A Two-Fold Approach to Addressing Water Insecurity and Vector Control: Water Disinfectants for the Control of Aedes Aegypti and Engineering Social Justice

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Table of Contents

List of Tables and Figures

I. Executive Summary

The objective of this dissertation to address unsafe water storage is twofold. Firstly, to address the risk of mosquito infestation caused by unsafe household water storage (HWS), laboratory studies were conducted to examine the effectiveness of water disinfectants (chlorine, silver, and copper) as a means of vector control. Inadequate access to safe drinking water within the household, experienced by 1 in 4 people, is a pervasive issue, often rooted in systemic causes. As a result of water insecurity, households cope by storing water inside or around the home. To address these engineering problems demands not only technical solutions but also a comprehensive, critical understanding of the interplay between the technical, social, economic, and political factors that contribute to the pervasiveness of the issue, Thus, the second objective of this dissertation was to establish a framework that would facilitate developing and applying these crucial social competencies in the engineering design process.

While all water disinfectants tested within drinking water quality guidelines showed potential to considerably reducing the *Aedes aegypt*i population, sodium hypochlorite exhibited the highest performance of decreasing survival of late first instar larvae while silver nitrate exhibited the highest effectiveness for inhibiting emergence of late third instar larvae. Since none of the treatments led to complete inhibition of the emergence of late instar mosquitoes, combinations of the water disinfectants were tested to determine the most effective approach. While the combination treatments did not always perform better than the individual chemical treatments against younger instar larvae, they achieved higher inhibition of emergence against older instar mosquitoes as compared to the water disinfectants used separately. The combination of silver and copper proved to be the most effective in this regard, resulting in inhibiting the emergence of roughly 97% of the *Ae. aegypti*. This research provides communities, organizations, and governments with guidance regarding chemical treatment alternatives that can serve as viable options for addressing both vector control and water treatment management of HWS containers.

To evaluate the undergraduate engineering students' perspective on the significance of incorporating a social justice-oriented lens in engineering education, a survey was conducted before and after the Social Justice in Engineering Design (SJ-ED) module and workshop. The survey results indicate that the vast majority of the students in the class were motivated to pursue engineering because they wanted to make a positive impact on people's lives and believed that promoting social justice is crucial. However, less than half of the students had previously participated in a class or workshop related to engineering social justice, even though approximately eighty percent of them recognized the relevance of social justice to engineering even before partaking in the SJ-ED workshop. After the SJ-ED intervention, students were significantly more likely to: think they will encounter social justice issues; see social justice as relevant to engineering; have an opportunity to address social justice issues within an engineering profession; and feel they knew more about social justice than before the module. The research offers valuable insights for engineering educators on how to effectively engage and retain young engineers effectively in the classroom by appealing to their social agency and furthermore, offers guidance to facilitate the growth of social agency within them.

II. Dedication

I would like to dedicate this work to the memory of two remarkable individuals who have had a profound impact on my life and academic journey. **Pastor Jesse Jerome Johnson**, who worked at the University of Virginia (UVA) for more than two decades, was my guiding light during my time as both an undergraduate and graduate student. He instilled in me the confidence to keep pursuing engineering and empowered me to believe in my ability to succeed. Secondly, I would like to dedicate this work to **Dr. Louise Stokes Hunter**, the first Black woman to graduate from UVA. a trailblazer whose pioneering achievements have paved the way for generations of Black women to graduate from UVA. Her legacy continues to inspire and motivate me to take up space and support my sistxrs.

III. Acknowledgments

First and foremost, I acknowledge that the University of Virginia is located on the ancestral and traditional land of the Monacan people. The **Monacan Nation** has lived in and cared for this land for thousands of years, and their historical and cultural connections to this region continue to this day. I acknowledge and honor the labor and humanity of the thousands of **enslaved people** who were forcibly brought to this land, including those who were enslaved at UVA. Their contributions to the development of this institution and the wider community are immeasurable, yet their stories and experiences have often been silenced and marginalized. I acknowledge the ongoing work of **descendants, scholars**, and **community members** in preserving the memory and legacy of these people. I recognize the harm and lasting legacy of white supremacy in our community, and commit to engaging in ongoing reflection, education, and action towards acknowledging and redressing historical and current injustices.

During my time at UVA, there were numerous people who played a significant role in contributing to my PhD journey. I would like to begin by thanking my advisor, **Dr. Jim Smith**, and committee, **Dr. Lisa Colosi Peterson, Dr. Garrick Louis, Dr, José G. Juárez,** and **Dr. Meara Habashi**, for their guidance and mentorship throughout the entire process. I am so grateful for the time and effort they dedicated to helping me pursue my research interests. I would also like to acknowledge the Department of Engineering Systems and Environment (ESE) Staff (**Dottie Gardner, Chantel Gross, Michelle Busby, Shelley Thomas, and Briana Dandridge**), the Center for Diversity (s/o to **James Bland** and **Katie Giwa-Osagie**), Office of African American Affairs (s/o to **Dean Michael Mason**), the Office of Diversity, Equity and Engagement (s/o Associate Dean for DEI **Dr. Meara Habashi**), the ESE DEI Committee (s/o **Dr. Garrick Louis**), UVA Library Staff (s/o to **Clay Ford** and **Erich Purpur**), and UVA SEAS Staff (s/o to **Dr. Brian Smith, Blake Jimenéz-Calhoun,** and **Priya Date),** for their unwavering support throughout this process, removing roadblocks, enabling me to pursue my aspirations. Their dedication to their job and encouragement were crucial in enabling me to achieve my academic goals and reach this point.

I am grateful to the funding I have received to pursue my research, including from the **American Association of University Women, Achievement Rewards for College Scientists Foundation Scholar Metropolitan Washington Chapter, the Raven Society, the Black Faculty and Staff Employee Resource Group (BFS ERG), Graduate Assistance in Areas of National Need Fellowship, and the Dean's Scholar Fellowship. The NSF Louis Stokes Alliance for Minority Participation Bridge to Doctorate Fellowship** funded my masters' research at Colorado State University (CSU) and ultimately prepared me for this next step. This financial support over the years made this research possible.

I am so appreciative of the connections I have made through working in UVA's Water Quality Lab. **Dr. Beeta Ehdaie**, who served as my first research mentor during my undergraduate studies, played a pivotal role in fostering my passion for research. Working alongside **Chloe Rento** and **Veronica Son** made my undergraduate research experience enjoyable and adventurous, even on the days of mundane, routine lab tasks. Throughout my doctoral studies, I received mentorship from senior researchers including **Dr. Courtney Hill, Dr. Charles Burgis**, **Dr. Gail Hayes,** and **Dr. Rekha Singh**. Additionally, I thoroughly enjoyed collaborating with my colleagues, **Dr. Ana Estrella-You, Dr. Jamie Harris, Dr. Wuhuan Zhang,** and (future Dr.) **Roshne Tahmina**

Ahmed, who consistently demonstrated a willingness to assist, connect, and motivate one another. My gratitude extends to the undergraduate research laboratory assistants, including **Sophie Howle, Karin Brett, Julia Davis, Lorin Bruno, Victoria Cecchetti,** and the lab managers, **Dr. Derek Henderson, Ryan Henry,** and **Dr. Tony Singh.**

One of the most significant and transformative aspects of my PhD experience was collaborating with fellow graduate students who shared a vision of a more just and inclusive engineering field, where social justice was at the forefront of our work. Together, we formed a collective, Social Justice in Engineering Design (SJ-ED). that challenged the status quo of engineering and worked towards creating a more equitable and accessible future for the profession and those it affects. I would like to express my sincere appreciation to the SJ-ED team, **Dr. Patrick Hancock, Dr. Bethany Gordon, Dr. Katelyn Stenger,** and (future Dr.) **Tomeka Carroll**, for their invaluable contributions to co-creating a welcoming space in engineering that values old and new knowledges and embraces diverse perspectives. I also want to thank our collaborators, including **Dr. Courtney Rogers, Dr. Sadegh Eghdami, Maggie Salomonsky, Sophie Howle, Kelly Schaefer,** and the **Engineering Social Justice and Peace (ESJP) Network.** I feel a great sense of pride to be surrounded by individuals who continuously motivate and inspire me. I look forward to our continued work together toward creating a more equitable and just world.

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How can I even begin to express the depth of my gratitude towards my family… My family has been my biggest source of love and support. Mom and Dad, your encouragement and belief in me built the foundation for my strong spirit, providing me with the conviction to pursue my passions. My parents allowed me opportunities to try new things, to succeed, to make mistakes, and to ultimately build trust in myself. When you have three siblings with graduate degrees, one who flies in the sky, one who dives to the bottom of the ocean, and another who added an entirely new dimension to life through artistic and creative abilities, you can see that my parents instilled in us that the sky was *not* the limit because there was no limit. **Bryce** & **Kim**, **Dr. Margaret** & **Abigail**, and **Marshal**, thank you for always being there for me and for being my best friends. I love that we are the same type of crazy. **Toni Lou, Birdy bell,** and **Tascha**, I am so grateful for the companionship and adventures we have been on together through it all.

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1. The Overview

To build resilient public health strategies in communities without access to safe drinking water, this dissertation addresses two main areas: A) the immediate needs of communities for safe storage of clean drinking water and B) the importance of educating engineers to acquire critical social competencies required to tackle the systemic causes of the lack of access to clean water.

Laboratory studies presented in *Chapters 2 and 3* focus on analyzing the efficacy of water disinfectants for mosquito larval source management of *Aedes aegypti,* designed to resemble the context of treating household water storage (HWS) containers simultaneously for waterborne pathogens and vector control.

While the results from the laboratory study demonstrate how a particular method may be effective in reducing the emergence of adult mosquitoes, the results from the study do not address (1) how people will interact with the technology due to cultural and behavioral practices (Figueroa & Kincaid, 2010), (2) how the most marginalized and vulnerable groups will get access to the technology (Workman et al, 2021; Ezbakhe, Giné-Garriga & Pérez-Foguet, 2019; Roller, 2019), and (3) the root causes of water insecurity that have led to unsafe water storage practices (Deitz & Meehan, 2019; National Academies of Sciences, 2017; Balazs & Ray, 2014). To engineer or design comprehensive solutions to public health problems, engineers must reflect upon and contextualize the work within a wide variety of domains (e.g. social, behavioral, cultural, political, ecological, biological, historical) in a holistic manner with a diverse set of people in order to produce positive and just outcomes (Lue et al, 2022; Workman et al, 2021; National Academies of Sciences, 2017; Quintero et al, 2014). Thus, in *Chapter 4*, the role of engineering education to prepare future engineers to design with a social justice-oriented lens is examined.

1.1. Discovering a Double-edged Sword: Water Disinfectants for Water Treatment and Vector Control

For regions experiencing water shortages and water stress, especially within communities without access to uninterrupted, reliable piped water connections (UN-Water, 2021, Deshpande et al, 2020), a common coping strategy is the storage of water within or around the home (Venkataramanan et al, 2020; Barrera, Ávila, & González-Téllez, 1993). Unsafe water storage practices can lead to adverse public health outcomes caused by water degradation and mosquito proliferation (Overgaard et al, 2021; Pinchoff, 2021; Akanda et al, 2021; Vannavong et al, 2017; Quintero et al, 2014). *Ae. aegypti* is a species of mosquito that is notorious for its role in transmitting diseases such as dengue fever, yellow fever, and Zika virus (European CDC, 2023; Leta et al, 2018). Typically found in tropical and subtropical regions, the *Ae. aegypti,* among other species, have demonstrated resistance to common insecticides currently employed in HWS containers, such as temephos, within the context of large vector borne disease mitigation efforts

(Dusfour et al, 2019; Vontas et al, 2012). Thus, the research presented within this dissertation assesses the efficacy of alternative chemicals to mitigate larval growth, reduce emergence of adult mosquitoes responsible for transmitting disease while simultaneously making sure water being treated is safe for human consumption.

