INVESTIGATING THE EFFECT OF BLUE LIGHT AGAINST FOODBORNE VIRUSES ON STAINLESS STEEL AND IN FRESHWATER

by

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(Under the Direction of Malak A. Esseili)

ABSTRACT

Human norovirus (HuNoV) and hepatitis A virus (HAV) are the two main viruses causing foodborne outbreaks. These viruses can be transmitted through the fecal-oral route via food, water, or food-contact surfaces. The objectives of this study were to investigate the effect of blue light (BL) at 405 nm on HuNoV and HAV on stainless steel (SS) and in freshwater at room temperature. At a dose of 1520 J/cm^2, infectious TV and HAV suspended in organic matter then dried on SS, were inactivated by ~0.6 and 0.8 log $TCID_{50}$/ml, respectively. In sterile water, infectious TV and HAV showed ~0.6 and 2.5 log reductions, while in pond freshwater, the reduction reached ~0.8 and 2.8 log, respectively. Furthermore, BL transiently affected pond water pH, conductivity, and total suspended solids while minimally reducing pond water indigenous bacteria. Overall, this study provided novel insights on the potential use of BL to inactivate foodborne viruses.

INDEX WORDS: Human norovirus, Tulane virus, Hepatitis A virus, blue light (405 nm), freshwater, stainless steel, indigenous bacteria, water quality

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DEDICATION

This work is dedicated to my mother, Joy Obioma Ugo-Ukegbu. Your faith and support in me is unwavering. You have been by my side through thick and thin, your words always guiding and encouraging; your love and prayers going ahead to strengthen me. *Nne'm oma, daalu.*

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CHAPTER 1

INTRODUCTION

Human norovirus and HAV are major causes of foodborne outbreaks and are responsible for hospitalizations and deaths around the globe (CDC, 2024). These are human viruses that infect the gastrointestinal tract and are transmitted via the fecal-oral route, contaminated food, water, and food-contact surfaces (Sattar et al., 2000). Viruses cause the most diseases from foodborne outbreaks; therefore, they are of utmost importance for food safety. Due to the notable rise in viral foodborne outbreaks over the previous 20 years, this concern is well founded (Olaimat et al., 2024). The more prevalent viruses linked to outbreaks of foodborne sickness or waterborne illness are human norovirus (HuNoV) and hepatitis A virus (HAV) (Olaimat et al., 2024). Presently, there are also potential emerging foodborne viruses. These viruses have long been recognized as pathogens but have now been demonstrated to spread through food (Koutsoumanis et al., 2014). Several novel viruses have recently been identified from food items; they could be classified as emerging foodborne pathogens, which is concerning due to the possibility of human transmission via the food chain. Humans may contract zoonotic viruses, such as avian influenza viruses, and certain coronaviruses, by consuming contaminated food, especially undercooked or raw meat products from infected animals (O'Shea et al., 2019; Todd & Greig, 2015).

Purpose of the Study: to investigate the effect of blue light (BL) at 405 nm on HuNoV and HAV on stainless steel (SS) and in freshwater at 25°C.

CHAPTER 2

LITERATURE REVIEW

Human norovirus characteristics

Human norovirus (HuNoV) is a single-stranded positive sense non-enveloped, small (23-40 nm) RNA virus with a genome length of about 7.5 kb in length belonging to the *Caliciviridae* family (Committee on Infectious Diseases et al., 2021; Green et al., 2020). Norovirus is classified into ten genogroups (GI-GX), which can be subdivided into more than 40 genotypes (De Graaf et al., 2016; Kroneman et al., 2013; Vinjé, 2015). The genogroups GI, GII, and GIV are most commonly associated with human infection. Human norovirus is a major cause of acute gastroenteritis with symptoms including vomiting, nausea, abdominal pain, and diarrhea. The study of HuNoV poses a problem due to the difficulty of in vitro cultivation of this virus (Green et al., 2020). The virus was recently reported to successfully replicate in 3D enteroid culture model (Ettayebi et al., 2016). However, this cell culture model is costly, labor-intensive, and not commercially available. In addition, infectious HuNoV from fecal samples of sick humans are not commercially available and cannot be generated using the enteroid model. Because obtaining infectious HuNoV that replicates in vitro in high titers is difficult, surrogate viruses like murine norovirus (MNV), feline calicivirus (FCV), Tulane virus (TV), and bacteriophage MS2 are still commonly used to study HuNoV.

Human norovirus burden, transmission and outbreaks

Globally, HuNoV is responsible for over 685 million cases of disease and 200,000 deaths annually (CDC, 2024). It is also the cause of more than half of all foodborne disease outbreaks in the US, as well as between 56,000-71,000 hospitalizations in the US from the 19-21 million cases of annual acute gastroenteritis cases (CDC, 2024). It is transmitted via the fecal-oral route, contact with infected persons, contaminated food and water bodies, and contaminated surfaces (CDC, 2024). Norovirus outbreaks have a worldwide annual economic burden of $60.3 billion in societal expenditure and $4.2 billion due to direct health system costs (Bartsch et al., 2016). Adults aged ≥ 45 years account for more than half of the burden, and sporadic community cases account for >90%, while productivity losses for 89% of the overall burden (Bartsch et al., 2020). Norovirus outbreaks have occurred in cruise ships, hospitals, prisons, daycares, and restaurants where surfaces are potentially involved in the transmission.

From 1992-2000, the most common place of HuNoV outbreaks occurred in healthcare facilities. Specifically, ~754 cases occurred in hospitals with 24 deaths while 724 cases were recorded in residential-care institutions with 19 deaths (Lopman et al., 2003). Based on the records reported to the CDC (Centers for Disease Control and Prevention) CaliciNet in the US, between 2009 and 2013 the most common settings of HuNoV outbreaks were long-term care facilities (62.5%), restaurants (9.8%), schools and communities (5.7%), events parties or events (5.4%), hospitals (3.6%) and cruise ships (1%). For example, multiple HuNoV outbreaks that occurred in three college campuses in Michigan, California, and Wisconsin, reported to the CDC about1000 cases and a minimum of 10 hospitalizations (CDC, 2009). In restaurants, especially for ready-to-eat products (salads, fruits, vegetables, fruits, desserts, snacks), the most common transmission pathway occurs through infected food handlers via poor hygiene (unwashed or dirty hands after

using the toilets), or through contaminated food-contact surfaces (Bidawid et al., 2004). Other foods that have been involved in HuNoV outbreaks include red meat, oysters, poultry, and soups (Lopman et al., 2003). In all these, food-contact surfaces are potentially involved in the transmission.

Human norovirus surrogate, Tulane virus

Tulane virus (TV) is commonly used as HuNoV surrogate to estimate HuNoV infectivity in response to a treatment or intervention (Tian et al., 2013). Tulane virus belongs to the *Caliciviridae* family and was first isolated in 2008 from the stool samples of rhesus macaques (Farkas, 2015; Yu et al., 2013). Similar to HuNoV, TV contains three open reading frames (ORFs), is a positive-sense, non-enveloped, small 40 nm RNA virus that is transmitted through the fecal-oral route (Farkas, 2015). The virus genome is ~6.7 kb long and is often used as a surrogate for HuNoV because it can be cultivable in vitro, and recognizes human histo-blood group antigens as cellular attachment factors, like HuNoV (Farkas et al., 2010; Farkas et al., 2008).

Hepatitis A virus characteristics

Hepatitis A virus (HAV) is a single-stranded positive-sense non-enveloped RNA virus in the family *Picornaviridae (Sattar et al., 2000)*. It is small (27 nm) and has a genome length of ~7.5 kb. HAV strains are genetically differentiated into seven genotypes (I, II, III, IV, V, VI, VII). Viruses of HAV with genotypes (I, II, III, IV) are associated with human infections while viruses with genotypes (V, VI, VII) are associated with simian species (Robertson et al., 1992). Nucleotide sequence divergence of 15-25% occurs among different HAV genotypes. The virus causes inflammation of the liver, diarrhea, and nausea (Nemes et al., 2023). It is transmitted mainly

through the fecal-oral route, from person-person contact, contaminated foods, surfaces, and water (Sattar et al., 2000). Wildtype HAV is difficult to grow in cell culture; however, a commercially available cell-culture-adapted HAV strain can be propagated in routine cell culture (FRhK-4 cells obtained from Rhesus monkey). HAV175/18f is a HAV strain that is cell culture-adapted and is usually used to evaluate the reduction in HAV infectivity in response to treatment or intervention (Kulka et al., 2003). However, the growth of this HAV strain in FRhK-4 remains relatively slow and takes at least 7 days to grow; with a total of 10-14 days for the infectious titer to be quantified (Brack et al., 1998).

Hepatitis A virus burden, transmission, and outbreaks

According to the CDC report from 2016-2023, a total of 44,926 cases of HAV, 27,457 hospitalizations, and 424 deaths occurred (CDC, 2017). The average cost of hospitalization for hepatitis A per person in the US in 2017 was $16,232 (Hofmeister et al., 2020). In 2007, 6.5% of total cases of HAV recorded in the US were due to food and water contamination (Annemarie et al., 2007; Daniels et al., 2009). Seafoods, fruits, and vegetables are usually the type of foods associated with HAV contamination, and this contamination can occur via water or at any point during processing, harvesting, preparation, or distribution. This is of huge concern because fruits (such as berries) and vegetables (such as lettuce and green onions) are usually eaten raw or are subjected to minimal cooking. In 1988, the biggest HAV outbreak occurred in Shanghai, where 300,000 people contracted the virus after eating raw clams (Halliday et al., 1991). In the United States, the first outbreak of HAV was reported during the Civil War in 1812 in Norfolk, VA with more than 40,000 confirmed cases. By World War II, there were approximately 16 million cases of HAV reported among civilians and soldiers (Ian D. Gust, 1988; Sherlock, 1984). In another

example, in Pennsylvania, after patrons at a restaurant consumed green onions, a significant foodborne outbreak was documented. Upon investigating the origin of the contamination, it was discovered that the green onions were imported from Mexico and had been contaminated with HAV either prior to or during the packing, irrigation, rinsing, processing, cooling, and icing steps or from transmission from infected workers (Wheeler et al., 2005).

Persistence of foodborne viruses on contaminated surfaces

Fomites (contaminated surfaces) may act as vehicles for the transmission of viruses. These include surfaces or objects such as stainless steel, door handles, clothing, paper, gloves, etc. The majority of fomite contaminations are caused by direct contact or the deposition of virus-containing aerosol particles. For instance, flushing the toilet or coughing, sneezing, vomiting, or talking can all produce aerosols (Boone & Gerba, 2007). Also, contamination of surfaces occurs when the virus is released in bodily secretions, such as blood, feces, urine, saliva, and nasal fluid, both during and after sickness (Bellamy et al., 1998; Hall et al., 1980). After that, the virus can spread to other fomites through human contact. It is believed that a major factor in the transmission of infection is virus transfer from hands to fomites and vice versa. It has been demonstrated that HuNoV can be easily transferred from fomites to hands and that contaminated hands can subsequently cross-contaminate up to seven other clean surfaces (Barker et al., 2004). In another study, TV with an initial titer of about 5×10^4 PFU (plaque forming unit)/mL persisted on stainless steel and acrylic-based solid surfaces for 14 days with only about 1 log reduction for both surfaces. There were no significant variations in TV persistence between the two surface types ($p > 0.05$) based on log decreases from days 0 to 7, although there were significant differences between the two surfaces ($p < 0.05$) at days 10 and 14 (Arthur & Gibson, 2016a). Viral RNA from HuNoV GI

and GII has been found to persist on stainless steel (SS), ceramic, and Formica surfaces for as much as 42 days after contamination with an initial inoculum of about $10^7 – 10^8$ genomic copies/g/g. An average reduction of 1.5-2 log was observed for HuNoV GI and GII under ambient temperature after 42 days (Escudero et al., 2012). Another study used MNV and FCV and showed that at 4 °C these viruses could persist up to 7 days on stainless surfaces with ~ 1.8 and 2.5 log reductions, respectively (Cannon et al., 2006). At room temperature (RT), MNV could survive up to 5 days on SS surfaces while FCV could survive up to 7 days with ~ 5.5 and 5 log reductions, respectively (Cannon et al., 2006). Another study corroborated the earlier studies that FCV was able to survive on SS surfaces for up to 7 days (Mattison et al., 2007). The authors found that FCV with an initial inoculum of 3×10^5 PFU/ml deposited on the surfaces and incubated at 4 °C and room temperature was found to remain on the surfaces at ~ 10^3 PFU and $10^{2.5}$ PFU, respectively, after 7 days. Although FCV survived a longer time at 4 °C than at room temperature, this difference was not statistically significant ($P > 0.05$) (Mattison et al., 2007).

