiments with foams and spore-forming protozoa (*M. cerebralis* or any surrogates).

One of the key obstacles in controlling *M. cerebralis* is the complex lifecycle that includes a TAM and spore life history stage, and a fish and worm host. Attempted eradication or control of *M. cerebralis* with fire-fighting foams must take into account all of the possible stages, some of which are highly resistant to disinfection. The TAM stage, released by the worm host, is more susceptible to chlorine inactivation relative to the spore stage released by the fish host. For example, 100% of TAMs were inactivated by 13 mg/L chlorine for 10 min at room temperature (Wagner et al. 2003); whereas, a 5-log removal of spore infectivity required 500 mg/L chlorine for 15 min (Hedrick et al. 2008). Other conditions and chemicals may require high doses to be effective on TAMs. For example, the previous authors found that a ten-fold higher dose (131 mg/L chlorine for ten minutes) was required to inactivate 100% of the TAMs in ice water (Wagner et al. 2003). In addition, complete inactivation (approximately 2-log) of TAMs required a ten minute contact to 10.2% hydrogen peroxide solution, while a 50% solution of povidone-iodine (5000 mg/l) for 60 minutes resulted in incomplete inactivation of approximately 2-logs (Wagner et al. 2003). The spore stage, released in large numbers by the fish host upon death, is considered the most resistant stage in the lifecycle of *M. cerebralis*. A recent study examining the infectivity of chemically treated spores to the worm host by measuring subsequent TAM production demonstrated only partial inactivation of 1.08-logs with 200 mg/L chlorine for 15 min (Hedrick et al. 2008). In addition, spore inactivation with a quaternary ammonium compound (alkyl dimethyl benzyl ammonium chloride) at 1500 mg/L for 10 min resulted in a 4.14 log-removal of *M. cerebralis* spore infectivity (Hedrick et al. 2008). Finally, little information is available in the literature on controlling the worm host, *T. tubifex*, by disinfection. Inactivation of the worm host is further complicated by the ability of *T. tubifex* to form cysts when exposed to drying conditions (Kaster and Bushnell 1981). Manipulation of water quality variables such as extreme high and low pH and high temperatures have been shown to inactivate *T. tubifex* adult worms (Wagner 2002), but there is no published information regarding disinfection of the cyst stage to our knowledge (Wagner 2002). There is evidence that worms exposed to temperatures above 25°C stop releasing TAMs after four days (El-Matbouli et al. 1999), but there are no published reports on whether TAMs (or spores) would be inactivated or simply expelled from worms treated with chemical disinfectants. A thorough summary of *M. cerebralis* disinfection literature can be found in the attached document.

The USFS firefighting foams did not display any disinfection properties against *E. coli* or coliphage, which are demonstrably easier to kill than spore-producing organisms. We can infer that the

foams will not be able to inactivate the TAM or spore stage in the lifecycle of *M. cerebralis*. However, we did not examine the capability of the firefighting foams to displace bacterial and viral organisms when acting as a surfactant. As surfactants, the foams may be able to reduce the number of organisms adhering to equipment with thorough scrubbing and rinsing away of viable organisms, but the foams would not inactivate invasive species when water or diluted foams were moved between watersheds. Currently, the USFS intermountain region technical guidelines to prevent the spread of invasive species suggests using 500 mg/L of chlorine for 10 minutes to inactivate whirling disease (<http://www.fs.fed.us/r4/resources/aquatic/guidelines/>). Given studies performed by Wagner et al. (2003) and Hedrick et al. (2008), this guideline would likely achieve complete inactivation of TAMs and up to a 5-log removal of spores. However, we conclude that further research into the use of diluted fire retardant foams to inactivate invasive microorganism species is not warranted.

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