More specifically, the laboratory studies were designed to assess the larvicidal effects of juvenile *Ae. aegypti* after their aquatic growth environment was dosed with common water disinfectants, silver, copper, and chlorine, at concentrations within drinking water quality guidelines (***Chapter 2***). After assessing the chemicals' efficacies in decreasing emergence of *Ae. aegypti* at concentrations below drinking water quality guidelines, the next stage of experimentation tested chemicals in combination to see if higher efficacy for inhibition of emergence could be achieved (***Chapter 3***). The research seeks to provide communities, organizations, and governments with guidance regarding chemical and point-of-use water treatment (POUWT) alternatives that may serve as viable options for addressing both vector control and water treatment management.

1.2. Addressing the Source of Engineering Problems

Societal contexts that underpin the laboratory studies in this dissertation include:

- poverty
- social inequality (e.g., disparities across gender, race/ethnicity, class, neurodiversity, age),
- marginalization (e.g., social, political, economic, and educational exclusion and disenfranchisement), and
- insufficient access to resources (e.g., historical exploitation of resources, low quality access to public services).

Laboratory studies can easily be criticized for their lack of relevance or application to real-world settings, primarily due to limitations that make conducting studies feasible and replicable (Chapanis, 2007; Ho, Gray & Spence, 2014). Furthermore, the field of engineering, typically characterized as the practical application of scientific and mathematical principles to design solutions for real world problems (Pawley, 2009), primarily focuses on technical solutions with little to no regard for: (1) the social, behavioral, and cultural factors that contribute to these problems (Nieusma, Dean & Tang, Xiaofeng, 2012) or (2) the ethical and moral implications of engineering work (Banks & Lachney, 2017). Ultimately, this brings into question whether engineers truly understand how their work will be applied in real world contexts (Cumming-Potvin & Currie, 2013) and if engineers are motivated to enhance public welfare (Garibay. 2015; Cech, 2014; Riley, 2008).

Across the many disciplines of engineering–civil, environmental, systems, chemical, mechanical, electrical, biomedical, and others–numerous instances, such as those listed next, illustrate the challenging nature of tackling the world's most pressing and complex problems:

- inequality via technology, e.g., the digital divide, bias in facial recognition and AI (Zajko, 2022; Khalil et al., 2020; Robinson et al, 2020; Benjamin, 2019),
- disproportionate displacement of communities due to large-scale engineering projects (such as highways, dams, and urban development) and/or climate change (Piggott-McKellar et al, 2020; Fleming, 2018; Lunstrum, Bose & Zalik, 2016; Anguelovski et al., 2016; Karas, 2015),
- disparities within disaster preparedness and recovery actions (Smith et al., 2022),
- discrimination in healthcare and bias in medical research (Obermeyer, Powers, Vogeli & Mullainathan, 2019; Williams & Cooper, 2019; Vazquez, 2018; Konkel, 2015; Lustick & Zaman, 2011),
- disproportionate exposure to chemicals and hazardous waste, e.g., toxic materials in consumer products and proximity of waste disposal (Henderson & Wells, 2021; Olden, Ramos & Freudenberg, 2009; Elliott et al., 2003),
- disparities within access to basic services (such as water, housing, electricity, transportation), clean energy, and sustainable materials (Hidayati, Tan & Yamu, 2021; Brosemer et al., 2020; Carley & Konisky, 2020; Balazs & Ray, 2014),
- barriers, biases, and exclusion mediated within the profession of engineering itself, e.g., gender and racial pay gaps, stereotypes, and implicit bias (Holly & Masta, 2021; Martin & Fisher-Ari, 2021; Sterling et al., 2020; Longe & Ouahada, 2019; Asplund & Welle, 2018).

Pertinent to the research in this dissertation is the topic of vector control. Consider the history of DDT (dichlorodiphenyltrichloroethane), acknowledged as both an engineering marvel and an ecological disaster. First introduced in the United States as a pesticide in the 1940s, DDT became quintessential in the fight against malaria, typhus, and the other insect-borne human diseases (US EPA, 2022). It was used extensively around the world; however, over time, unintended consequences of the widespread use of DDT (e.g., toxicity to non-target wildlife, the environment, and humans) became increasingly more evident (Mansouri, 2017; Beard, 2006), especially after the publication of Rachel Carson's *Silent Spring* (1962) ignited an environmental movement (Griswold, 2012). As a result, the use of DDT was banned or severely restricted in many countries, although the Stockholm Convention on persistent organic pollutants (POPs) allowed for the singular use of DDT for the mitigation of malaria (US EPA, 2022; van den Berg, 2009). Both the positive and negative environmental and health impacts of DDT were not equally distributed, revealing stark inequities in the fight against malaria (Donley et al., 2022; Stratton et al, 2008). This is a common theme within the public health field, demonstrated further by WHO’s World Health Report of 1998 conclusion that the world’s greatest risk factor for disease was poverty (Lucas & McMichael, 2005).

This calls for a more comprehensive approach to engineering that centers the underlying social, economic, and political factors that contribute to building and maintaining our society’s inequities. Examples such as these have led many scholars to believe that engineers are inadequately prepared to address the complex problems that many in society perceive engineers have a professional and ethical responsibility to try to resolve (Hancock & Turner, 2023; Gunckel & Tolbert, 2018; Leydens & Lucena, 2017; Walther, Miller & Sochacka, 2017; Karwat et al. 2015; Cech, 2013; Riley, 2008).

1.3. Examining Engineering Education Through a Social Justice Lens

For these reasons, the laboratory studies presented within this dissertation are followed by a study at the intersection of engineering education and social justice (***Chapter 4***). By incorporating a social justice lens into engineering practice, engineers can promote equality and respect for human rights and dignity through their work (Karwat, 2019). This can help to ensure that the benefits of technology are shared equitably and that the potential negative impacts are minimized (Hancock & Turner, 2023). Objective 3 of this dissertation involves the creation of an online learning module that teaches engineering students about the social justice implications of their work. The effectiveness of the module was evaluated through a pre- and post-survey to understand the students' perceptions of social justice in relation to engineering and how those perceptions changed after taking the module.

1.4. Concluding Remarks

Chapter 5 of the dissertation provides an overview of the key findings, broader impacts, strengths, and limitations of the studies.

1.5. Dissemination of Knowledge

Chapter 4 of this dissertation on engineering social justice has been published with the American Society for Engineering Education (ASEE): Turner, S., Hancock, Gordon, B., P., Carroll, T., Stenger, K., 2022). A second manuscript for this work is currently under review. Building knowledge within the field of engineering social justice, I have also shared first authorship on two other published peer-reviewed journal articles (Hancock & Turner**,** 2022; Carroll, T., Gordon, B., Hancock, P., Stenger, K., and Turner, S., 2022). This work has been presented at many conferences, such as the ASEE Annual Conference and the Engineering Social Justice and Peace Conference.

The manuscripts for Chapter 2 and Chapter 3 are currently under preparation to be submitted to peer reviewed journals. This work has been presented in many different settings including the University of North Carolina Conference on Water & Health and University of Michigan's Conference on Sustainability & Development.

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2. A Double-edged Sword: Analyzing the Efficacy of Water Treatment Disinfectants as Vector Control

This chapter is currently being prepared as a manuscript for publication.

Abstract

In 2020, around one in four people lacked safely managed drinking water in their homes (UNICEF, 2021). For communities without access to uninterrupted, piped water, household water storage (HWS) practices can lead to adverse public health outcomes caused by water degradation and mosquito proliferation. Eighty percent of the world's population is at risk of vector-borne disease with over 700,000 deaths caused by vector-borne diseases annually (WHO, 2020a). The objective of this study was to determine whether water disinfectants, at concentrations deemed safe for human consumption, are effective in reducing the emergence of adult mosquitoes that transmit disease. The laboratory study presented within this paper assesses the larvicidal effects of juvenile *Aedes aegypti* after their aquatic environment is dosed with sub-lethal concentrations of silver, copper, and chlorine at concentrations within drinking water quality guidelines. All water disinfectants that were tested within the guidelines for drinking water quality demonstrated the ability to significantly reduce the population of *Ae. aegypti.* Sodium hypochlorite was found to be the most effective in decreasing the survival rate of late first instar larvae. Silver nitrate exhibited the highest effectiveness in inhibiting the emergence of late third instar larvae. None of the treatments resulted in the complete inhibition of emergence of late instar *Ae. aegypti* larvae into adult mosquitoes. Ultimately, this study aims to provide more information regarding the potential of different water treatment options to improve public health outcomes within communities, advocating for a more integrated approach to Water, Sanitation, and Health (WASH) solutions and vector control.

2.1. Introduction

A vector-borne disease (VBD) is one caused by the spread of viruses, bacteria, and parasites from a contaminated animal to a human. VBDs account for more than 17% of the global burden of infectious diseases, and 80% of the world's human population is at risk of one or more VBDs (World Health Organization [WHO], 2017). The vectors, or the infected animal spreading the disease, are often blood-sucking insects (WHO, 2020a), the most prolific of which are mosquitoes (de Almeida et al., 2021). A resurgence and growing burden of certain VBDs across the world has been credited to many factors, such as globalization, international trade, climate change, and urbanization (Baker, et al., 2022; Pley et al., 2021; Shragai et al., 2017; Institute of Medicine. 2008).