Hepatitis A virus was shown to persist at a half-life of 103 h (more than 4 days) when placed on SS disks and kept at 5 °C (Sattar et al., 2000). Also, HAV can persist on SS disks at a half-life of about 8 days, under low relative humidity and at 20 °C (Sattar et al., 2000). In a study on the survival of HAV on six food-contact surfaces (wood, rubber, stainless steel, ceramic, glass, and plastic), HAV survival and infectivity remained on SS coupons for up to 28 days under room temperature (Bae et al., 2014). Stainless steel had the greatest log reduction of 2.3 log PFU/coupon while wood had the lowest log reduction of 1.4 log PFU/coupon after the 28th day. The initial HAV titer inoculated on the different surfaces was 5 log PFU/mL (Bae et al., 2014). These studies have demonstrated that HuNoV and HAV can survive for prolonged periods on a variety of surfaces, with surface material, temperature, and humidity all having an impact on survival.

Chemical disinfection of foodborne viruses on surfaces

Because fomites serve as a means of transmission of human pathogenic viruses, effective disinfection of surfaces is key to controlling foodborne virus outbreaks (Taranisia MacCannell, 2011). Disinfection can be carried out using either chemical or non-chemical disinfectants.

Effective disinfection has been carried out with chemical disinfectants such as chlorine and chlorine-based compounds. Chlorine and chlorine-based compounds used in disinfection come in liquid form as chlorine solution or in gaseous form as chlorine dioxide. They are strong oxidizing agents that inactivate viruses by degrading viral protein and genomic RNA, which then disrupts the viral structure (Yeap et al., 2016). Chlorine dioxide (ClO_2) gas has been shown to inactivate MNV when the virus was inoculated on SS coupons at 10^7 PFU/coupon (Yeap et al., 2016). The virus infectivity was assessed after the samples were exposed to ClO_2 gas at 1, 1.5, 2, 2.5, and 4 mg/liter for a maximum of five minutes at 25°C and 85% relative humidity. At least a 3-log reduction of MNV was observed when the SS coupons were treated with ClO_2 gas at 2 mg/liter for 5 minutes and 2.5 mg/liter for 2 minutes and no infectious virus was recovered when the coupons were treated with 4 mg/liter of ClO_2 gas within 1 minute of treatment (Yeap et al., 2016). In another study, 50 µL of chlorine solution (a dilution of commercial Clorox, which contains 6% sodium hypochlorite) at 200 ppm or 1000 ppm was used to inactivate different dried HuNoV surrogates, FCV, MNV, TV, and porcine enteric calicivirus (PEC) on SS surfaces (Cromeans et al., 2014). Less than one log reduction was observed for all the viruses when the SS were treated at 200 ppm for 5 minutes, while a 5-log reduction was observed when the FCV was treated with 1000 ppm chlorine and ~1 log reduction was observed for the other viruses (Committee on Infectious Diseases et al., 2021; Cromeans et al., 2014). Although these methods are effective for the

disinfection of surfaces, chlorine leaves chemical residues that are unsafe for humans (Cromeans et al., 2010).

Light-based disinfection of foodborne viruses on surfaces

Disinfection of viruses has also been carried out with non-chemical disinfectants like light-based technologies such as UV-C and pulsed UV light. UV-C uses radiation between 250-280 nm to inactivate microorganisms while pulsed UV light uses low energy input, short and high-peak-energy light pulses with a wide range of wavelengths for inactivation microorganisms.

Using UV-C for disinfection, MNV and HAV on SS surfaces have been shown to inactivate in a dose-dependent manner. For MNV, a 1-log reduction in infectivity was achieved on SS exposed to UV-C at a dose of 33.3 mWs/cm^2, while for HAV, a higher dose was required, ~55.4 mWs/cm^2 (Park et al., 2015). The latter indicated a difference in susceptibility to UV-C between different viruses. In another study, 5 log reduction was observed on SS surfaces contaminated with MNV and HAV (at ~ 10^5 PFU/ml) suspended in phosphate buffered saline (PBS), after treatment with pulsed UV light for 2 and 3 seconds at a dose of 0.060 mWs/cm^2 and 0.091 mWs/cm^2 and 10.5 cm from the light source (Jean et al., 2011). When the virus matrix included organic matter in the form of fetal bovine serum (5% FBS), a 3.6 log reduction was observed for MNV, and complete inactivation (i.e., 5 log reduction) was observed for SS contaminated with HAV after similar pulsed UV light treatment (Jean et al., 2011).

Other technologies such as ozone and hydrogen peroxide vapor have been used to inactivate HuNoV implicated in outbreaks (Maclean et al., 2015). Although these methods are effective, they are time-consuming and laborious in food facilities and clinical wards as both patients and workers will have to evacuate the premises when in operation due to the deleterious effects of these

technologies (Otter et al., 2013). Hence, they can only be used for terminal disinfection when no one is present.

Persistence of foodborne viruses in freshwater surfaces

Freshwater contaminated with enteric viruses used in produce irrigation or post-harvest washing can lead to contamination of the produce. Many enteric viruses, including HuNoV, have been found in irrigation water, such as; surface water, groundwater, and drinking water samples (Gibson et al., 2011). In a meta-analysis of the prevalence of HuNoV in water sources, 61 studies were reviewed. Although the prevalence of HuNoV varied by type of water source, the highest estimate was 43.5% for river water, followed by estuarine water (30.6%), composite water (27.9%), marine water (25.9%), groundwater (19.7%), and lake water (2.2%). In natural water, the genogroups GI, GII, and GI & GII were present in 16.4%, 20.6%, and 12.8%, respectively (Ekundayo et al., 2021). Water contamination usually happens by overflowing sewage or septic tanks (Takuissu et al., 2023). A study has shown that HuNoV GI (Norwalk virus) can remain infectious for 61 days in groundwater as assessed by human volunteers (Seitz et al., 2011). The virus RNA remained detectable for three years when the contaminated groundwater was stored at room temperature in the dark. In this case, the virus infectivity was assumed by first treating the water sample with RNase before RT-qPCR analysis to be able to quantify only possibly infectious virions with intact capsids. (Seitz et al., 2011). A previous study on virus survival in freshwater (sterilized surface water and groundwater) showed that TV with an initial titer that ranged from 10^5 to 10^6 PFU/ml and a volume of 20 ml was inactivated at RT in surface water by 0.24 log after 28 days and $\geq 3.5 - 4$ log in groundwater after 21 days (Arthur & Gibson, 2016b).

Studies have shown that HAV survives for up to 12 weeks in untreated groundwater, which was incubated in the dark at 5 °C with a ~ 1 log reduction of the virus and a 1-2 log reduction of HAV

at 25 °C (Sobsey et al., 1986). Another study showed that at 4 °C, HAV survived and maintained its infectivity for up to 360 days in mineral water with < 1 log reduction of the initial titer of 10^7 $TCID_{50}$/ml (Biziagos et al., 1988). In contrast, at room temperature, HAV survived and maintained its infectivity for up to 330 days with a 5-log reduction of initial titer (10^7 $TCID_{50}$/ml). A meta-analysis review involving 144 articles and 200 HAV prevalence data from six distinct water sources from 1986-2020 showed that the prevalence of HAV is 16.7% worldwide. The prevalence of HAV depended on the type of water sources and was as follows: treated wastewater (18.0%), surface water (15.0%), groundwater (2.4%), drinking water (0.4%), and untreated wastewater (31.5%). In other types of water, the prevalence was (8.5%) (Takuissu et al., 2023). Taken together, these studies highlight the critical need for proper treatment of freshwater sources to reduce HuNoV and HAV transmission from contaminated freshwater to irrigated crops.

Waterborne outbreaks due to HuNoV and HAV

Noroviruses caused five outbreaks in recreational water in the United States from 2007 to 2008, affecting 121 individuals (Hlavsa et al., 2011). Two outbreaks were caused by HuNoV GII strains and happened in treated waters. Two of the three that happened in lakes with untreated water were caused by HuNoV GI strains. Human norovirus was the source of four of the 36 drinking water outbreaks that occurred during that time, resulting in 265 instances of sickness (Blackburn et al., 2004). Another HuNoV outbreak was observed among employees of the restaurant and a group of tourists due to contaminated water from the restaurant where the tourists had stopped 33 to 36 hours earlier to eat. This was due to the HuNoV GII strains, which contaminated the well that supplied water to the restaurant (Beller et al., 1997). In August 1980, 1,500 people in a village in northern Georgia suffered gastroenteritis for one week due to a

Norwalk virus outbreak that was correlated with the consumption of contaminated drinking water from the municipal water system (Kaplan et al., 1982). A major outbreak in Czech Republic in 1979 caused 28,880 people to be ill, which was caused by HAV-contaminated frozen strawberries due to sewage presence in irrigation water (Legge, 1997).

Factors affecting virus persistence in freshwater

Enteric viruses survive for prolonged periods in different types of water. Enteric viruses' persistence in water is affected by various conditions including temperature, exposure to light (UV), virus association with solids, and the presence of endemic microbiota (Bosch et al., 2006; John & Rose, 2005). These factors are expected to influence viral inactivation in water. For example, in a microcosm study of raw or filter-sterilized creek water inoculated with HuNoV and incubated between 10 to 20 °C in the dark, HuNoV decay rates were 0.69 to <0.01 day^{-1}, respectively (Kennedy et al., 2024). The latter indicated that when the water had biological and inert particles (raw freshwater), HuNoV exhibited a higher decay rate. In addition, at higher temperature, the HuNoV decay rate was faster. Variable viral decay rate constants may result from environmental events and processes that alter temperature, biological and inert particles in surface water, or both. Models of virus fate and movement in surface water may be improved by including the effects of particles, temperature, and their interactions (Kennedy et al., 2024). In another study, virus persistence was monitored in groundwater and surface water that had been spiked with HuNoV GI (Norwalk virus) and HuNoV surrogates- FCV and MNV and incubated at 25 and 4 °C (Bae & Schwab, 2008). Temperature played a role in virus inactivation in both waters. Specifically, at 25 °C, the rates of infectious virus reduction were significantly higher than those of nucleic acid reduction for FCV and MNV, but these rates were not statistically significant at 4

°C (Bae & Schwab, 2008). The type of water also affected virus infectivity reduction. For example, the infectivity of MNV and FCV, as well as RNA for HuNoV and MNV, declined significantly faster in surface water than in groundwater (Bae & Schwab, 2008). Other factors, such as indigenous freshwater bacteria and dissolved oxygen, were observed to affect the persistence of viruses in freshwater. For example, in a previous study, HuNoV was spiked into freshwater microcosms taken from the three freshwater ponds and incubated in an environmental chamber with a 12-hour photoperiod, 20–15 °C, and 50–80% relative humidity (daily–night) for two weeks (Esseili et al., 2025). In the three freshwater microcosms, infectious HuNoV showed variable persistence of ≤1 day to ≥7 days. However, RNA from intact HuNoV capsids showed longer persistence, ~4.2 to 57.5 days for 1 log reduction. Among the three ponds, the RNA from intact HuNoV had the quickest inactivation in a pond that had significantly higher conductivity, turbidity, total suspended particles, and salinity, suggesting a potential role for these parameters in virus inactivation in freshwater sources (Esseili et al., 2025).

Disinfection of foodborne viruses in freshwater

Effective virus disinfection is crucial in mitigating foodborne viruses in freshwater. Chlorine-based treatments have been shown to reduce HuNoV and HAV in freshwater sources. Sodium hypochlorite at different concentrations has been used to disinfect raw groundwater artificially inoculated with HuNoV GII.4 (4 log genome copies/µL) and HAV (5.5 log genome copies/µL). (Jeon et al., 2024). Specifically, using propidium monoazide (PMA)/reverse transcription quantitative real-time PCR (RT-qPCR), HuNoV and HAV were estimated to be inactivated by 1 log at ~116 to 99 ppm, respectively, during a 10 minutes contact period with sodium hypochlorite (Jeon et al., 2024). In another study, chlorine at concentrations of 10 or 20 ppm and after an

exposure time of 30 minutes completely inactivated HAV with an initial concentration of $10^{5.75}$ $TCID_{50}$/ml in sterile water (Li et al., 2002). Taken together, the type of water matrix can affect the inactivation of viruses in response to chlorine, as well as contact time and concentration.

Definition of blue light:

Blue light (BL) is light in the visible region defined within the wavelengths ranging between 380-500 nm (Zeiss, 2024). Its ability to inactivate microorganisms is due to the presence of the porphyrin structure within the pathogen. Porphyrin is an endogenous photosensitizer that has a multi-ringed structure (Amin et al., 2016). When microorganisms are treated with BL, this leads to the formation of reactive oxygen species upon porphyrin's excitation, which in turn leads to the death of the microorganism (Maclean et al., 2008; Murdoch et al., 2013).

The intensity or irradiance of BL emitted is a function of both the wavelength and the distance from the light to the sample (Bernardy & Malley, 2023). This intensity is measured using a spectroradiometer. The dose is obtained by multiplying the intensity of the BL by the time of exposure (in seconds) of the sample to BL.

$$\text{Dose (J/cm}^2\text{)} = \text{Intensity (mW/cm}^2\text{)} \times \text{Time (s)} \times 0.001$$

Blue light application in clinical settings

Blue light has been used in treating dermatological problems like acne vulgaris and improving skin texture (Leanse et al., 2022). Additionally, BL can be used for the treatment of several localized infections like eye infections and skin infections (Leanse et al., 2022; Zhu et al., 2017). Exposure of mice to BL (415 nm) for 6 h at a dose of 84 J/cm^2 gave a 3-log reduction of

Pseudomonas aeruginosa ex vivo and a 2-log reduction at 36 J/cm^2 in vivo (Zhu et al., 2017). Ex vivo here means enucleated mice eyes were used here to monitor the infection progression using bioluminescence imaging. In contrast, for the in vivo studies, live mice were used to monitor the infection's progression in the eye cornea (Zhu et al., 2017). Previous studies showed that BL (405 nm) has been effective in the inactivation of bacteria and certain fungi important in clinical settings and hospitals (Tomb et al., 2017). For example, BL at 450 nm at a dose of 500 J/cm^2 has been found to cause a 2.11, 2.35, and 6.88 log reduction to the biofilms of *Candida albicans*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Pseudomonas aeruginosa* respectively grown in brain heart infusion (BHI) broth (Ferrer-Espada et al., 2019). Another study analyzed the effect of exposure to BL (405 nm) for 18 h on steel surfaces (worktable and sink) and plastic surfaces containing MRSA at a concentration of 2×10^4/mL in a hospital setting. The BL irradiance was 1.5 W/cm^2 on the steel surface and 8.5 W/cm^2 on the plastic surface. A significant level of reduction was observed on both steel and plastic surfaces (2 log), with greater reduction on the steel surfaces (2.5 log) (Amodeo et al., 2023).

Blue light applications in the food industry

The surfaces of equipment used in the food industry are prone to microbial contamination. *Escherichia coli*, *Salmonella enterica*, and *Campylobacter* spp. are among the bacteria known to linger in food production facilities and on uncooked foods like fruits, vegetables, and raw seafood (WHO, 2020). Studies on the effect of BL at 405 nm on meat (packaged hot dogs) at three doses (30, 60, and 100 J/cm^2) showed a 75.61 - 96.34% inactivation of *Salmonella* while at a wavelength of 464 nm and light doses of 6, 12, and 18 J/cm^2 produced 80.23–100% significant inactivation of the bacterium (Guffey et al., 2016). BL at 405 nm with a dose of 2,672 J/cm^2 at 16-hour exposure

time has been shown to reduce the bacterium *Listeria monocytogenes* on stainless steel (SS) by 3 log CFU/cm^2 (Olszewska et al., 2023). BL with wavelengths of 420 and 460 nm on SS at doses that did not exceed 1,000 J/cm^2 did not give greater than 2 log CFU/cm^2 reduction *Listeria monocytogenes (Olszewska et al., 2023)*. Therefore, significant bacteria inactivation on food-contact surfaces has been demonstrated using BL treatment at different wavelengths and light dosages, and the efficacy of inactivation varies according to the duration of exposure and energy intensity.

Blue light against viruses

Due to the drawbacks of the previously listed virus-inactivation approaches as being unsafe either to humans or the environment, there is a need for effective, yet safe technology to combat these viruses. Hence, there is interest in the use of BL to potentially inactivate viruses because it has been shown to inactivate bacteria and other organisms. However, viruses do not possess endogenous porphyrin structures that are present within bacteria, which causes their inactivation on exposure to BL (Hessling et al., 2017; Rathnasinghe et al., 2021). Some studies found that BL may inactivate viruses in the presence of photosensitizers (CDC, 2024; Tomb et al., 2017). Photosensitizers are multi-ring structured compounds that have an identical chemical structure to the porphyrin ring (Guffey et al., 2016). For example, TV on blueberries that were coated with 0.1% of food-grade photosensitizers, rose bengal or riboflavin, showed ~ 1 and 0.5 log reduction, respectively upon exposure to 405 nm BL at 4.2 mW/cm^2 for 30 minutes (Kingsley et al., 2018). Another study used BL at 405 nm to investigate the inactivation of a HuNoV surrogate, FCV, in the presence of organically rich media, including secretions from humans –like artificial saliva, blood samples, feces, and vomit (Tomb et al., 2017). The authors showed that a dose of 421 J /cm^2

resulted in 5.1 log inactivation of FCV when suspended in a mixture containing riboflavin, tyrosine, tryptophan, pyridoxine, and folic acid. In addition, the virus showed a 5.1, 4.8, and 4.5 log reduction when it was suspended in artificial saliva, blood plasma, and artificial feces, respectively (Tomb et al., 2017). The inactivation of FCV in these media was suggested to be due to the presence of certain proteins in the media that interact with BL and lead to photosensitization (Guffey et al., 2016). Therefore, BL inactivation of surfaces is particularly important in decontaminating viruses in clinical settings where surfaces are prone to contamination with blood, vomit, and saliva.

Blue light at 420 nm has been shown to completely inactivate both enveloped virus (SARS-CoV-2 and respiratory syncytial virus) and non-enveloped virus (adenovirus) with an initial titer of 10^{10}, 10^{6}, and 10^{5} respectively within 15 minutes at an irradiance of 120 mW/cm² in the presence of photosensitizers like porphyrin or respiratory droplets (Guffey et al., 2016). This shows that the presence or absence of a viral envelope is not always required for the virucidal effects of BL; however, the presence of photosensitizers is important. The RT-PCR analysis showed that the viruses lost their infectivity but retained their viral genome (Terrosi et al., 2021). The latter suggests that the mechanism of inactivation of viruses by BL is due to oxidative damage in the presence of photosensitizers by the formation of ROS, which damages the viral envelope for enveloped viruses or capsid proteins for non-enveloped viruses (Bumah et al., 2017; Terrosi et al., 2021). However, the authors used viruses in suspension (cell culture media), which in real-life scenarios is not fully present around viruses.

Another study showed that BL with a wavelength of 455 nm and a dose of 7200 J/cm² in the presence of photosensitizers caused more than 3 log reduction of Phi6, which is an enveloped bacteriophage virus used as a surrogate of SARS-CoV-2 (Vatter et al., 2021). Phi6 was used at an

initial titer of 1.5×10^7 PFU/ml in phosphate-buffered saline (PBS) solution and BL was used at an exposure time of 40 h with a temperature maintained at 20 °C (Vatter et al., 2021).

In another study, the inactivation of feline infectious peritonitis virus (FIPV), an enveloped virus, by BL 405 nm in growth media (suspension) showed approximately 4.2 and 4.5 log reduction at 60 and 90 minutes, respectively, at a distance of 25 cm from the virus and irradiance of 16 mW/cm^2 (Gardner et al., 2021). The initial titer of FIPV tested ranged from 3.56×10^5 to 1.12×10^7 and differed with batches. Different surfaces (metal, paper, and plastic) were also tested for wet and dried FIPV for 405 nm BL (16 mW/cm^2). A significantly higher log reduction for dried FIPV on metal surfaces occurred when the virus was in artificial saliva as compared to no addition of saliva (~1 versus 0.1 log, respectively) (Gardner et al., 2021). In the presence of photosensitizers, BL has demonstrated promising virucidal effects, with its effectiveness varying based on the virus matrix with liquid suspensions demonstrating higher inactivation than when viruses are dried on surfaces.

Another research compared the viricidal efficacy of 405nm BL on surfaces to inactivate MS2 bacteriophage, a positive-sense single-stranded RNA virus, sometimes used as a surrogate for HuNoV (Bernardy & Malley, 2023). The surfaces used were ceramic, polytetrafluoroethylene (PTFE), and SS disks, and the experiments were done to test the effect of the environment- dew point [high (18 ˚C) and low (4 ˚C)]. The high-dew-point conditions yield higher levels of inactivation of the MS2 bacteriophage on all the surfaces tested at 50, 100, and 200 J/cm^2. The highest level of inactivation due to 405 nm BL (3.9 log) was observed on the PTFE surface, in the high-dew-point conditions at 200 J/cm^2 due to the higher reflectivity, lower porosity, and higher contact angles of these surfaces. In contrast, the ceramic surface showed the lowest virus inactivation (0.3 log) at 50 J/cm^2 (Bernardy & Malley, 2023). Also, no log reduction was observed for SS at low dew points at 0, 50, 100, and 200 J/cm^2. However, 0, 1.5, 2.3, and 3.76 log reductions

at the same time points were observed for the high dew point (Bernardy & Malley, 2023). The inactivation of viruses with BL varied based on BL dosage, type of surface, environmental factors such as temperature and humidity. Inactivation increases with higher BL dose and in the presence of photosensitizers. Furthermore, metal and PTFE surfaces showed greater inactivation than other surfaces at a higher-dew point.