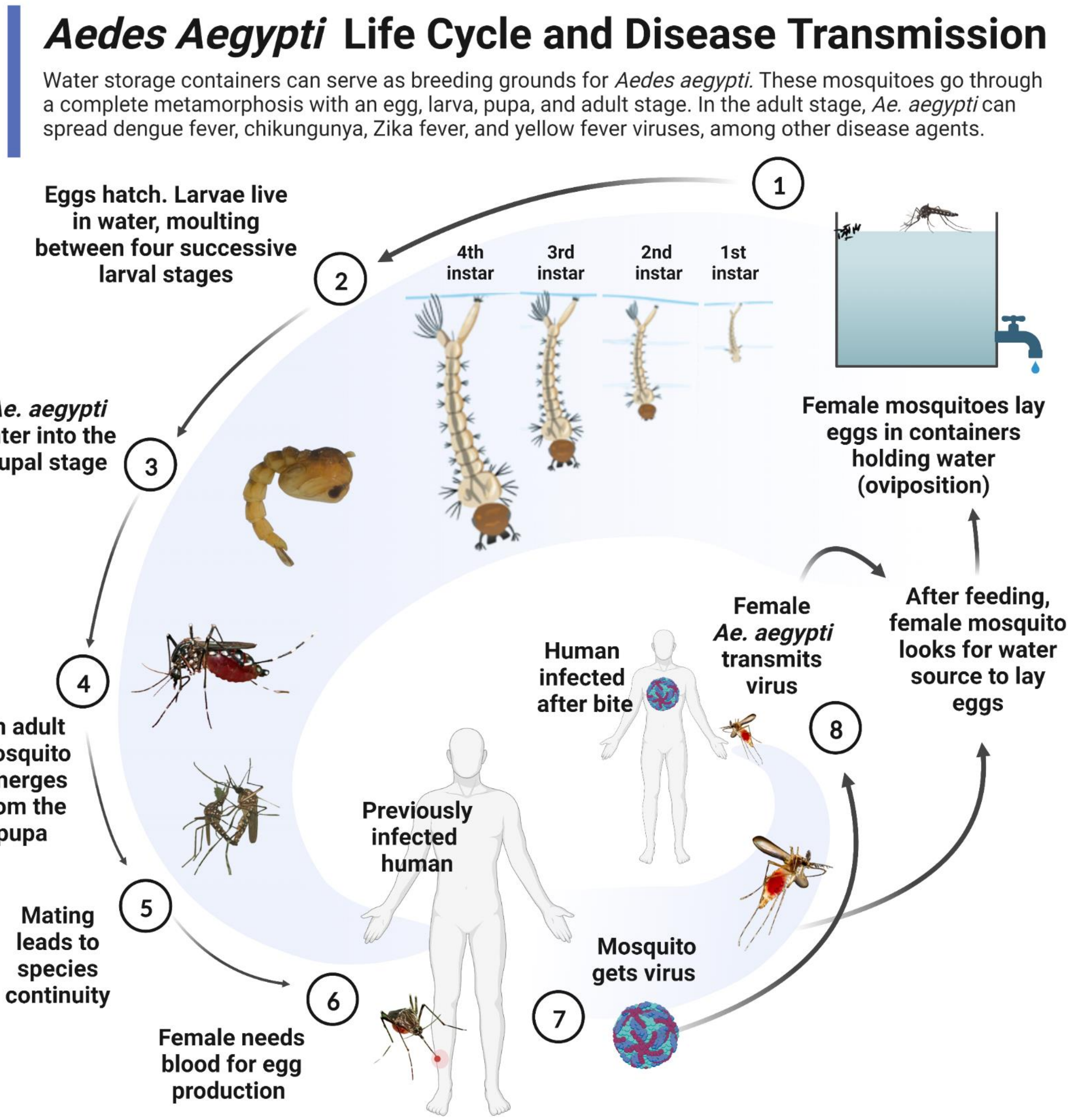
The goal of vector control management is to reduce the risk of disease transmission by control of the mosquito vector population or interruption of human–vector contact (Wilson et al., 2020; WHO, 2009). Different vector control management strategies, as classified in Erlanger, Keiser & Utzinger (2008), are presented in **Table 2.1**. Vector control programs often face challenges in securing adequate funding and prioritization, especially since many VBDs tend to be significant public health threats in primarily low- and middle-income regions where resources for vector control programs may be limited (Wilson et al., 2020). Thus, many of these VBDs are classified as neglected tropical diseases (NTDs), as they primarily impact populations that are underserved or marginalized, especially communities lacking access to basic healthcare, clean water, and sanitation (Boisson et al., 2021; Tidman, et al., 2021). Sustained global efforts backed by investments in alleviating poverty will be required to reduce the burden of VBDs (WHO, 2023a; Boisson et al., 2021; WHO, 2020b). The World Health Organization published their first draft of a Vector Response Plan in 1982 in an attempt to create a global and sustainable solution for VBDs. The WHO has periodically updated their Vector Response Plans as the presence and transmission of VBDs worldwide fluctuates due to climate and societal factors (WHO, 2017a).

Aedes aegypti, one of the two most abundant mosquito species, is responsible for transmitting several arboviral diseases, such as Chikungunya, Dengue, Rift Valley fever, Yellow fever, and Zika (WHO, 2020a). Understanding the life cycle of this mosquito species, as well as its behaviors and habitats, is crucial for developing effective strategies to prevent and control the spread of vector-borne diseases (McGregor & Connelly, 2021; Smith et al, 2014). *Ae. aegypti* are found in close association with humans and lay their eggs in water or on damp surfaces. They can only transmit VBDs as adults, the final of four life cycle stages for the mosquito (see **Figure 2.1** for depiction of *Ae. aegypti* life cycle). Studies have shown that *Ae. aegypti* do not fly far from their breeding grounds, with one study suggesting a coverage of 200 m for the use of integrated vector control management approaches (Juarez et al., 2020).

Table 2.1 Categories of vector management practices. Descriptions from Erlanger, Keiser & Utzinger (2008) and Keiser, Singer, & Utzinger (2005)

Major categories of vector management practices
Chemical Control
Spraying chemicals
Chemicals introduced within the treatment of water
Biological Control
The introduction of larvivorous organisms such as fish, copepods, and insect larvae into water containers
The release of transgenic vectors with reduced capacity to transmit disease and reproduce
Environmental Management
Environmental modification—describes measures aiming to create a permanent or long-lasting effect on land, water, or vegetation to reduce vector habitats (e.g., the installation and maintenance of drains)
Environmental manipulation—describes methods creating temporary unfavorable conditions for the vector (e.g., water or vegetation management)
Modification or manipulation of human habitation or behavior to reduce human–vector contact (e.g., screening doors and windows, using insecticide-treated nets, covering and screening of water containers)
Integrated vector management
Using a variety of approaches together, usually facilitated through community-based approaches

Dynamic models of mosquito-borne pathogen transmission (MBPT) have attempted to build theoretical frameworks that capture epidemiological and entomological concepts and metrics for measuring transmission in order to illuminate vulnerabilities in the transmission cycle to guide disease control programs (Smith et al, 2014, Smith et al., 2012). Models, such as expansions to the popular Ross–Macdonald MBPT model, have made evident that vector control management needs to target different life stages, including: (1) *larval and pupal control*, involving the stages at which the living organism is typically aquatic, and (2) *adult control*, involving the stage which is responsible for transmitting diseases. For example, insecticides, even at sublethal doses, have been shown to affect the different mechanisms associated with the mosquitoes ability to reproduce such as their fecundity (Antonio et al., 2009; Firstenberg & Sutherland, 1981), egg hatching (Giusti et al., 2014; De Coursey et al., 1953), immature development (Wijeyaratne, 1976), adult longevity adult size (Kelada et al., 1981) and blood feeding (Belinato & Valle, 2015 & Liu et al. 1986). **Figure 2.2** illustrates examples of different control methods (chemical, biological, mechanical/environmental) based on the development of the *Ae. aegypti*. These different layers of vector control management can be used in combination or in a tailored approach to the specific context and needs of a given community or region.



***Aedes Aegypti* Life Cycle and Disease Transmission**

Water storage containers can serve as breeding grounds for *Aedes aegypti*. These mosquitoes go through a complete metamorphosis with an egg, larva, pupa, and adult stage. In the adult stage, *Ae. aegypti* can spread dengue fever, chikungunya, Zika fever, and yellow fever viruses, among other disease agents.

Figure 2.1 Life cycle of the *Aedes aegypti* and disease transmission. Figure created with Biorender.com.

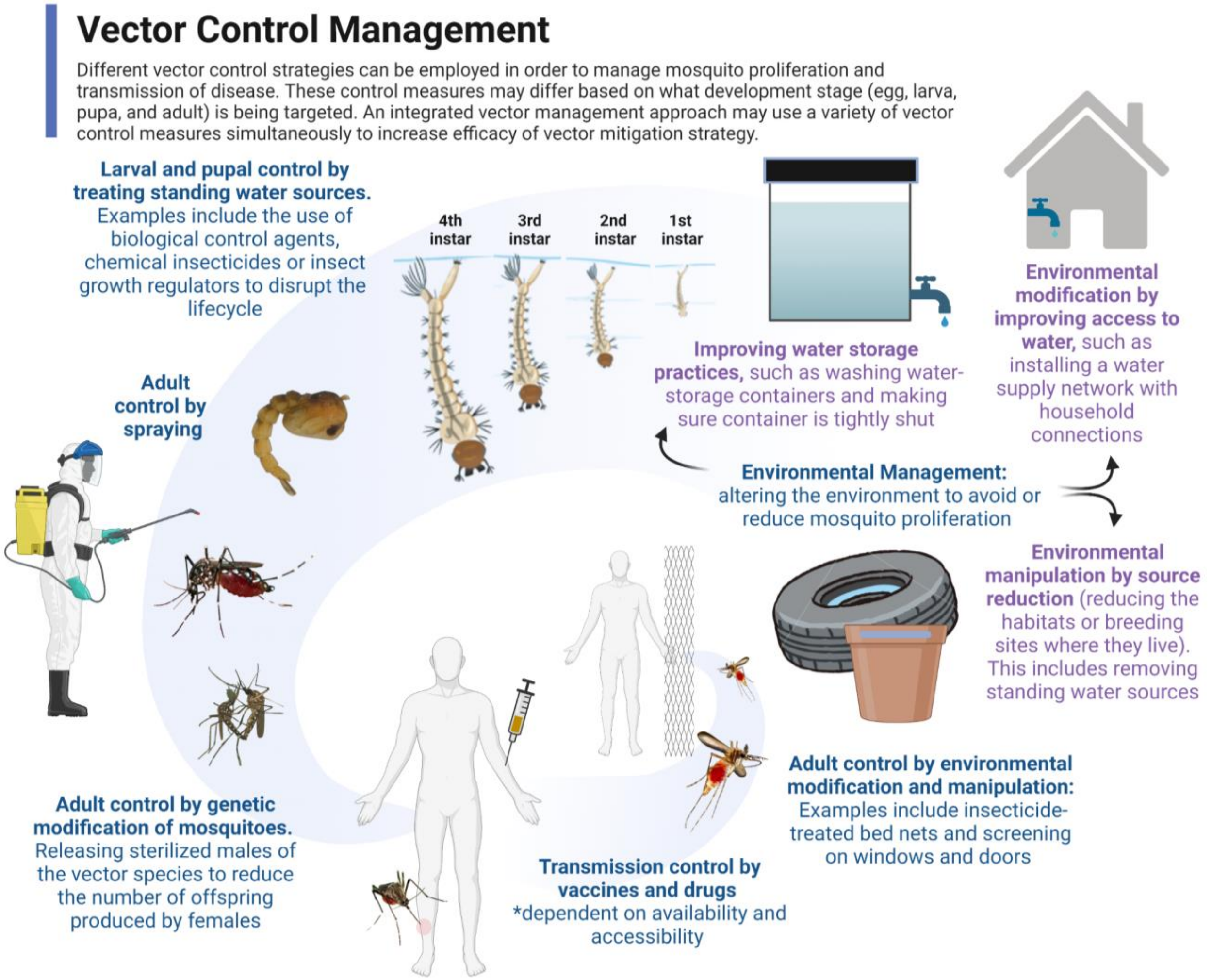


Figure 2.2 Vector control strategies that target different developmental phases of the *Aedes aegypti*. Vector control examples (biological, chemical, environmental management, and integrated practices) informed by Agyemang-Badu et al (2023); Erlanger, Keiser & Utzinger (2008); and Keiser, Singer, & Utzinger (2005). Figure created with Biorender.com.

Household water storage containers, such as buckets, drums, or jars, are commonly used to store and access water for daily use particularly in poorly resourced settings without access to a reliable, piped clean water supply (see **Figure 2.3)**. When not managed properly, household water storage containers become contaminated with pathogens that can cause illness (Wright, Gundry, & Conroy, 2004) and also can become a common breeding ground for *Ae. aegypti* (Padmanabha et al, 2010; Barrera, Ávila, & González-Téllez, 1993), presenting a higher risk scenario for the contraction of VBDs. For these reasons, it is important to implement proper water storage and hygiene practices, such as covering containers and using treated water, to reduce the risk of disease transmission (Quintero et al., 2014).