To my knowledge, no previous studies explored the use of BL against viruses in irrigation water but given that these waters are usually rich in organic matter, BL may offer a way to reduce viral load in these matrices.

CHAPTER 3

MATERIALS AND METHODS

Generation of stock viruses

Tulane virus stock preparation was done by propagating TV in the kidney epithelial cell line of Rhesus Monkey (LLC-MK2) cells. The LLC-MK2 cells were grown in M199 media (Thermo Fisher Scientific, USA) supplemented with 5% horse serum and 1% antibiotic-antimycotic (AA) (Corning, VA, USA). When the cells reached 90-95% confluence, they were infected with TV. The TV inoculum was added to the infection media (M199 supplemented with 2% fetal bovine serum (FBS), and 1% AA) at 1:100 dilution. The old media was discarded, and the cell culture flasks were washed with Dulbecco's Phosphate Buffered Saline (DPBS). After this, the new TV inoculum was added to the cell culture flasks, and the flasks were incubated for 48-72 h at 37 °C until at least 80 % cytopathic effect (CPE) was seen. The flasks were subjected to three freeze-thaw cycles, after which the cells were harvested and centrifuged for 10 minutes at 3000 rpm at 4 °C. Some of the supernatants were aliquoted and stored at -80 °C while the remaining supernatants were ultra-filtered using Amicon ultra centrifugal filters (Millipore Sigma, Burlington, MA, USA) at 3000 rpm for 30 minutes to remove any remaining cell lysates and to concentrate the virus by a factor of 10 x. The ultra-filtered virus was aliquoted and stored at -80 °C until needed.

Hepatitis A virus stock preparation was done by propagating HAV on the FRhK-4 (Fetal Rhesus Monkey Kidney) cell line. The FRhK-4 cells were grown in Dulbecco's modified Eagle medium (DMEM; Corning, VA, USA) media supplemented with 10% FBS and 1% AA (Corning, VA, USA). When the cells reached 95% confluency, they were ready to be infected with the HAV

stock (HAV P4 PID 7). The HAV stock was added to the infection media (DMEM supplemented with 2% FBS, and 1% AA) at 1:100 dilution. The old media was discarded, and the cell culture flasks were washed with DPBS. After this, the HAV inoculum was added to the flasks, and the flasks were incubated for 7 days at 37 °C until at least 80 % cytopathic effect (CPE) was seen. The flasks were subjected to three freeze-thaw cycles, after which the cells were harvested and centrifuged for 10 minutes at 3000 rpm at 4 °C. The supernatant was also ultrafiltered as mentioned above, and aliquots were stored at -80 °C until needed.

Investigating the effect of BL against viruses on stainless steel

The effect of BL against viruses on SS was investigated at room temperature (RT) over time (1, 2, 4, and 12 h). Inside a biosafety cabinet, TV and HAV droplets (50 µL total) were pipetted on separate triplicate stainless steel (SS) coupons (7.6 by 2.5 cm) for the control and treatment groups for each intended time point. The SS coupons were purchased from Biosurface Technologies, (Bozeman, MT, USA). The viral droplets on the coupons were left to dry inside a biosafety hood for ~1 hour. Three 405 nm light-emitting diode (LED) array lamps (FASTTOBUY Resin Curing Light 405 nm with 20W Power) were used. Under each lamp, one replicate of SS designated for each time point (1, 2, 4, and 12 h) was placed at 11.2 cm from the lamps. Another set of control coupons containing dried viral droplets was placed beside the lamps but covered with aluminum foil (i.e., control unexposed group). This experimental design was repeated using ultrafiltered TV and HAV mixed at 1:1 (v/v) ratio with organic matter made of 10% filter-sterilized suspension of a human fecal sample in PBS (Lee Biosolutions, MO, USA).

The viruses were eluted from SS at 1-, 2-, 4-, and 12-hour time points using M199 (supplemented with 1% anti-anti and 2% FBS) for TV and DMEM (supplemented with 1% anti-

anti and 2% FBS) by pipetting up and down 10 times until no dried spots were visibly seen on the coupons. The eluted liquid was transferred into sterile 1.5 ml tubes and saved at -20 ºC until testing using the $TCID_{50}$ assay (as described below). At each time point, the average intensity of BL was measured using a spectroradiometer (Honle UV Technology, Germany).

Investigating the effect of BL against viruses in water

Tap water was collected and sterilized by autoclaving at 121 ºC for 30 minutes. The viruses, TV, and HAV, were spiked separately into the water at a 1:10 (v/v) ratio in 1 L glass bottles. The bottles were vortexed for 1-2 minutes to mix the viruses and then 30 ml aliquots were transferred into sterile plastic cups (Globe Scientific Collection Cup, NJ, USA). Immediately, 1 ml aliquots were taken in triplicate and saved to test later (this will constitute time 0 hour).

A set of virus-spiked water microcosms was placed under the BL lamps while another set of microcosms was placed nearby but unexposed to BL and remained capped (Controls). At each time point (1, 2, 4, and 12 h), 1 ml was taken from each replicate water microcosm and placed in 1.5 ml Eppendorf tubes. An equal volume of sterile water was added back to each replicate container. At each time point, the average intensity of BL was measured using a spectroradiometer, and the temperature was measured using a thermometer.

A similar experimental design was followed as the sterile (autoclaved) water above. However, the water used was collected from three natural freshwater ponds located in Griffin, Georgia, USA. Water samples were collected using a sterile water sampler and placed inside sterile bottles, which were taken to the lab and spiked immediately with TV and HAV.

Simultaneously, replicate water microcosms containing only raw freshwater (i.e. no viruses spiked) were either placed under BL lamps or not (control) to monitor the effect of BL on indigenous freshwater bacteria at 0, 1, 2, 4, and 12 h. General bacteria were tested at each time point, by plating several ten-fold serial dilutions of the water samples onto R2A agar plates (R2A agar from Oxoid Ltd. Hampshire, England). The plates were incubated for 3-4 days at RT. Plates with colonies between 3-300 were counted. Water quality parameters such as pH, conductivity, total dissolved solids, and temperature were monitored using the H19829 Multiparameter Meter (HANNA Instruments, USA).

$TCID_{50}$ assay for virus infectivity quantification

The $TCID_{50}$ assay is an endpoint dilution assay used to measure infectious viral titers. This happens by measuring the virus titer where the infected cells show at least 50% cytopathic effect. In this assay, the samples tested were first 10-fold serially diluted in a 96-well plate, then each dilution was tested on quadruplet wells of a 96-well plate containing the respective cell line for each virus (LLC-MK2 for TV or FRhk-4 plates for HAV). The cell culture plates were then incubated at 37 °C for 5-6 days for TV and 10-14 days for HAV.

Statistical analyses

Each experiment was conducted thrice independently, with a minimum of three technical replicates for each treatment or condition tested. Virus titers ($TCID_{50}$/ml) and bacterial counts (CFU/100 ml) were log_{10}-transformed. Mean values and standard errors were derived from all technical replicates. Statistical analyses were performed using GraphPad Prism version 10.4 (Graph Pad Software, USA). Analysis of variance (ANOVA) was utilized for comparing multiple means. Significant difference in means was determined when the P value was less than 0.05.

CHAPTER 4

RESULTS

Survival of viruses on stainless steel with and without BL treatment.

Viruses were either left in their culture media or mixed in organic matter, spot-inoculated on SS coupons, and then allowed to dry. In the control group, TV in media persisted on SS for 12 h with a significant decrease after 4 h, but then the virus infectivity titers stabilize till at least 12 h (Figure 1A). Under BL treatment, TV started to decrease significantly at 2 h and continued to significantly decrease till the 12 h time point (Figure 1A). In contrast, HAV remained stable throughout the 12 h period in the control group and showed a non-significant decrease in infectivity titers under BL-treatment (Figure 1B).

When the viruses were suspended in organic matter and then dried on SS, TV in the control group was stable throughout the 12 h period and only showed significant decreases under BL-treatment at the 12 h time point (Figure 1C). Similarly, in the control group, HAV infectivity titers were stable on SS throughout the 12 h period; however, under BL-treatment, HAV showed a non-significant decrease at the 12 h time point (Figure 1D). Except for HAV in media, both TV and HAV showed significant differences under BL-treatment as compared to control non-treated SS at the 12 h-time point (Asterisks in Figure 1).

Virus infectivity log reductions on stainless steel with and without BL treatment

On SS, BL doses were calculated based on irradiance and exposure time in seconds, and the virus log reductions were calculated from the control at each dose of BL. For TV in media on SS, there was a significant increase in virus infectivity log reduction at the dose of 1520 J/cm^2

(~0.6 log) in comparison to the 130 J/cm^2 dose (Figure 2A). Similarly, for TV in the organic matter matrix, there was a significant increase in virus log reduction at the dose of 1520 J/cm^2 (~0.6 log) (Figure 2B). For HAV in media on SS, there was a non-significant increase in virus infectivity log reduction, reaching 0.6 log at 1520 J/cm^2 (Figure 2C). In contrast, for HAV in organic matter, there was a significant increase in HAV infectivity log reduction, especially at the dose of 1520 vs.130 J/cm^2 (reaching 0.8 log) (Figure 2D). When comparing the log reductions of TV and HAV on SS exposed to any specific BL dose, there were no significant differences between the viruses (Table 1). The matrix of TV and HAV on stainless steel had no significant effect on virus inactivation under BL (Table 1).

Survival of viruses in sterile distilled water microcosms with and without BL treatment

Both TV and HAV were suspended in sterile distilled water microcosms of 30 ml, and then their infectivity titers were monitored with and without BL-treatments for 12 h. In control water microcosms, TV and HAV infectivity titers showed no significant changes in their infectivity titers over the 12 h period (Figure 3A and B). In BL-treated water, TV infectivity showed no significant changes whereas HAV infectivity showed significant decreases starting at 4 h, and further significant decreases at 12 h (Figure 3B). Significant differences occurred between control and BL-treated water for TV at 12 h and for HAV at 4 and 12 h (Figure 3).

1. **Virus infectivity log reductions in sterile distilled water microcosms**

For TV in sterile water, there was a significant increase in infectivity log reduction at the 1520 vs. 130 J/cm^2 dose (Figure 4A). The highest log reduction at the 1520 J/cm^2 dose was ~ 0.6 log (Figure 4A). In contrast, for HAV in sterile water, there was a significant dose-dependent increase starting at the 530 J/cm^2 dose (Figure 4B). At the highest dose of 1520 J/cm^2, HAV

infectivity showed ~2.5 log reduction (Figure 4B). When comparing TV and HAV exposed to a specific BL dose, there was significantly higher HAV inactivation than TV at the 530 and 1520 J/cm^2 doses in sterile water (Table 2).

Survival of viruses in raw freshwater microcosms with and without BL treatment

In the control freshwater microcosms, TV survival showed some variation between ponds. Specifically, for TV in pond I, there was a significant decrease in its infectivity titers starting at the 4 h time point, whereas in pond II and III, TV infectivity titers remained stable (Figure 5). Similarly, TV infectivity titers in BL-treated freshwater microcosms showed some variation among ponds. For pond I, TV infectivity titers significantly decreased in response to BL treatment starting at 4 h time points (Figure 5A). While in pond II, TV infectivity only showed a significant decrease at 12 h (Figure 5B). However, in pond III, TV infectivity under BL treatment remained stable like the control group (Figure 5C). When comparing TV infectivity between control and BL-treated freshwater, significant differences were found at 4 and 12 h for pond I and at 4 h only for pond II (Figure 5). For HAV in control freshwater microcosms, the virus infectivity titers were stable in all pond waters (Figure 6). In contrast, under BL treatment and in all ponds, HAV showed significant decreases in its infectivity titers starting at 1 h, with further significant decreases at 12h (Figure 6).

Virus infectivity log reductions in freshwater water microcosms

Virus log reductions were calculated from the control group at each dose and then were combined across the three ponds. For TV in freshwater, increasing the BL dose to 1520 J/cm^2 did not significantly increase virus infectivity reduction as compared to the other doses tested (Figure 7A). At the highest dose of 1520 J/cm^2, TV showed ~ 0.8 log reduction (Table 2). In contrast, for

HAV in freshwater, there was a significant dose-dependent increase starting at the 530 J/cm^2 dose (Figure 7B). The highest log reduction for HAV was ~2.65 log achieved at the BL dose of 1520 J/cm^2 (Table 2).

When comparing different matrices (sterile versus raw freshwater), HAV log reduction in infectivity was significant between sterile and freshwater microcosms at all doses except the highest dose (Table 2). In contrast, the matrix did not significantly affect TV log reduction (Table 2). This suggests that HAV inactivation in water is affected by the water matrix, whereas TV is less affected by the water matrix.

Survival of heterotrophic bacteria in raw freshwater microcosms with and without BL treatment

The indigenous freshwater bacteria count responded differently across the three ponds in the control group as well as under BL-treatment. Specifically, in pond I, the bacteria count in control freshwater microcosms showed a significant 0.3 log increase starting at 4 h and further significant increases by 0.6 log at the 12 h time point (Figure 8A). Although the bacteria count in BL-treated freshwater followed the same trend as the control group, the overall changes through the 12 h period were not significant (Figure 8A). There was a significant difference between control and BL-treated bacteria counts at the 4 h time point only (Figure 8A). In pond II, the bacteria count remained the same through the 12 h period in control freshwater microcosms; however, in BL-treated freshwater, there was a significant 0.2-log decrease at the 2 and 4 h time points (Figure 8B). There was a significant difference between control and BL-treated bacteria counts only at the 4 h time point (Figure 8B). In pond III, the bacteria in the control untreated raw freshwater microcosms behaved like in pond II i.e. they did not show any significant change in count through

the 12-h period (Figure 8C). In contrast, the bacteria in the BL-treated freshwater showed a significant ~0.3 log reduction in counts at 4 h, which further got reduced significantly by 0.5 log reduction at 12 h (Figure 8C). There were significant differences between control and BL-treated bacteria at the 4 and 12 h time points (Figure 8C).

The overall log reduction for bacteria across the three ponds, reached ~0.2 log CFU/100ml at the highest BL dose, but was not significantly different than other tested BL doses (Figure 9).

Physiochemical parameters of freshwater microcosms with and without BL treatment

The pH values of the water in pond I and III freshwater microcosms showed a similar significant increasing trend in both control and BL-treatment groups. Specifically, for pounds I and III, the pH increased significantly from 7.4 to 8 and from pH 7 to 7.8, respectively (Figure 10, A and C). In contrast, in pond II, the trend in pH values seemed to decrease over time from pH 8.5 to pH 8; however, the changes were only significantly different for the BL-treatment group (Figure 10 B,). There were significant differences between control and BL at 1 h for pond I and 1 and 2 h for pond III. However, for all ponds, the pH values returned to similar values as the control group at 12 h.

For water conductivity, there were non-significant variations across time for all three control freshwater microcosms (Figure 10, D, E, and F). Similarly, under BL-treatment, water conductivity varied slightly but not significantly (Figure 10, D, E, and F). There was only a transient significant difference between control and BL-treatment groups in ponds I and III at the 1 h time point (Figure 10, D, E, and F). Total suspended solids followed the same trend as water conductivity across all three freshwater microcosms for control and BL-treated water (Figure 10, G, H, and I).

The overall temperature variation combined from all three experiments for the various freshwater microcosms showed that in the control group, there was a significant 1.2 °C increase in water temperature starting at the 4 h time point (Figure 11). However, in BL-treated water, variation in temperature was not significant despite a 1 °C increase at the 1 h time point (Figure 11). When comparing control and BL-treated water, there was a 0.5 °C significant increase in the temperature of BL-treated water at the 2 h time point; however, by the 12 h time point, both waters were at a similar temperature (Figure 11). The latter indicates that overall, BL only transiently increased the water temperature by 0.5 °C.

Figures

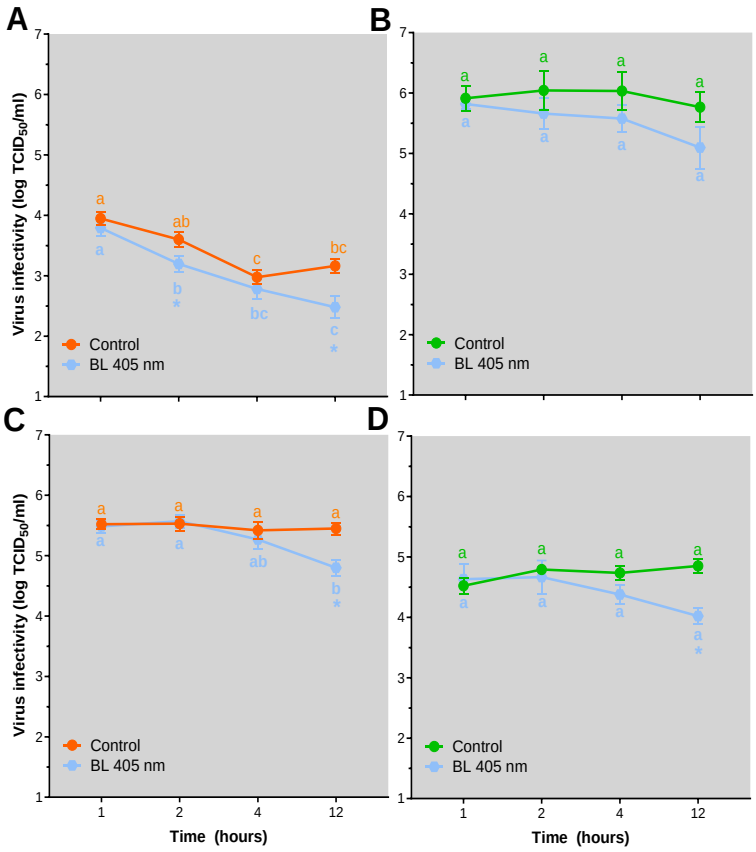

Figure 1: Survival of viruses on stainless steel (SS) coupons incubated at room temperature (RT) after treatment with blue light (BL). Viruses were either left in their original culture media: (A) TV and (B) HAV or suspended in organic matter: (C) TV and (D) HAV, before being spot inoculated on SS coupons. Means with different letters indicate significant differences ($p < 0.05$). The presence of asterisks indicates a significant difference between control and BL-treatment at a specific time point.

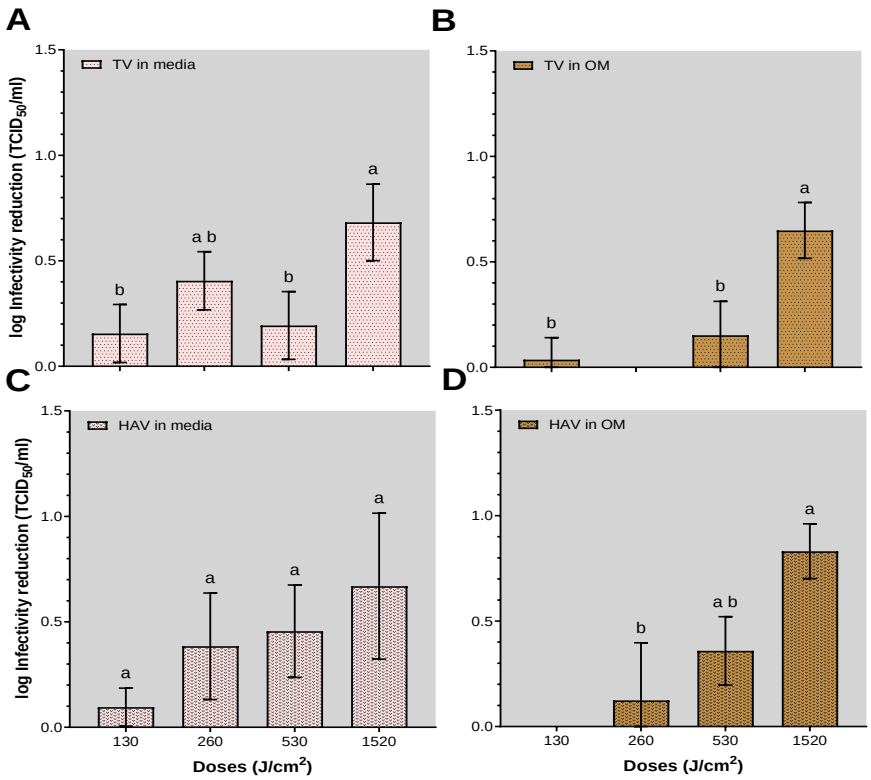

Figure 2: Virus infectivity log reductions on stainless steel (SS) coupons incubated at room temperature (RT) after treatment with blue light (BL) at different doses. Viruses were either left in their original culture media (A and C) or suspended in organic matter (B and D) before being spot inoculated on SS coupons. Means with different letters indicate significant differences ($p < 0.05$).

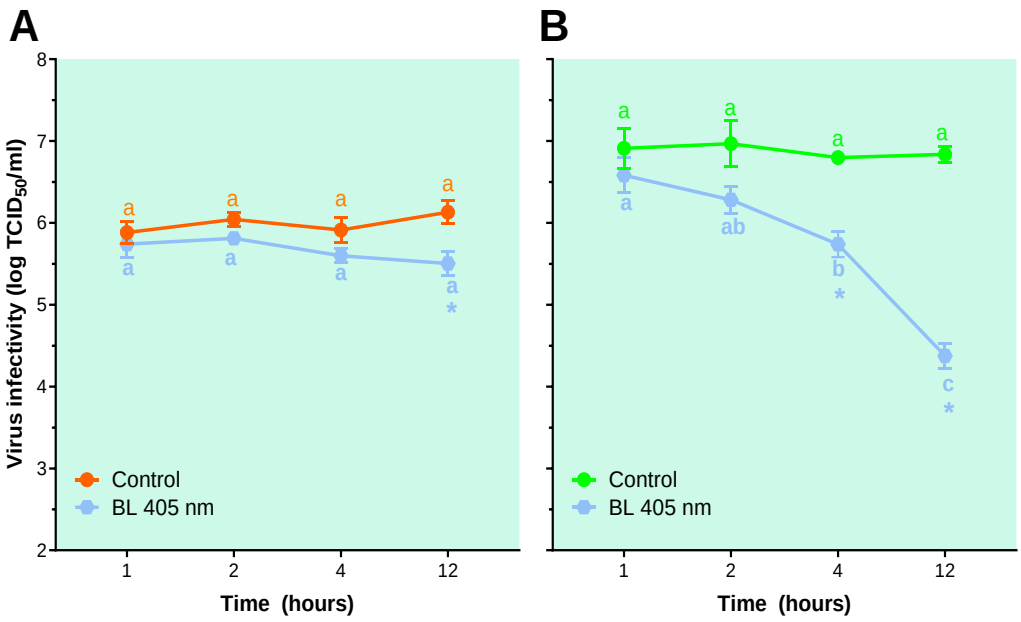

Figure 3: Survival of viruses, (A) TV and (B) HAV, on sterile distilled water microcosms incubated at room temperature (RT). Means with different letters indicate significant differences ($p < 0.05$). The presence of asterisks indicates a significant difference between control and blue light (BL)-treatment at a specific time point.