Figure 2.3 Observations of water storage containers in Dzimali, South Africa. Photos captured by Sydney Turner in 2020.

Within the study presented in this paper, we focus on reducing transmission risk by mitigating the emergence of larvae into adult mosquitoes by larval control. The goal is to disrupt the life cycle of the vector, preventing it from reaching maturity and being able to spread disease. The focus on larval control in this study is important, as it addresses a crucial stage in the vector's development. It is important to note that this is just one piece that must fit in a larger strategy of vector control management, as multiple layers are necessary for comprehensive control. As stated in Erlanger, Keiser & Utzinger (2008), integrated vector management provides a tailored, holistic approach to vector control management that involves the integration of multiple control methods, such as chemical, and environmental management, to achieve the most effective and sustainable control of vectors. By reducing the number of adult mosquitoes that emerge, the risk of disease transmission can be reduced in support of a larger, more comprehensive approach to vector control management within communities.

Types of chemical control include (Bellinato et al, 2016):

1. Insecticides and larvicides, referring to chemical substances used to control insects by killing them. These neurotoxic compounds fall into four primary classes: carbamates, organochlorates, organophosphates, and pyrethroids;
2. Spinosyn, referring to a class of natural insecticides derived from the fermentation products of the soil bacteria *Saccharopolyspora spinosa* that disrupt the normal functioning of the insect nervous system; and
3. Insect growth regulators (IGRs), referring to chemicals that interfere with the normal growth and development of insects, preventing them from reaching maturity. This class can be further divided into two main classes: juvenoids and chitin synthesis inhibitors.

Table 2.2 lists examples of different chemical compounds and formulations commonly applied in vector control programs. Larvicides are often used to quickly reduce insect populations but can have negative impacts on non-target species and lead to insecticide resistance. Benefits of IGRs include having a more targeted approach which typically results in less harm to the environment and slower development of insecticide resistance; however, IGRs are often less effective in controlling established insect populations (Karunaratne & Surendran, 2022). Nation-scale vector control programs predominantly utilize larvicides on WHO Pesticide Evaluation Scheme's (WHOPES) Prequalified Vector Control Products list, which includes temephos, pirimiphos-methyl, polydimethylsiloxane, diflubenzuron, pyriproxyfen, novaluron, spinosad, pyriproxyfen, and BTI strain AM65-52 (WH0, 2023b).

The development of resistance to common chemicals by mosquitoes, such as temephos, has had significant implications for the control of vector-borne diseases, as the efficacy of temephos as a control tool has diminished in many regions around the world. **Table 2.3** provides a list of chemicals currently used in water storage containers and instances of resistance that have been observed among the *Ae. aegypti* species to those chemicals (Bharati & Saha, 2021; Guedes et al., 2020; Valle et al., 2019; Moyes, et al., 2017). The laboratory study presented in this paper assesses the efficacy of common water disinfectants, chlorine, silver, and copper, as alternative chemicals that may be useful in treating potable water containers.

Table 2.2 Compounds and formulations for control of mosquito larvae

Insecticide	Chemical Type	Dosage of active ingredient (g/ha)	Formulation	WHO Hazard Classification of Active Ingredient (a)	Use in drinking water: Recommended (Y/N)	Maximum recommended dosage for use in potable water (mg/L)	Acceptable Daily Intake (ADI: mg/kg bw)
Fuel oil	–	b	Solution	–	N	–	
B. thuringiensis israelensis [Bti]	Biopesticide	c	WG	III ****	Y	1-5 *****	–
Diflubenzuron	Insect growth regulator	25-100	DT, GR, WP	U	Y	0.02-0.25 *****	0–0.02 *****
Methoprene	Insect growth regulator	20-40	EC	U	Y	1 *****	0–0.09 *****
Novaluron	Insect growth regulator	10-100	EC	NA	Y	0.01–0.05 *****	0–0.01 *****
Pyriproxyfen	Insect growth regulator	5-10	GR	U	Y	0.01 *****	0–0.1 *****
Chlorpyrifos	Organophosphate	11-25	EC	II			
Fenthion	Organophosphate	22-112	Emulsifiable concentrate granules	II			
Pirimphos-methyl	Organophosphate	50-500	EC	III	N	1 *****	0–0.03 *****
Temephos	Organophosphate	56-112	EC, GR	U	Y	1 *	0.023 *****
Spinosad DT	Biopesticide		DT, GR, SC	III ****	Y	0.25–0.5 ***	0–0.02 *****
Malathion	Organophosphate			III ****			

DT, tablet for direct application; EC, emulsifiable concentrate; GR, granule; SC, suspension concentration; WG, water dispersible granule; WP, wettable powder

a Class II = moderately hazardous; class III = slightly hazardous; class U = unlikely to pose an acute hazard in normal use; NA = not available.

b 142–190 1/ha, or 19–47 1/ha if a spreading agent is added

c To open bodies of water at dosages of 125–750 g of formulated product per hectare, or 1–5 mg/l for control of container-breeding mosquitoes

Recommendations from: * WHO (2006); ** WHOPES, personal communication (2006) ***WHO (2008a) **** WHO (2022) *****WHO (2019)

Table 2.3 Observations of efficacy of chemicals introduced in water storage containers for the purpose of vector control

Chemicals	**Observations of efficacy** **[*location of field study if applicable*]**	**Observations of resistance of *Aedes aegypti* to chemical**
Temephos	● George, Lenhart, Toledo, Lazaro, Han, Velayudhan, Ranzinger, Horstick, 2015 [*review article*]; ● Garelli, Epinosa, Weinberg, Trinelli, & Gürtler, 2011 [*Argentina*]; ● World Health Organization, 2009 [*overview document*]; ● Tawatsin, Thavara, Chompoosri, Bhakdeenuan, & Asavadachanukorn, 2007; ● Chen, Nazni, Lee, & Sofian-Azirun, 2005 [*Malaysia];* ● Thavara, Apiwat, Kong-Ngamsuk, Mulla, 2004 [*Thailand*]; ● Pinheiro & Tadei, 2002 [*Brazil*]; ● Laws, Sedlak, Miles, Joseph, Lacomba et al., 1968 [*Puerto Rico*]	● Saeung, Ngoen-Klan, Thanispong, Muenworn, Bangs & Chareoviriyaphap, 2020 [*Thailand and Surrounding Countries*]; ● Valle, Bellinato, Viana-Medeiros, Lima & Martins, 2019 [*Brazil*]; ● Khan & Akram, 2019 [*Pakistan*]; ● Morales, Ponce, Cevallos, Espinosa, Vaca & Quezada, 2019 [*Ecuador*]; ● Garelli, Espinosa, Weinberg, Trinelli, Gürtler, 2011 [*Argentina*]
Diflubenzuron	● Marcombe, Chonephetsarath, Thammavong & Brey, 2018 [*Laos*]; ● Bellinato, Viana-Medeiros, Araújo, Martins, Lima & Valle, 2016 [*Brazil*] ● Lau, Chen, Lee, Norma-Rashid & Sofian-Azirun, 2015 [*Malaysia*]; ● Neto, Cavalcanti, Pontes & Lima, 2013 [*Brazil*]; ● World Health Organization, 2008 [*overview document*]; ● Thavara, Tawatsin, Chansang, Asavadachanukorn, Zaim, & Mulla, 2007 [*Thailand*];	
Pyriproxyfen	● Juarez, Garcia-Luna, Roundy, Branca, Banfield & Hamer, 2021 [*United States*]; ● Hustedt, Boyce, Bradley, Hii & Alexander, 2020 [review]; ● Oo, Thaung, Maung, Aye, Aung, Thu, Thant & Minakawa, 2018 [*Myanmar*]; ● Maoz, Ward, Samuel, Müller, Runge-Ranzinger, Toledo, Boyce, Velayudhan & Horstick, 2017 [*review article*]; ● Seng, Setha, Nealon, Socheat & Nathan, 2008 [*Cambodia*]; ● World Health Organization, 2008b [*overview document*] ● Sihuincha, Zamora-perea, Orellana-rios, Stancil, López-sifuentes, Vidal-oré & Devine, 2005 [*Perú*]	● Campos, Martins, Rodovalho, Bellinato, Dias, Macoris, Andrighetti, Lima & Obara, 2020 [*Brazil*]; ● Darriet, Marcombe, Etienne, Yébakima, Agnew, Yp-Tcha & Corbel, 2010 [*Martinique*]
Bti	● Ritchie, Rapley & Benjamin, 2010 [*Australia*]; ● Setha, Chantha & Socheat, 2007 [*Cambodia*]; ● Mulla,, Thavara, Tawatsin, & Chompoosri, 2004 [*Thailand*]; ● Phan-Urai, Kong-ngamsuk & Malainual, 1995 [*Thailand*]	● Boyce, Lenhart, Kroeger, Velayudhan, Roberts & Horstick, 2013 [*review*]; ● Bonin, Paris, Férot, Bianco, Tetreau & Després, 2015; ● Paris, Marcombe, Coissac, Corbel, David & Després, 2013; ● Paris, Tetreau, Laurent, Lelu, Després & David, 2011
Spinosad DT	● Marina, Bond, Muñoz, Valle, Quiroz-Martínez, Torres-Monzón & Williams, 2020 [*Mexico*]; ● Marcombe, Chonephetsarath, Thammavong & Brey, 2018 [*Laos*]; ● Tomé, Pascini, Dângelo, Guedes & Martins, 2014; ● Marcombe, Darriet, Agnew, Etienne, Yp-Tcha, Yébakima & Corbel, 2011 [*Martinique, French West Indies*]; ● World Health Organization, 2010 [*overview document*]; ● Pérez, Marina, Bond, Rojas, Valle & Williams, 2007 [*Mexico*]	● Pérez, Marina, Bond, Rojas, Valle & Williams, 2007
Plant oils	● Njoroge & Berenbaum, 2019	

2.1.1. Existing Literature: Mosquitoes Versus Water Disinfectants Silver, Copper, and Chlorine

This section presents a literature review that comprehensively analyzes the current state of research on the effects of water disinfectants on mosquitoes. In this study, the objective was to investigate the survival and emergence of juvenile *Ae. aegypti* mosquitoes when exposed to low concentrations of silver nitrate, copper sulfate, and sodium hypochlorite, which are commonly used as water disinfectants. The concentrations of water disinfectants used in this study were intentionally kept below the recommended guidelines for drinking water quality. This was because the aim of the research was to investigate the effectiveness of these disinfectants as an alternative method for controlling mosquito populations in drinking water storage containers. Within the literature, there have been few studies that have tested the efficacy of water disinfectants in terms of vector control as will be discussed below. The majority of previous studies investigating the effects of water disinfectants on mosquitoes did not focus on treating water storage containers for vector control. Instead, these studies had different objectives, and therefore, higher concentrations of the chemicals were often used to achieve better efficiency and shorter exposure times. Many of these studies measured the LC50 or LC90 values, which represent the concentration of a chemical required to kill 50% or 90% of the exposed population, respectively, within a relatively short time period of 24 to 48 hours. The comparison of this research to other studies will help to identify similarities and differences between the results of this study and those of previous research help to provide a deeper understanding of the factors influencing survival and emergence of the population.

2.1.1.1. Silver

According to Ratte (1999), small aquatic invertebrates, especially during their embryonic and larval stages, are considered to be the most susceptible organisms to the toxic effects of silver. In the Shanmugasundaram & Balagurunathan (2015) study, silver nitrate ($AgNO_3$) concentrations ranging from 10–50 mg/L (ppm) were tested for larvicidal effects between on *Anopheles subpictus, Culex quinquefasciatus,* and *Ae. aegypti*. The lethal concentration in which 50% of the larvae exposed die (LC_{50}) was determined for a 24 hr period. Expressed in terms of LC_{50}, the toxicity of silver nitrate for *Anopheles subpictus, Culex quinquefasciatus,* and *Ae. aegypti* was determined to be 42.544 ppm, 44.922 ppm, and 39.664 ppm respectively. Similarly in the Velayutham, Ramanibai, & Umadevi (2016) study, an aqueous $AgNO_3$ 1 Mm solution (Ag concentration in the solution is 1 mg/L assuming a density of 1 g/mL for the solution) was tested against third instar of *Ae. aegypti* and *Culex quinquefasciatus*. The LC_{50} for *A. aegypti* was found to be 76.96 mg/mL and the LC_{50} for *Culex quinquefasciatus* was found to be 84.06 mg/L. These concentrations are *notably higher* than the maximum concentration evaluated in this study. Nonetheless, the LC50 magnitude observed in the Shanmugasundaram and Balagurunathan (2015) study sheds light on why a concentration roughly 495 times lower may not be particularly effective within the initial

24-hour contact. Given that the concentration of silver nitrate capable of killing half of the larval population within 24 hours (i.e., LC_{50}) is not safe for human consumption, the design of this study, involving lower concentrations of silver, assesses its efficacy over an extended period of exposure or contact time in order to see if it could still achieve its desired outcome for vector control.

2.1.1.2. Copper

The results of this study are consistent with other studies conducted by Rayms-Keller et al., (1998); El-Sheikh et al. (2010); Perez & Noriega (2012); Reza, Yamamoto, & Matsuoka (2014); Reza & Ilmiawati (2020); Miah et al. (2021), Neff & Dharmarajan (2021); and Miranda et al. (2022), that have all demonstrated that copper can have negative effects on mosquito development. Rayms-Keller et al., (1998) identified a dose dependent relationship to copper in terms of the mortality of third instar *Aedes aegypti* and reported an LC_{50} of 33 ppm. El-Sheikh et al. (2010), demonstrated that heavy metals were also toxic to mosquito species *Culex pipiens*, reporting that the LC_{50} of copper sulfate for second instar *Culex pipiens L.* was 5.1 ppm. Reza, Yamamoto, & Matsuoka (2014) tested copper concentrations 1.1 ppm, 3.3 ppm, and 10 ppm for larvicidal effects on first instar *Aedes albopictus, Anopheles stephensi*, and *Culex pipiens* over a 96-hour period (4 days). The lowest concentration of copper tested (1.1 ppm) caused a mortality of roughly 10%, 40%, and 50% of the *An. stephensi, Ae. albopictus*, and *Cx. pipiens pallens* larvae, respectively, at 96 hours. In Reza & Ilmiawati (2020), low concentrations of copper (150 ppb, 300 ppb, and 600 ppb) were tested with first instar larvae of *Ae. albopictus, Anopheles stephensi,* and *Culex pipiens.* The results showed that exposure to copper ($CuSO_4$) at varying concentrations had different effects on the mortality of larvae from different mosquito species. At 300 ppb of copper, roughly 50% mortality was observed in larvae from all three species after seven days of exposure. When exposed to 600 ppb of copper, larvae of *Ae. stephensi* and *Cx. pipiens* showed 100% mortality within a week, while some *Ae. albopictus* larvae survived. Neff & Dharmarajan (2021) also observed that copper levels below 1300 ppb increased larval mortality and furthermore, provided evidence that metal exposure is linked to a mosquito's ability to transmit parasites.

Miranda et al. (2022) found how the exposure to copper sulfate decreased the lifespan and impaired the developmental time of *Ae. aegypti*. Among other experiments that considered midgut morphology, blood-feeding and fecundity, survival bioassays were performed on third instar larvae at copper concentrations 1.5 ppb; 15 ppb; 150 ppb; 1,500 ppb; and 15,000 ppb. Unlike the study presented in this paper and the Reza & Ilmiawati (2020) study where larvae were continually exposed to the chemical treatment, the larvae in the Miranda et al. (2022) study were only exposed to the copper sulfate for 24 hours. While other aspects of the study designs also differed (e.g., *Ae. aegypti* strain tested, environmental conditions, etc.), this particular variation in exposure time may explain the large differences in larvicidal effect seen between the studies at concentrations of the same magnitude. This portrays that the effectiveness of the water disinfectants as vector control may be highly dependent on contact time the larvae have with the disinfectant. This is further portrayed in the study by Miah et al. (2021), which evaluated the larvicidal efficacy of copper sulfate, with concentrations between 1 to 20 ppm, on third instar *Ae. aegypti, Culex quinquefasciatus,* and *Anopheles quadrimaculatus.* In both laboratory and in semi-field conditions, larval mortality in the study showed concentration and time dependent correlations, specifically larval mortality was higher with increasing concentration and exposure time. After 72 hours of exposure, the larval mortality of *Ae. aegypti, Anopheles quadrimaculatus,* and *Culex quinquefasciatus* in contact with 1 ppm $CuSO_4$ in laboratory conditions was 5%, 35%, 42.5% respectively.

A possible mechanism of action for the negative effects of copper sulfate on mosquito larvae may be related to its impact on the larval gut microbiota, with some studies suggesting that this can lead to gut dysfunction impaired nutrient absorption. This, in turn, could reduce the amount of energy available for larval development, including molt, metamorphosis, and adult development. (Miranda et al., 2022; Reza & Ilmiawati, 2020; Strand, 2018; Beaty et al., 2002). Other potential mechanisms of action for the effects of copper sulfate on mosquito larvae could include direct toxicity to the larvae (Bellini et al., 1998), interference with physiological processes such as respiration or ion transport, or disruption of key enzymes or proteins involved in development (Muttkowski, 1921). Further research is required to fully understand the underlying mechanisms of the observed effects.

In the Perez & Noriega (2012) study, the mortality of mosquito larvae in a metal stressed environment (1 ppm Cu) was not evenly distributed across the four instar developmental stages. Instead, most of the mortality observed occurred during the 1st instar stage, suggesting that younger larvae are particularly sensitive to copper exposure. Perez & Noriega (2012) also suggested that rapid tolerance to copper exposure develops in the survivors. The study also considered how a longer duration of pharate 1st instar quiescence (developed 1st instar larvae dormant state in an egg) can reduce larval fitness and decrease the ability of newly hatched larvae to tolerate metal stress.

2.1.1.3. Chlorine

The Sherman et al. (1998) study estimated time required to reach 50% mortality in third and fourth instar larvae and pupae of *Ae. aegypti* exposed to different concentrations of detergent and chlorine bleach. The chlorine bleach tested was Magia Blanca (Industrias Magna SA, San Pedro Sula, Honduras, sanitary registration number V-00016), which listed sodium hypochlorite as the active ingredient of the product (5.25%). Doses tested of the chlorine bleach were 2 ml, 10 ml, 26 ml, and 52 ml per liter of water. Assuming 5.25% sodium hypochlorite is equivalent to 5.00% available chlorine (i.e., amount of chlorine available for disinfection), 2 ml of bleach in 1 L can produce a chlorine solution of roughly 100 ppm. For 2 ml of the bleach, the LC_{50} for 3rd/4th instar larvae was achieved in 4 hours and the LC_{50} for the pupae were achieved in 15 hrs. This concentration is not safe or pleasant for human consumption. This study provided evidence that sodium hypochlorite would be able to produce larvicidal effects; however, it was still unclear whether concentrations 100 times lower would demonstrate any larvicidal effects.

The Shahen et al. (2020) study provided insight on lower, sublethal concentrations of sodium hypochlorite in their study that compared the toxicity of calcium and sodium hypochlorite against 1st, 2nd, 3rd and 4th instar *Culex pipiens* larvae. The concentrations tested ranged between 4 and 100 ppm for sodium hypochlorite and between 0.1 and 20 ppm for calcium hypochlorite. The study found that both chemicals were effective in reducing the survival rate of the larvae, but sodium hypochlorite had a higher toxicity than calcium hypochlorite. Estimated LC_{50} values, over a 24-hour experimental period, for sodium hypochlorite were 12.24, 46.2, 65.33 and 99.5 ppm for 1st, 2nd, 3rd and 4th instar larvae, respectively. Both hypochlorite compounds were found to significantly prolong the duration of the development period. Additional observations by the researchers illuminated that hypochlorite treatment had adverse effects on the integument (outer covering of larvae) development of the larvae as well as abnormalities with the siphon (essentially their breathing tube). Many of the treated larvae were unable to shed their skin and failed to complete the metamorphosis process.

2.2. Materials and Methods

This section describes the methods used to: culture and rear *Aedes aegypti* mosquitoes in the laboratory (*2.2.1*), create test concentrations (*2.2.2*), test various water treatment disinfectants against the larvae (*2.2.3*), and perform the data analysis (*2.2.4*).

2.2.1. *Aedes aegypti* Culturing

The *Ae. aegypti* eggs were obtained commercially from Benzon Research, Inc. The colony, derived from the USDA "Gainesville" strain, has been continuously colonized at Benzon Research since 1994. Eggs procured from Benzon were 2-3 weeks old. These eggs were stored until used, with unused eggs discarded after 1.5 months. The mosquitoes were reared in the Water Quality Laboratory at the University of Virginia on a 12:12 hour light cycle. The Extech RHT20 Humidity and Temperature Datalogger was used to monitor the environmental conditions. The *Ae. aegypti* eggs and larvae were cultured at 27.9 ± 0.2 (82.2°F) in Sterlite plastic trays (35.6×27.9×8.3 cm) containing deionized (DI) water. The larvae were fed daily with ground larval food, a 3:1 mixture of Liver Powder:Brewer’s Yeast (MP Biomedicals™). Five grams of this mixture was added to 400 ml water. The DI water was deoxygenated by adding 1/8 oz of the food slurry to the rearing trays. Twenty-four hours later, eggs attached to strips of paper were submerged into the trays. Larvae of an intended instar were collected for each experiment.

No food was added to rearing trays on the day of the hatch. The larvae were fed 0.25, 0.5, and 1 oz on the first three days post hatching respectively. After day 3, larvae feed between 1 to 1.5 oz/day until pupation. Larvae were strained and put into new water when the water became cloudy, or a film was present.

2.2.2. Water Treatment Disinfectants

The motivation behind the present study was to find the water treatment disinfectant that would be the most efficient at reducing the emergence of *Ae. aegypti* in **drinking** water storage containers. *Ae. aegypti* larvae were assessed against varying concentrations of silver nitrate, copper sulfate, and sodium hypochlorite (see **Table 2.4**). Since water in a HWS container must be safe to drink, established drinking water guidelines provided an upper boundary condition for the concentrations tested within the study. Therefore, all concentrations tested in this laboratory study were safe to drink as per the guidelines established by public health agencies—the World Health Organization (WHO), the US Environmental Protection Agency (EPA), and the Center for Disease Control and Prevention (CDC).