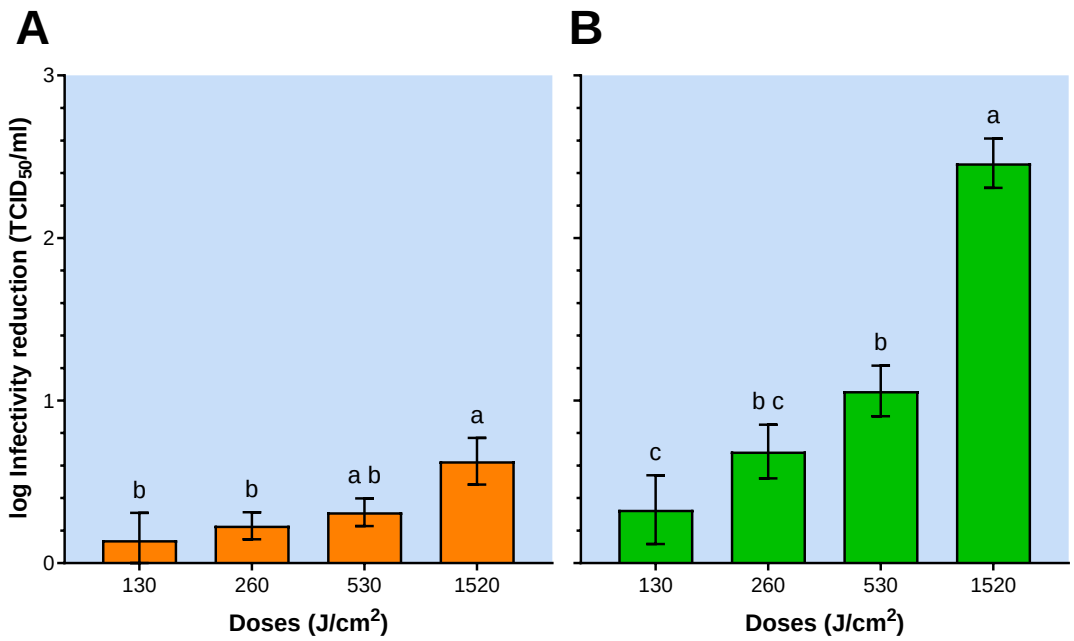

Figure 4: Infectivity log reductions for (A) TV and (B) HAV in sterile water microcosms incubated at room temperature (RT). Means with different letters indicate significant differences ($p < 0.05$).

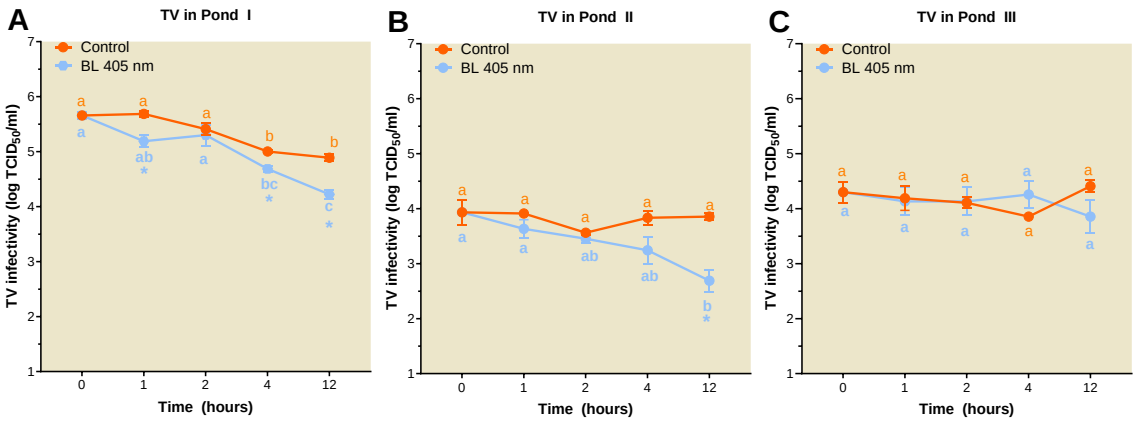

Figure 5: Survival of TV in freshwater microcosms incubated at room temperature (RT). Water was sampled from three freshwater ponds across Georgia. Means with different letters indicate significant differences ($p < 0.05$). The presence of asterisks indicates a significant difference between control and blue light (BL)-treatment at a specific time point.

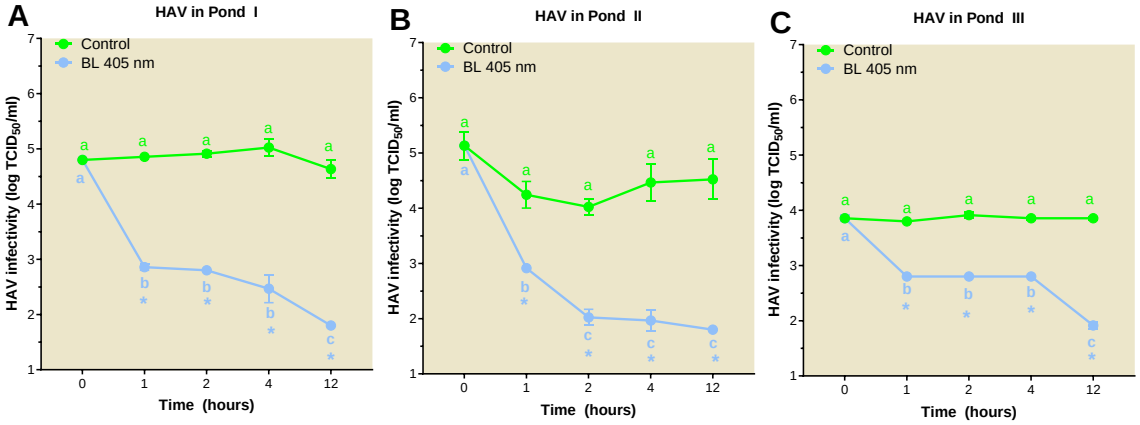

Figure 6: Survival of HAV in freshwater ponds microcosms incubated at room temperature. Water was sampled from three freshwater ponds in Georgia. Means with different letters indicate significant differences ($p < 0.05$). The presence of asterisks indicates a significant difference between control and BL-treatment at a specific time point.

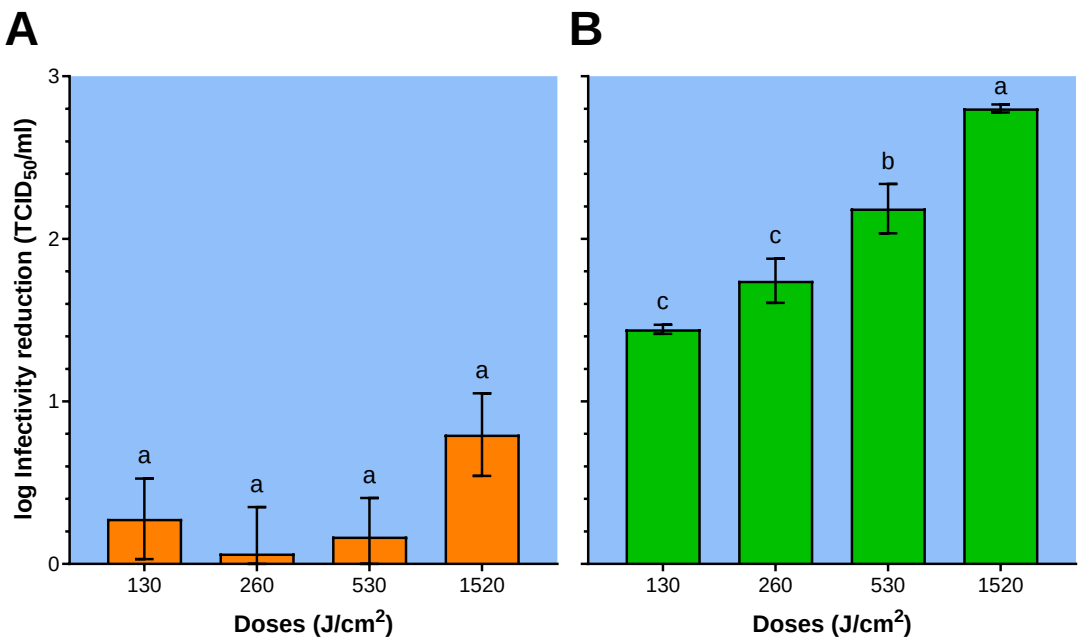

Figure 7: Infectivity log reductions for (A) TV and (B) HAV in freshwater ponds microcosms incubated at RT. Water was sampled from three freshwater ponds across Georgia. Means with different letters indicate significant differences ($p < 0.05$).

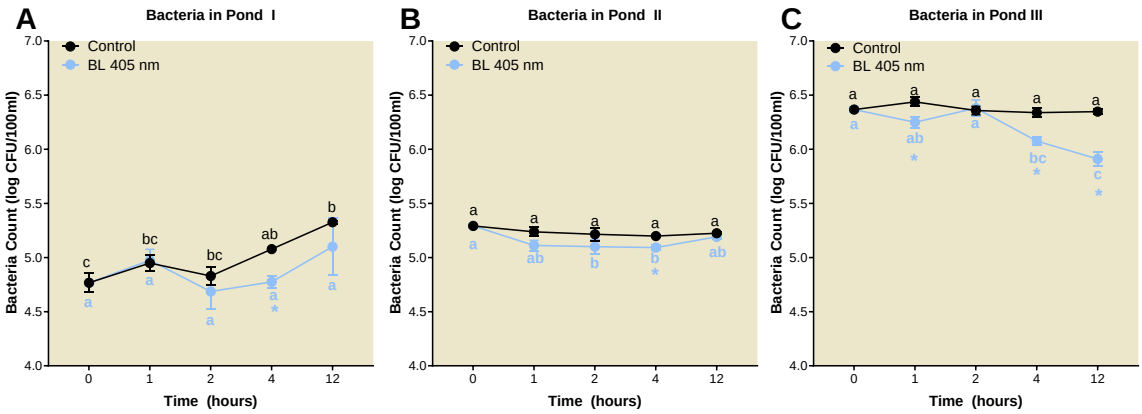

Figure 8: Survival of indigenous heterotrophic bacteria in freshwater ponds microcosms incubated at RT. Water was sampled from three freshwater ponds across Georgia. Means with different letters indicate significant differences ($p < 0.05$). The presence of asterisks indicates a significant difference between control and blue light (BL)-treatment at a specific time point.