To avoid health effects that can occur above the MCL or recommended doses for consumption (e.g. high levels of chlorine may cause eye and nose irritation and stomach discomfort) and to anticipate social acceptability factors (e.g. chlorine taste thresholds in different populations, cost), a range of concentrations were tested, none of which were directly at the uppermost limit of the drinking water quality recommendation or limit.The concentration ranges chosen for this study represent ***high***, ***mid***, and ***low*** range dosing for water treatment. For example, the drinking water quality guideline set by the United States Environmental Protection Agency (EPA) for silver is 100 ppb and the concentrations tested in the present study represent 20%, 40%, and 80% of the drinking water quality standard. When more than one drinking water guideline was identified, the more conservative concentration was used to develop concentrations within the high, middle, and low dose ranges to ensure for broad applicability of work as well as to maintain an added safety margin.

Table 2.4 Concentrations tested in larvicidal bioassays

	Drinking Water Quality Guidelines	Concentrations Tested		
		High	Mid	Low
Silver (Ag): AgNO3	**100 parts per billion [ppb]** (WHO & EPA)	**20 ppb Ag**	**40 ppb Ag**	**80 ppb Ag**
Copper (Cu): CuSO4	**1.3 parts per million [ppm]** (EPA) **2 ppm** (WHO)	**300 ppb Cu**	**600 ppb Cu**	**1200 ppb Cu**
Chlorine (OCl^- /HOCl): NaOCl	**4 ppm** (EPA) **<2 ppm Free Cl Residual @ 30 min** (CDC Safe Water System Program)	**500** (0.5 ppm) **Free Chlorine**	**1000** (1 ppm) **Free Chlorine**	**2000** (2 ppm) **Free Chlorine**

2.2.2.1. Silver Nitrate

The stock solution was made by dissolving 16.99 g of $AgNO_3$ powder (Artcraft Chemicals, CAS No. 7761-88-8) into 1000 mL of DI water. One milliliter of this 100 mM $AgNO_3$ stock solution (10.79 g of silver in 1000 mL) was added to 1,077 ml of DI water to make a 10 ppm silver solution. Nominal silver concentrations were confirmed by inductively coupled plasma mass spectrometric analysis (ICP-MS) using the Agilent 7900 ICP-MS instrument (Agilent, Santa Clara, CA). Samples for the ICP-MS were prepared by adding 2% HNO3. Serial dilutions of the stock solution were performed to make test concentrations 80 ppb (*high dose*), 40 ppb (*mid dose)*, and 20 ppb (*low dose*).

The United States EPA's drinking water standard for silver is 100 parts per billion (ppb). The World Health Organization set their guideline value for silver at 100 ppb as well.

2.2.2.2. Copper Sulfate

The stock solution was made by dissolving 16.0 g of $CuSO_4$ powder (Alfa Aesar, CAS No. 7758-99-8) into 1000 mL of DI water. One milliliter of this 100 mM $CuSO_4$ stock solution (6.35 g of copper in 1000 mL) was added to 634 ml of DI water to make a 10 ppm copper solution. The copper level was confirmed using the ICP-MS. Serial dilutions were performed to make test concentrations 1,200 ppb (*high dose*), 600 ppb (*mid dose*), and 300 ppb (*low dose*).

The guideline value for copper in drinking water set by the WHO is 2 mg/L or 2000 ppb. This is higher than the maximum contaminant level (MCL) for copper in drinking water established by the US EPA, which enforces the standard of below 1,300 ppb.

2.2.2.3. Sodium Hypochlorite

The stock solution of sodium hypochlorite with 10-15% available chlorine was procured from Sigma-Aldrich (CAS No. 7681-52-9). Serial dilutions were performed to create a new stock solution of an estimated concentration of 25 ppm. Further serial dilutions to make free chlorine test concentrations 0.5 ppm (*low dose*), 1 ppm (*mid dose*), and 2 ppm (*high dose*) were performed directly before the start of an experiment. Concentrations of free chlorine were measured directly, utilizing the USEPA DPD Method 8021 for low range free chlorine, before and after the addition of larvae, as well as at 4 and 8 hrs, using a digital colorimeter (HACH).

In this study, free chlorine is measured because it is an indicator of the overall chlorine concentration in the water available for disinfection and is thus used as the metric for ensuring that the disinfectant concentration is high enough to effectively kill microorganisms and prevent the spread of waterborne diseases. Sodium hypochlorite ($NaOCl$) is a powerful oxidizing agent that can react with water to release free chlorine. Free chlorine is in a solution as $HOCl$ or OCl^-. When sodium hypochlorite is added to water, the hydrolysis reaction occurs. These processes are represented in the following equations:

$$NaOCl \rightleftharpoons Na^+ + OCl^- \qquad \textit{(Eq. 1)}$$

$$HOCl \rightleftharpoons H^+ + OCl^- \qquad \textit{(Eq. 2)}$$

Depending on factors such as pH, temperature, and pressure, the ratio of OCl^- and HOCl will change:

$$NaOCl + H_2O \rightleftharpoons Na^+ + OH^- + HOCl + OCl^- \quad (\textit{unbalanced}) \qquad \textit{(Eq. 3)}$$

The resulting mixture of free chlorine, OCl^- and HOCl, can then react with and disinfect microorganisms present in the water, making it safe for consumption or other uses.

The U.S. EPA (2019) recommends a minimum free chlorine residual of 0.4 mg/L in drinking water to ensure disinfection. To prevent water from developing an unpleasant taste or odor, the Centers for Disease Control and Prevention (CDC) Safe Water Storage (SWS) program suggests that 30 minutes after the addition of the sodium hypochlorite, no more than 2.0 mg/L of free chlorine residual should be present in water stored in containers. To guarantee microbiologically safe water, the CDC advises that containers used by households to store water should have a free chlorine residual level of at least 0.2 mg/L 24 hours after the introduction of sodium hypochlorite. (CDC, 2005).

2.2.3. Survival Bioassays: Evaluation of Dose Response to Water Treatment Disinfectants

The main objective of the laboratory study was to test and determine the optimum application dosage of common water disinfectants silver, copper, and chlorine against *Aedes aegypti* to create recommendations for water treatment within household water storage containers for the purpose of vector control. Experimental methods for the laboratory study were influenced primarily by WHO's *Guidelines for laboratory and field testing of mosquito larvicides* (2005).

For each concentration, 25 larvae were placed in 200 ml of solution within a 250 ml beaker. Control beakers were filled with DI water only. The larvae were transferred to test beakers by means of disposable transfer pipettes. The test beakers were held at the same environmental conditions described above (27.9 ± 0.2°C, photoperiod of 12L:12D). Larvae were fed the 3:1 LP:BY slurry during the long exposure term to avoid high mortality in the controls. Larvae within the control and treated beakers were fed in the same manner.

Larval activity (surviving larvae, the emerging pupae, and adult mosquitoes) was recorded in both treated and control beakers every 24 hours. Observations were made every day until completion of adult emergence in all treatment groups or until the experiment reached Day 16. After observing the emergence of pupae, test and control beakers were covered with netting in order to prevent successfully emerged adults from escaping. The number of successfully emerged adults were confirmed by observations of the empty pupal cases left in the solution in the instance that an adult mosquito may have escaped. The water disinfectant's inhibitory effect on mosquito emergence was indicated by the observation of adult mosquitoes that failed to fully separate from their pupal cases, and the presence of moribund or dead larvae and pupae. Larvae were considered moribund or dead when they did not move when their aquatic environment was disturbed and furthermore, could not be induced to move even after being probed. Larvae matching this description were counted as dead larvae for calculating survival. For the purpose of calculating mortality, any larvae that successfully completed their metamorphosis into adult mosquitoes were considered alive for the duration of the experiment.

The experiment was conducted with three replicates of each concentration, in addition to a control. Each test was carried out three times on different days, using fresh solutions and batches of larvae each time. The resulting data, which included counts of larvae, pupae, and adult mosquitoes in each container, were subject to statistical analysis.

Within this study, testing larvae at different stages allowed the researchers to understand if susceptibility of the larvae to the water disinfectants' effects was dependent on the age of the larvae. Other studies have demonstrated higher susceptibility of younger instar to larvicides. For example:

- a study by Ong & Jaal (2018) showed that younger instar *Culex quinquefasciatus* were more susceptible to temephos than older instar larvae,
- a study by Nartey et al (2013) showed that younger instar *Anopheles gambiae* were more susceptible to *BTi* than older instar larvae,
- Patil et al. (2012) showed that younger instar *Aedes aegypti* and *Anopheles stephensi* were more susceptible to silver nanoparticles synthesized using *Pergularia daemia* plant latex than older instar.

This study examined two distinct larval stages: late third instar larvae and late 1st instar larvae. By testing these stages, the study aimed to illustrate two potential scenarios within the context of household water storage. The first scenario involves a mosquito laying eggs in a water storage container, resulting in newly hatched larvae interacting with newly applied disinfectant. This scenario considers if a POUWT technology inputted into the water storage container would effectively reduce the survival emergence of newly hatched larvae. The second scenario could involve either the larvae already inhabiting the source water supplying the HWS container or a later introduction of POUWT technology to the HWS container, allowing the larvae to grow into later instars before encountering the disinfectant.

The older instar experiments took place over 16 days (when the control larvae reached full emergence and larvae in the treatment groups either emerged, died, or were moribound). If adult emergence in the control was less than 80%, the test was discarded and repeated. For the younger instar experiments, larvicidal data was collected for 3 days. Combining the two datasets provides a more comprehensive understanding of the impact of exposure to disinfectants at different larval stages.

The older larvae were fed after observations on day 4 and the younger instar larvae were fed after observations on day 2. Two drops of the slurry were added per test beaker at each feeding which occurred every other day until the experiment was terminated. While delaying the feeding, as described above, is suboptimal for typical lab-grown *Ae. aegypti* growth rates as observed in the literature, this particular food regiment was chosen for this particular study for two main reasons:

1. Simulate the scenario when the water within the household water storage container has been disinfected/safe to drink,
2. To decrease the confounding variable of the water treatment disinfectant interacting with the food slurry (e.g., volatility of the chlorine, interactions between silver or copper ions and brewer’s yeast or liver powder that could affect toxicity).

Further discussion on the feeding regimen can be found in Chapter 5 (*Section 5.1.2*).

2.2.4. Data Analysis

Larvicidal activity was calculated using the WHO (2005) bioassay protocol and with slight modifications (see Ngonzi et al., 2022 for a method to the one presented in this study). Data from all replicates in an experiment were pooled for analysis. Where the emergence was between 80% and 95%, mortality was calculated using Abbott's (1925) formula.

$$Survival\ (\%) = 100 - ((C - T) / C * 100) \qquad (Eq.\ 4)$$

where C = percentage survival in the untreated control and T = percentage survival in the treated sample. Larvae that developed into successfully emerging adults was expressed in terms of emergence:

$$Emergence\ (\%) = 100 - ((C - T) / C * 100) \qquad (Eq.\ 5)$$

where C = percentage emergence in the untreated control and T = percentage emergence in the treated sample. Inhibition of emergence (IE) was calculated on the basis of the number of larvae exposed. IE% is calculated using the following formula:

$$Inhibition\ of\ Emergence\ (IE\%) = 100 - (T * 100)/C \qquad (Eq.\ 6)$$

where T = percentage emergence in treated batches and C = percentage emergence in the control. Abbot’s correction was also applied when appropriate according to the WHO (2005) guidelines.