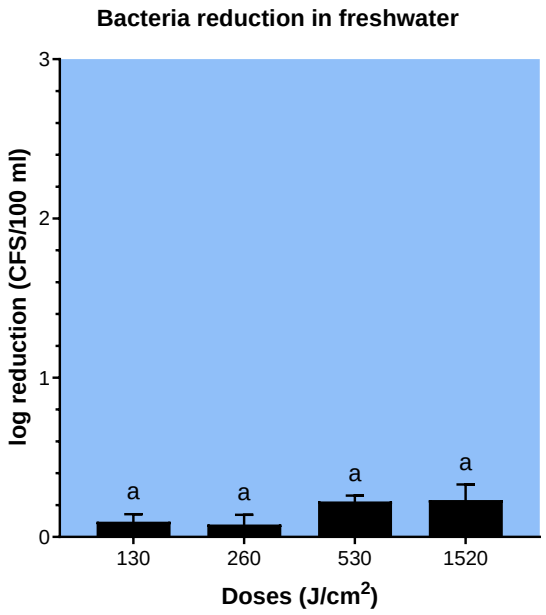

Figure 9: Overall log reductions in indigenous heterotrophic bacteria counts in freshwater ponds microcosms. Water was sampled from three freshwater ponds in Georgia. Means with different letters indicate significant differences ($p < 0.05$).

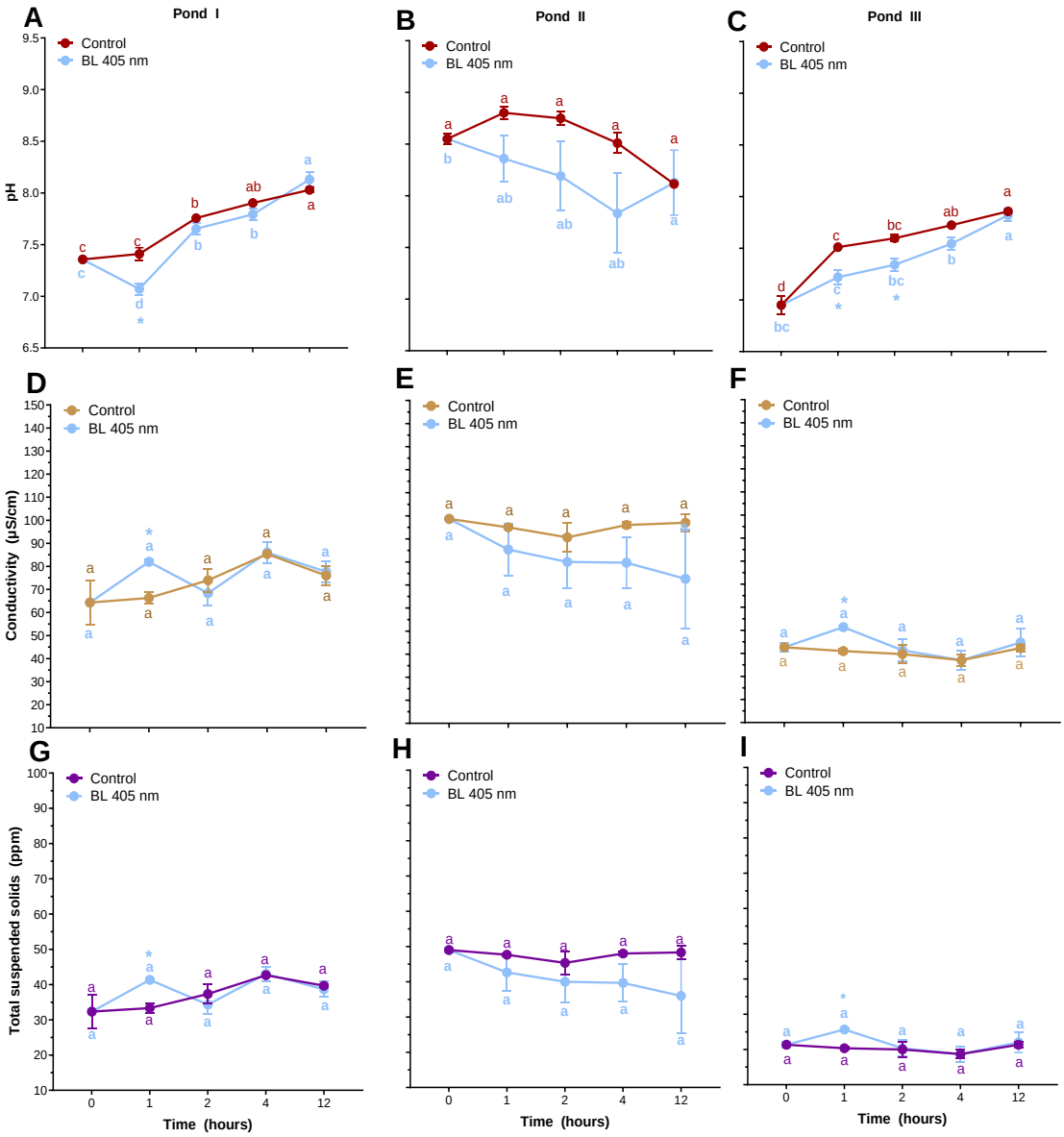

Figure 10: Freshwater ponds physiochemical parameters. The pH (A, B and C), conductivity (D, E and F), and total suspended solids (G, H and I) were measured across time in control as well as blue light (BL)-treated microcosms. Means with different letters indicate significant differences ($p < 0.05$). The presence of asterisks indicates a significant difference between control and BL-treatment at a specific time point.

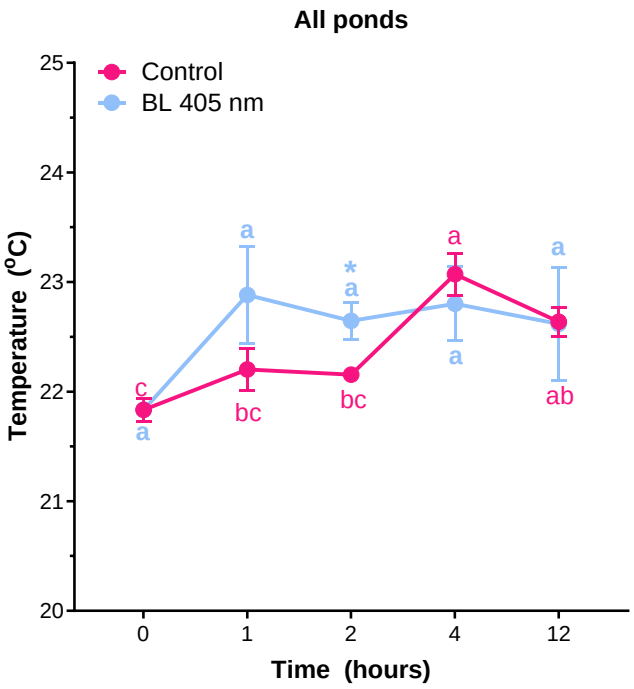

Figure 11: Overall temperature variations in the freshwater pond's microcosms. Means with different letters indicate significant differences ($p < 0.05$). The presence of asterisks indicates a significant difference between control and BL-treatment at a specific time point.

Tables

Table 1: Summary of infectivity log reductions for viruses dried on stainless steel and exposed to various doses of BL. Statistically significant ($p < 0.05$) means across a specific dose is indicated by different capital letter alphabets (Row). Means with different small letters indicate significant differences across a specific matrix ($p < 0.05$) (Column).

Stainless steel	Infectivity log reduction			
Virus	**TV**		**HAV**	
Matrix	Culture media	Organic matter	Culture media	Organic matter
Dose(J/cm^2)130	0.15 ± 0.13 Ab	0.03 ± 0.1 Ab	0.09 ± 0.09 Aa	-0.11 ±0.24 Ab
250	0.4 ± 0.13 Aab	-0.04 ± 0.1 Ab	0.38 ± 0.25 Aa	0.12 ±0.27 Ab
530	0.19 ±0.16 Ab	0.15 ± 0.16 Ab	0.45 ± 0.21 Aa	0.35 ±0.16 Aab
1520	0.68 ± 0.18 Aa	0.64 ±0.13 Aa	0.66 ± 0.34 Aa	0.83 ±0.12 Aa

Table 2: Summary of infectivity log reductions for viruses suspended in either sterile or raw freshwater and exposed to various doses of BL. Statistically significant ($p < 0.05$) means across a specific dose is indicated by different capital letter alphabets (Row). Means with different small letters indicate significant differences across a specific matric ($p < 0.05$) (Column).

Water	Infectivity log reduction			
Virus	**TV**		**HAV**	
Matrix	Sterile water	Raw freshwater	Sterile water	Raw freshwater
Dose(J/cm^2)130	0.14 ± 0.16 Bb	0.27 ± 0.24 Ba	0.32 ± 0.21 Bc	1.44 ± 0.02 Ac
250	0.23 ± 0.08 BCb	0.06 ± 0.28 Ca	0.68 ± 0.16 Bbc	1.74 ± 0.13 Ac
530	0.31 ± 0.08 Cab	0.16 ± 0.23 Ca	1.0 ±0.15 Bb	2.18 ± 0.15 Ab
1520	0.62 ± 0.14 Ba	0.79 ± 0.25 Ba	2.46 ± 0.15 Aa	2.8 ± 0.02 Aa

CHAPTER 5

DISCUSSION

Contamination of food-contact surfaces with pathogenic microbes is considered a significant health hazard because these surfaces can result in cross-contamination of food and subsequent foodborne outbreaks. It is important to ensure proper cleaning and disinfection of food-contact surfaces to reduce the spread of HuNoV. In this study, we explored the use of BL to inactivate HuNoV surrogate, TV, and HAV on SS, a food-contact surface commonly used in the food industry. In general, the stable persistence observed in our study for infectious TV and HAV on SS during the 12 h incubation period at RT was not surprising. This is because HuNoV has been shown to persist on food-contact surfaces such as SS with <0.5- 2 log reductions over weeks, depending on the virus genotype, initial level of virus titer, relative humidity, and temperature (Cook et al., 2016). Similarly, TV was shown to persist on SS for 2 weeks, showing only ~ 1 log reduction (Arthur & Gibson, 2016a). In addition, HAV has been shown to persist on SS under RT for weeks, showing ~2.3 log reduction after 4 weeks (Bae et al., 2014). In our study, only when the TV in media was used at a relatively lower initial titer on SS, a 1 log reduction was observed at 4 h. However, under a more relevant virus matrix, i.e. fecal suspension, both TV and HAV were stable on SS without any significant changes in infectivity titers through the 12 h period.