All statistical analysis was performed using R (version 4.2.2) and RStudio (2022.07.2 Build 576). To assess the dose response of silver, copper, and chlorine on the number of larvae, pupae, and emerged adult mosquitoes, the sample mean, standard deviation (SD), standard error of mean (SEM), was calculated on the observed data.

Probit analysis, a method first published in Science by Chester Ittner Bliss in 1934 and then popularized by the work of Finney (1971, 1952), is commonly used in toxicology in order to analyze the relationship between a dose and a response (Postelnicu, 2011). To investigate the impact of the treatments on the probability that a larva survives and/or has emerged, a mixed-effect probit regression model was fit to the data in R. The response variable was the classification of percent survival or percent emergence, while the independent variables were time (days), dosage, and their interaction. A natural spline with two degrees of freedom was applied to time to account for non-linearity in the rate of change per day. Each experiment was treated as a random effect. The function *glmer* (which stands for “generalized linear mixed effects regression”), from the lme4 package, was employed to produce predictive models for each of the treatments. The function *emmeans* (which stands for "estimated marginal means") was used to perform pairwise comparisons of the estimated means.

For the inhibition of emergence predictive model, day 16 values for the control were obtained from the emergence model. Bootstrapping was used to generate a confidence interval using the upper and lower limits of the 95% confidence interval for emergence. The *bootMer* (which stands for "bootstrap for mixed effects models") function simulated 100 samples based on the bounds of the model.

2.3. Results and Analysis

The results portray data collected from testing silver nitrate, copper sulfate, and sodium hypochlorite on older juvenile *Ae. aegypti* (*Section 2.3.1*) and younger larvae (*Section 2.3.2*). For older instar experiments, survival and emergence data is presented over 16 days. For the younger larvae, results reflect survival over the course of 72 hours of exposure to the treatments.

2.3.1. Older Instar Experiments

This section reports the survival and emergence results for the experiments conducted on late 3rd instar *Ae. aegypti* utilizing silver nitrate, copper sulfate, and sodium hypochlorite treatments. Each of the following sections will start with observed data, which will serve as input for probit regression models. Significant differences will then be examined through analysis of the models. Observed data results are expressed in terms of *Percentage Mean ± SEM* and the model data results are expressed in terms of *Predicted Probability Mean [Upper 95% Confidence Interval, Lower 95% Confidence Interval].*

2.3.1.1. Silver Nitrate

Introducing silver nitrate into the aquatic environment of juvenile *Ae. aegypti* negatively impacted the growth and development of the larvae at all concentrations tested (20, 40, and 80 ppb Ag) when compared to the controls by the end of the experimental period ($p_{control\text{-}AgTreatments} < 0.001$), as shown in **Table 2.5**. Both survival and emergence observations of the controls were around 90% by the end of the experiment while the survival and emergence for the *Ae. aegypti* submerged in 20 ppb Ag was 26.85±*3.61%* and 25.01±*3.72%* respectively. This demonstrated that continued exposure to a low concentration of silver nitrate is quite lethal. When the dose of silver was four times greater (80 ppb), roughly only a tenth of the *Ae. aegypti* emerged or survived. Thus, an exposure–response relationship was detected, where higher concentrations of silver nitrate increased mortality or decreased transformation of a larva to an adult mosquito. As seen in **Table 2.5,** emergence and survival values are comparable, suggesting that almost all of the surviving larvae at the end of the experimental period had emerged into adult mosquitoes. The discrepancy between the values highlights that a few larvae managed to survive beyond the 16-day observation period.

Table 2.5 Observed data for silver nitrate ($AgNO_3$) treatments of 20, 40, and 80 ppb. Standard deviation (St. dev) and standard error of mean (SEM) is presented with the mean percentage survival, emergence percentage, and inhibition of emergence (IE) percentage of older instar *Ae. aegypti* larvae on day 16. Data is corrected with Abbot's formula (1925).

Treatment	*Ag (20 ppb)*			*Ag (40 ppb)*			*Ag (80 ppb)*			*Control*		
Variable	*Mean*	*St. dev*	*SEM*	*Mean*	*St. dev*	*SEM*	*Mean*	*St dev*	*SEM*	*Mean*	*St dev*	*SEM*
Survival (%)	26.85	6.25	3.61	17.09	4.58	2.64	10.75	8.64	4.99	91.11	0.77	0.44
Emergence (%)	25.01	6.44	3.72	16.69	5.24	3.02	10.29	8.94	5.16	90.67	1.33	0.77
IE (%)	72.40	7.16	4.13	81.56	5.86	3.39	88.66	9.87	5.70			

Inhibition of emergence is another way to depict the potency of a treatment and its efficacy of decreasing the adult emergence of *Ae. aegypti*. The WHO guidelines use the inhibition of emergence as the primary measure of the effectiveness of larvicides as adult mosquitoes are responsible for transmitting disease. **Figure 2.4** depicts the inhibition of emergence of *Ae. aegypti* due to the various treatments of silver nitrate. The error bars reflect the SEM. From the data, it is clear that a silver nitrate treatment has great potential to inhibit emergence within these particular environmental conditions. As mentioned before, the data reflects a dose-response relationship, showing that 80 ppb achieves the highest inhibition of emergence of 88.66±*5.70%* on day 16, followed by 40 ppb at 81.56±*3.39%* and 20 ppb at 72.40±*4.13%*.

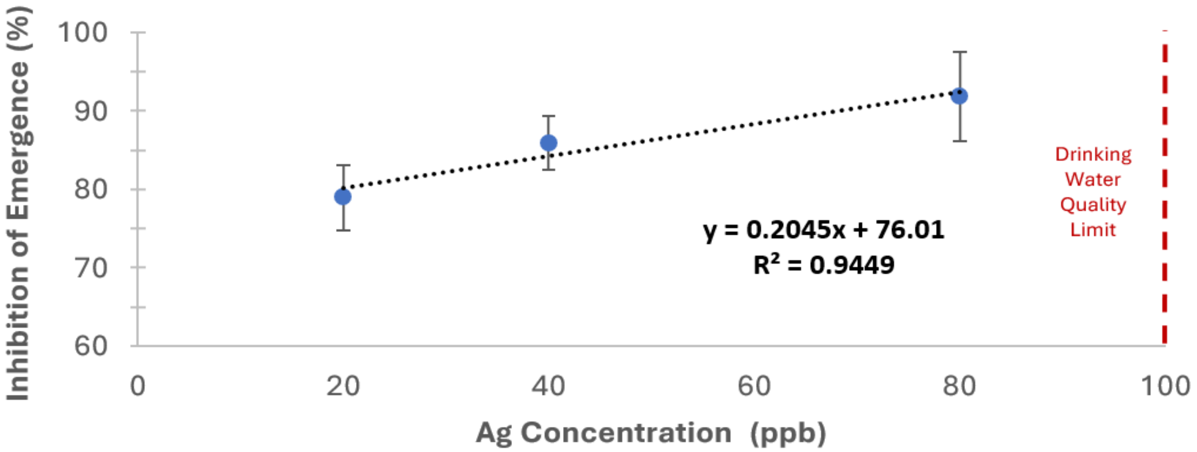


Figure 2.4 Percentage inhibition of emergence for 20, 40, and 80 ppb silver nitrate treated older instar larvae in regards to untreated larvae on day 16. Error bars represent SEM.

Depicted in **Figure 2.5** is the experimental data for survival with curves fitted by probit regression overlaid. This figure illustrates the relationship between the model and observed experimental data. The probit regression curve represents the probability of the binary response variable taking on the value of 1 (“alive” or ”has emerged”) as a function of the predictor variables with a 95% confidence interval. Thus, the probit regression model predicts the probability of survival for the juvenile *Aedes aegypti* over the course of the experiment. The probit regression curves for probability of survival across the different treatments are plotted together on a single graph in **Figure 2.6**. The model predicted that 11.21% [*8.25, 14.29*] of the juvenile *Ae. aegypti* treated with 80 ppb survived by day 16, followed by 15.91% [*11.49, 20.01*] at 40 ppb, and 23.57% [*18.10, 28.57*] at 20 ppb. **Table 2.6** presents data for day 16 of the model data.

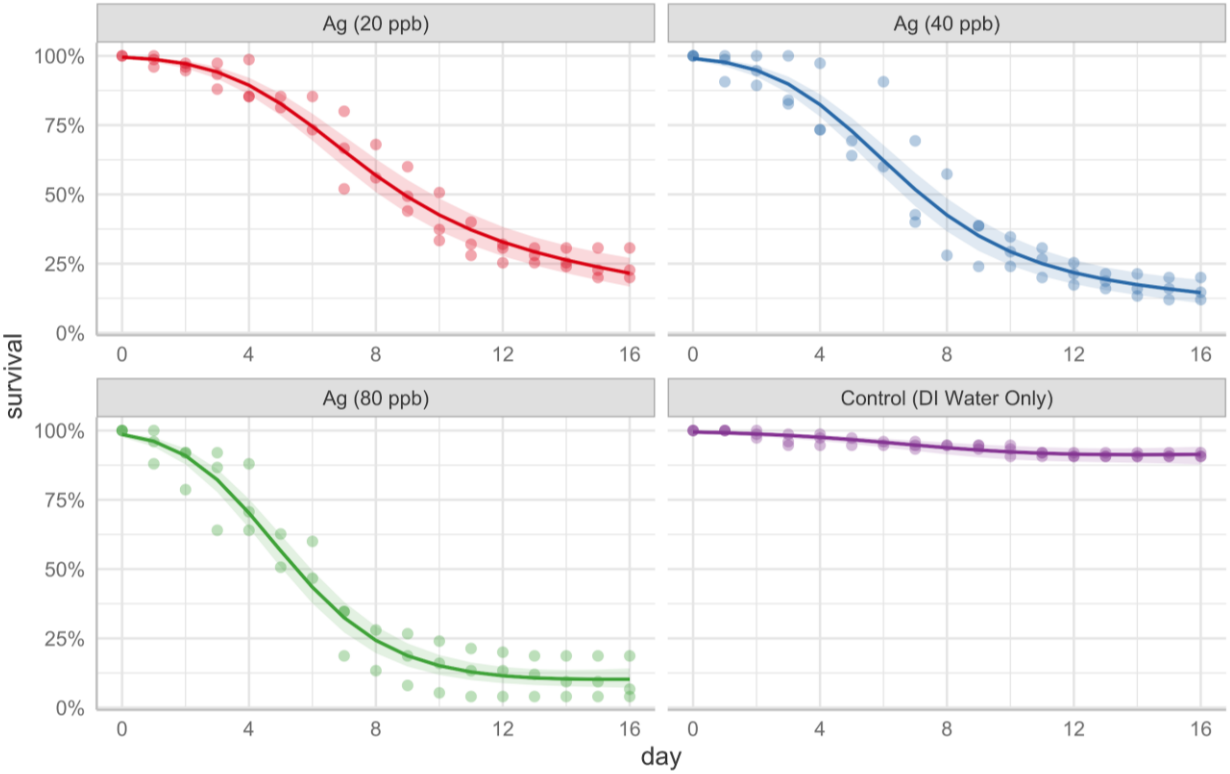


Figure 2.5 The predicted probabilities of survival of older instar *Ae. aegypti* during the experimental period for larvae treated with silver nitrate (20, 40, and 80 ppb) and controls. The shaded areas represent the 95% confidence interval.