Two virus matrices were used in our research on SS surfaces: each virus's own cell culture media and organic matter made from fecal suspension. The culture media, M199 and DMEM, were used in the preparation of TV and HAV, respectively. These are routine media used to provide

LL-CMK2 and FRhK-4 cells with the necessary nutrients; otherwise, the viruses cannot be propagated in cell culture. The culture media contain several amino acids, inorganic salts, vitamins, and other nutrient components in varying amounts. Among these vitamins, is riboflavin, about 0.01 mg/L for M199 and 0.2 mg/mL for DMEM. According to the study by Kingsley et al. (2018), exposure of blueberry surfaces to BL (405 nm) at 4.2 mW/cm^2 (i.e. 7.5 J/cm^2) for 30 minutes resulted in 0.5 log reduction of infectious TV. A 0.1% of food-grade riboflavin (i.e. 1000 mg/L) was added as a photosensitizer to cause the inactivation of TV on blueberries. Though the amount of riboflavin in our culture media is small compared to that used by Kingsley et al. (2018), there could have been interactions, though limited, with other components of the culture media that caused even the small to moderate inactivation that was observed when TV and HAV in media were exposed to 405 nm BL at increasing doses on SS. Furthermore, another main difference between the two cell culture media is the use of 5% horse serum for TV and 10% FBS for HAV. Serum is used in some of the previous studies as an inherent organic matter naturally found in the cultured virus matrix. Because the log reductions at the highest BL doses (1520 J/cm^2) were not significantly different for TV and HAV in culture media on SS, this means that the difference in the composition of the two culture media, including serum % did not affect the response of these viruses to BL on SS. Otherwise, HAV (prepared in DMEM with 0.2 mg/mL riboflavin and 10% serum) should have shown more inactivation than TV (prepared in M199 with 0.01 mg/mL riboflavin and 5% serum). Furthermore, at lower BL doses (130, 250, 530 J/cm^2), the log reduction ranged between 0.09 to 0.45 for HAV and 0.15 to 0.4 log for TV. This is consistent with a previous study reporting that 405 nm BL doses from 50 to 200 J/cm^2 under RT, showed no reduction for bacteriophage MS2 (used as a HuNoV surrogate) on SS (Bernardy & Malley, 2023). In another study, only 0.1 log reduction was observed for FIPV dried on SS and subjected to 405 nm BL at a

dose of ~10 J/cm^2 under RT. Furthermore, this is consistent with previous studies, which showed that BL alone has limited effectiveness against inactivating non-enveloped viruses due to their lack of endogenous photosensitizers (Hessling et al., 2017; Rathnasinghe et al., 2021). Taken together, these studies suggest that at low BL doses limited inactivation of viruses dried on SS is expected. Future research should explore testing higher doses of BL to inactivate viruses in SS.

The organic matter used in this research is 10% filter-sterilized fecal suspension and would contain proteins, lipids, amino acids, carbohydrates, vitamins and other nutrient contents in different proportions. In this study, the SS surfaces spiked with HAV supplemented with organic matter, to act as an external photosensitizer, had a significant inactivation at 1520 J/cm^2 of 0.8 log. This corroborates with previous findings by Tomb et al. (2017) that in the presence of external photosensitizers like artificial saliva, blood samples, plasma, and feces, 405 nm BL gave higher reduction for FCV. However, for TV, the presence of organic matter had no significant enhancement giving similar results to those of TV in media at different light dosages. This suggests that TV may be more resistant to inactivation by BL than HAV. Also, even in the presence of photosensitizers, the virus structure, the interaction of the virus with organic matter, and susceptibility to oxidative damage play a role in the inactivation of the virus. This is consistent with another research which showed that the composition of the surrounding matrix affects the efficacy of BL in inactivating viruses (Bernardy & Malley, 2023; Gardner et al., 2021). Studies by Jean et al. (2011) showed that MNV and HAV exhibited a 5-log reduction when treated with UV light at 0.060 mWs/cm^2 and 0.091 mWs/cm^2 (J/cm^2). However, when organic matter (5% FBS), was left in the virus matrix, a 3.6 log reduction was observed for viruses on SS. In this case UV's effectiveness was reduced by the presence of organic matter which contrasts with our finding that

BL inactivation of TV and HAV on SS was not significantly affected by the type of organic matter. In general, in our study virus matrix on SS (whether media or fecal suspension) did not significantly affect virus inactivation in response to BL. The latter is very important, because the presence of organic matter on surfaces is known to reduce the effectiveness of some chemical disinfectants such as chlorine. Blue light from this perspective may provide an advantage as it did not seem to be affected much by the presence or type of organic matter for virus inactivation on SS. However, from another perspective, the virus log reductions on SS in response to BL were not high (< 1 log). All photosensitizers are organic matter, but not all organic matter are photosensitizers. The organic matter used for this study did not enhance viral inactivation; rather, it may have shielded the viruses from inactivation by BL, which might be due to the presence of lipids and proteins in the organic matter that absorb and scatter light, causing limited interactions with the viruses. This could also be that the amount of light-absorbing or photosensitizing materials e.g. riboflavin, tyrosine, tryptophan, and others in the organic matter, is small to cause sufficient photosensitization and formation of ROS, which will consequently lead to limited inactivation of the viruses on SS. Further research is needed to investigate different types of photosensitizers that can be sprayed on surfaces to enhance BL inactivation of viruses.

Freshwater resources contamination with fecal viruses such as HuNoV and HAV is a serious public health issue. Previous studies indicated that HuNoV and HAV are prevalent in different water matrices (Ekundayo et al., 2021; Takuissu et al., 2023), such as lake and river water, which can be used to irrigate fresh produce. Thus, ensuring safe freshwater for crop irrigation, especially of minimally processed produce, is important. Our research explored the effect of 405 nm BL on TV and HAV in water samples – sterile and raw freshwater. For the four BL doses tested in this study, no significant dose-dependent virus inactivation was observed for TV in sterile

water, which is expected. Moreover, only a modest inactivation was observed for TV in raw freshwater, with a significant inactivation of about 0.79 log at the highest dose of 1520 J/cm². A greater reduction of HAV in raw freshwater at 530 J/cm² and 1520 J/cm², giving about 2.18 and 2.8 log reduction, respectively, is due to the presence of organic materials (such as suspended solids) in the water, which may act as an exogenous photosensitizer in the raw freshwater. In previous studies, more than 3 log reduction of Phi6, a surrogate of SARS-CoV-2, was observed after exposure to BL at 455 nm, and a dose of 7200 J/cm², but this is expected because this was carried out in suspension. BL is more effective against viruses in liquid matrices compared to solid surfaces (Gardner et al., 2021; Vatter et al., 2021). This also explains why we found more inactivation of HAV in the freshwater samples than in the dried HAV on the SS surface. The 1 and 2.4 log reduction observed for HAV in sterile water at 530 J/cm² and 1520 J/cm² is unexpected because sterile water does not contain particles or organic matter that can react with BL to cause external photosensitization. However, the consistently greater susceptibility of HAV to TV across all matrices, regardless of water parameters for water samples, indicates an inherent viral susceptibility difference and not just matrix or environment alone. This can be due to differences in capsid structure and genomic composition. HAV is a member of the *Picornaviridae* family and therefore has a smaller, compact, and acid-stable capsid, which might render it more susceptible to ROS-mediated oxidation under continuous exposure to light, especially if suspended in aqueous matrices or environments. Conversely, TV, a member of the *Caliciviridae* family, might contain capsid proteins or dynamics that are less reactive to ROS, or that might lack the components or residues that promote photosensitization in comparison to HAV (Guffey et al., 2016). Furthermore, it was recently shown that HAV grown in cell culture may also be generated with a bilayer of lipids that is hijacked from that of the cell membranes (Feng et al., 2013). Thus, HAV is currently

described as a quasi-enveloped virus, because the lipid bilayer is not a typical envelope that other enveloped viruses have, and which typically contains viral proteins (Verbrugghe et al., 2024). Therefore, this may explain the inherent higher susceptibility of HAV in comparison to TV when exposed to BL treatments, whether in sterile or freshwater microcosms.

Although BL has previously shown promising results for the inactivation of foodborne bacteria on surfaces and in suspension (Amodeo et al., 2023; Olszewska et al., 2023)In our study, freshwater indigenous bacteria showed limited ~0.2 log reduction in response to various BL doses. No previous studies explored BL effect on indigenous freshwater bacteria. Our speculation is that these bacteria may be better adapted to environmental stressors such as sunlight exposure and thus be more resistant to BL-treatment than other lab-adapted foodborne bacterial strains used in previous studies. Part of the sunlight, especially the ultraviolet region (UV-B) is known to harm the biota of oceans and surface freshwaters; however, the visible region of sunlight (also called photosynthetically available radiation 400-700 nm) in general can have both positive (stimulation) and negative (inhibition) effects on heterotrophic bacteria due to the huge diversity of freshwater bacterial community and their wide responses to sunlight (Ruiz-González et al., 2013). This was also observed in our three freshwater microcosms, which showed a wide response under BL-treatment, ranging from increasing, stable, and decreasing counts. Further research is needed to uncover how BL over longer periods of time affects freshwater bacteria communities.

The pH is a measure of hydrogen ions (H^+) in a sample, and it is an important water quality indicator for freshwater bodies. The recommended water criteria for pH by the United States Environmental Protection Agency (EPA) is 6.5- 9 to sustain the life of various aquatic organisms (EPA, 2021). The pH of lakes naturally fluctuates throughout the day/night cycle due to the

photosynthetic activity of aquatic plants and algae during light conditions (pH 8.4) and respiration during night conditions (pH 7) (EPA, 2021). In our raw freshwater microcosms, the pH in the control group showed a variable response, ranging from significant increases (pond I and III: 7.4 to 8 and 7 to 7.8, respectively) to non-significant decreases (pond II: 8.5 to 8). In addition, BL treatment followed the same trend as the control, suggesting that BL only transiently affects the freshwater pH. Overall, the pH values in the control or BL-treatments were still within the EPA acceptable range.

Freshwater conductivity and total suspended solids are also among the important water quality indicators of freshwater sources. The EPA defines water conductivity as the ability of the water to pass an electric current, which is related to the amount of dissolved ions, such as salts and inorganic chemicals (EPA, 2025). Therefore, conductivity is directly related to total suspended solids and will often show the same trend as we observed in our results. Determining the baseline conductivity and total suspended solids of a pond is important as future significant changes in these levels can be used as indicators of new pollution sources (EPA, 2025). In our three freshwater microcosms, the overall trends for conductivity and total suspended solids were not significantly different across time. Also, our results showed only a transient effect for BL at 1 hour on water conductivity and total suspended solids, suggesting that BL-treatments used did not significantly affect these water quality indicators.

In this study, a slight significant increase in temperature (~0.5°C) was observed under BL as compared to control group, specifically at the 2 h time point. However, by 12 h both control and BL-treatment groups were at similar average temperatures (22.6 °C), which was close to the starting average temperature (21.8 °C). The latter suggest that temperature is unlikely to be a

contributing factor in virus infectivity or indigenous bacteria count reductions observed in our study. One limitation of this study is that it was carried out at RT, which would not mimic the diurnal changes in temperature experienced in actual pond water. Further studies should compare different day/night temperatures to investigate how viral inactivation varies with BL-treatments under field conditions.

CHAPTER 6

CONCLUSIONS

Contaminated SS surfaces exposed to the highest BL dose tested (1520 J/cm^2) resulted in infectivity log reduction for TV that was similar to HAV at ~ 0.6 and 0.8 log, respectively. The type of virus suspension matrix (media versus organic matter) on SS had no significant effect on virus inactivation under BL doses tested. Contaminated raw freshwater exposed to a BL dose of 1520 J/cm^2 resulted in significantly higher log reduction for HAV than TV at ~2.8 versus 0.8 log, respectively. The water matrix (sterile versus raw) significantly affected the inactivation of HAV but not TV under BL treatment. The indigenous bacteria seemed to be either transiently affected by BL treatments or reduced significantly within the 12 h tested period. Further research is required to determine what role these bacteria play in virus reduction in freshwater in response to BL treatment. Water quality parameters such as pH, conductivity, and total suspended solids either changed transiently or did not change significantly in response to BL-treatments. This is the first study to explore the effect of varying doses of BL (405 nm) against TV and HAV on SS as well as in raw freshwater sources. Overall, BL showed some promising results for virus inactivation on surfaces and in freshwater; however, more research into other photosensitizers and higher BL doses should be explored to further enhance the effect of BL against viruses on SS and in freshwater.

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