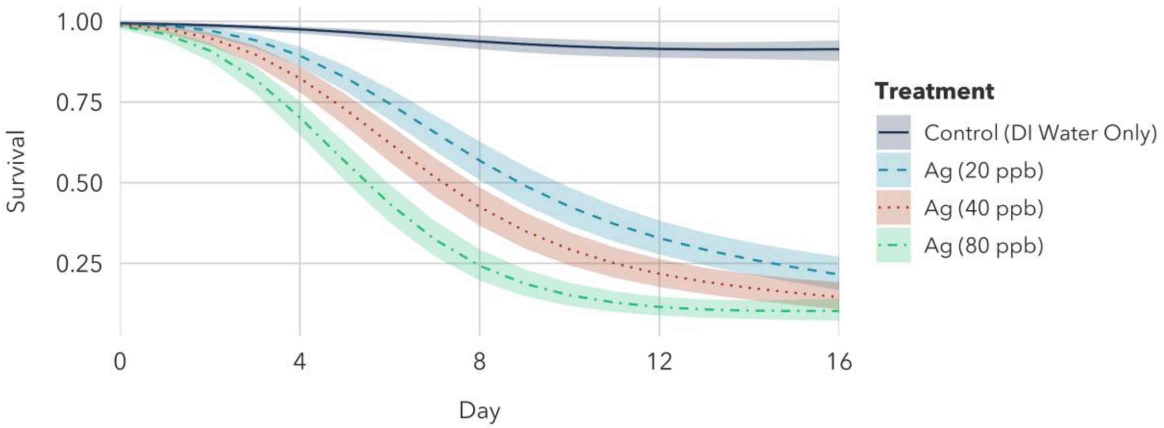


Figure 2.6 Predicted probabilities of survival of *Ae. aegypti* larvae in contact with silver nitrate at concentrations 20, 40, and 80 ppb. Shaded areas represent the 95% confidence interval.

Table 2.6 Predicted mean probability of survival and predicted probability of emergence of older instar *Aedes aegypti* on Day 16 after contact with silver nitrate treatments of 20 ppb, 40 ppb, and 80 ppb. Modeled inhibition of emergence (IE) percentage derived from the probit regression model for emergence. Data corrected by Abbot's formula (1925).

Treatment	*Ag (20 ppb)*	*Ag (40 ppb)*	*Ag (80 ppb)*	*Control*
Variable		*Predicted mean [95%CI]*		
Survival (%)	23.57 [18.10, 28.57]	15.91 [11.49, 20.01]	11.21 [8.25, 14.29]	91.39 [87.75, 94.15]
Emergence (%)	21.04 [15.48, 29.37]	14.10 [8.79, 20.55]	8.20 [4.81, 11.96]	92.84 [88.22, 95.93]
IE (%)	78.96 [71.87, 85.19]	85.90 [78.13, 90.51]	91.80 [86.72, 94.84]	

As seen in **Figure 2.7,** there is clear indication that the final proportion of individuals that have emerged into adult mosquitoes by day 16 in the treatment groups is significantly lower than that of the control ($p_{Cntrl\text{-}20Ag} = <0.001$, $p_{Cntrl\text{-}40Ag} = <0.001$, $p_{Cntrl\text{-}80Ag} = <0.001$). The visual interpretation of the model shows separation between all treatment groups from the control by day 7, with the first four days of the experiment characterized by minimal emergence in the controls. While the predicted probability of emergence for the controls by day 16 was 92.84% [*88.22*, *95.91*], the emergence predicted for larvae in water containing 20, 40, and 80 ppb Ag was 21.04% [*15.48*, *29.37*], 14.10% [*8.79*, *20.55*], and 8.20% [*4.81*, *11.96*] respectively. In the model, the 20 ppb and 40 ppb Ag treatments were found to be significantly different [$p_{40Ag\text{-}20Ag} = 0.032$] for the predicted probability that a larva has emerged by day 16. The proportion of larvae that are predicted to successfully transition into adulthood after exposure to 80 ppb Ag is significantly lower than that exposed to 20 ppb Ag ($p_{80Ag\text{-}40Ag} = 0.032$) and 40 ppb Ag ($p_{80Ag\text{-}20Ag} = <0.001$).

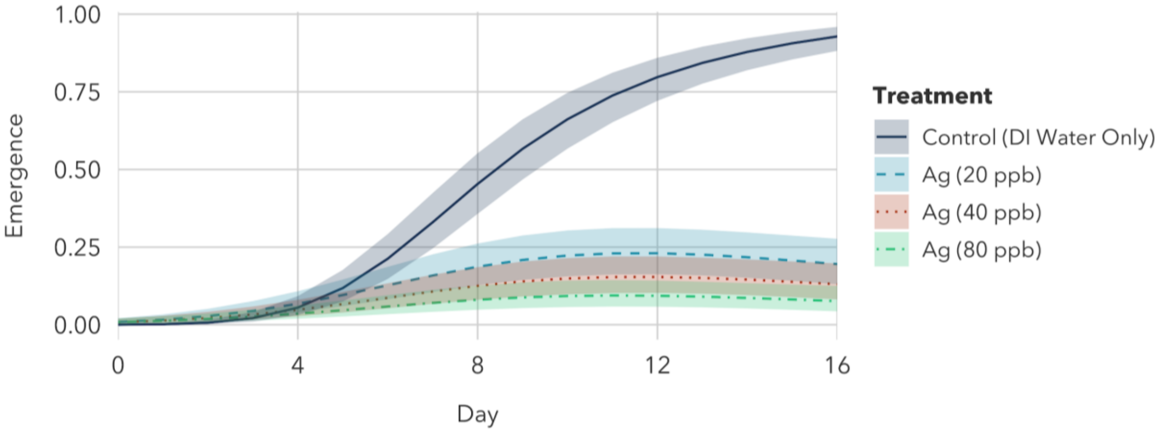


Figure 2.7 Predicted probabilities that *Ae. aegypti* larvae have emerged into adult mosquitoes after being exposed to silver nitrate at concentrations 20, 40, and 80 ppb Ag

Lastly, to measure the impact of silver nitrate on the emergence, the inhibition of emergence (IE%) was calculated. The goal of this type of analysis is to predict inhibition of emergence based on the concentration of disinfect applied as well as to optimize interventions for water storage containers to achieve a desired level of IE%. While the ultimate objective of vector control is to achieve a 100% inhibition of emergence (IE%=100%), excessive use of disinfectants beyond what is necessary to achieve this goal may lead to diminishing returns in terms of cost-effectiveness. Thus, it is important to balance the concentration of the chemical used with the desired outcome.

To calculate the IE% for each silver nitrate treatment, the day 16 emergence values for the control group from the observation data was applied to the IE% equation (*Eq. 6*). Then, bootstrapping generated an estimate for the range of values for the confidence intervals. With the bootstrapped values, a probit regression analysis was performed to determine the relationship between the treatment and the IE%. This process resulted in the model for inhibition of emergence depicted in **Figure 2.8**. Extrapolation of the IE% model outside of this study's experimental range suggests that 100% inhibition of emergence on day 16 would occur at a concentration of 117.32. ppb Ag which is slightly greater than the drinking water quality guideline. To validate this prediction, more experiments would need to be conducted within a wider range of silver concentrations, including those concentrations not safe for human consumption, to validate whether a linear regression line is the most appropriate model for this relationship between concentration of the larvicide and percent inhibition of emergence. Prolonged exposure of the larvae to higher concentrations of the water disinfectant may lead to compound effects (e.g. threshold toxicity concentration reached for the larvae or cumulative developmental effects over the duration of the experiment) that may cause the relationship to be more nonlinear in fit.

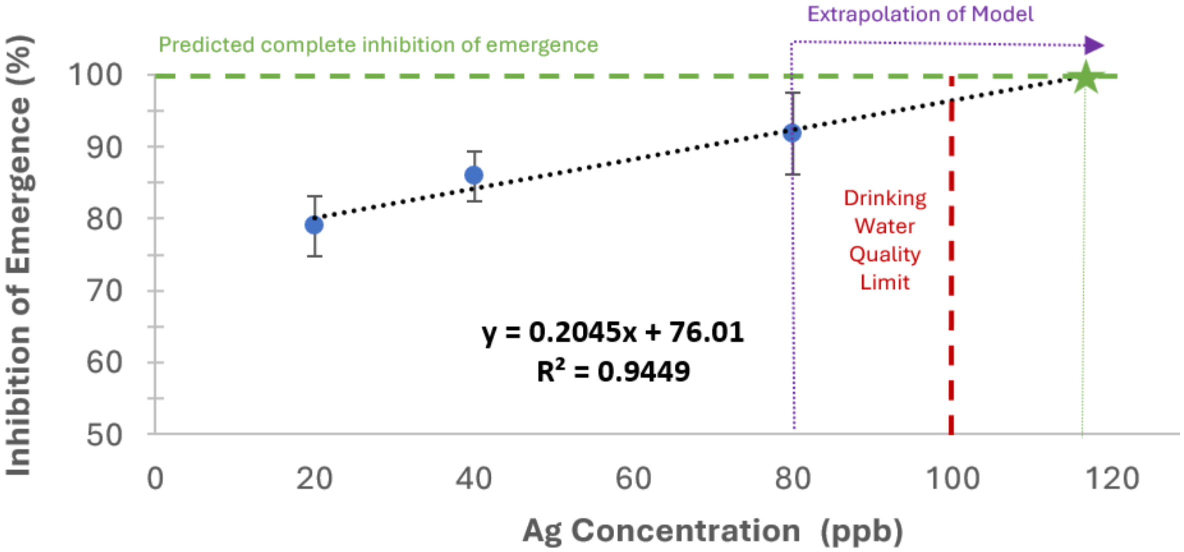


Figure 2.8 The modeled inhibition of emergence of *Ae. aegypti* larvae on day 16 of exposure to silver nitrate, with concentrations 20, 40, and 80 ppb Ag. Observed error bars reflect the standard deviation whereas the model error bars represent a 95% confidence interval.

2.3.1.2. Copper Sulfate

All copper concentrations tested (300, 600, and 1200 ppb Cu) resulted in lower survival and emergence percentages of juvenile *Ae. aegypti* compared to the control group by the end of the experimental period ($p_{control\text{-}CuTreatments} < 0.001$), as depicted in **Table 2.7.** The control group exhibited a survival and emergence of roughly 90%, while those that came into contact with 300 ppb Cu had a survival and emergence of only 42.55±*4.12%* and 40.30±*5.38%* respectively. Increasing the dose to 1200 ppb resulted in a more drastic reduction in both the emergence and survival of the *Ae. aegypti*, with on average 11.44±*4.34%* having emerged by day 16 and 11.92±*4.33%* probability of survival. A dose response is reflected within the inhibition of emergence data, with 300, 600 and 1200 ppb Cu, resulting in IE% on day 16 of 54.89±*3.48*%, 72.15±*6.42*%, and 87.19±*4.86%* respectively.

Table 2.7 Observed data for copper sulfate ($CuSO_4$) treatments of 300, 600, and 1200 ppb. Standard deviation (St. dev) and standard error of mean (SEM) is presented with the mean percentage survival, emergence percentage, and inhibition of emergence (IE) percentage of older instar *Ae. aegypti* larvae on day 16. Data is corrected with Abbot's formula (1925)

Treatment	*Cu (300 ppb)*			*Cu (600 ppb)*			*Cu (1200 ppb)*			*Control*		
Variable	*Mean*	*St. dev*	*SEM*	*Mean*	*St. dev*	*SEM*	*Mean*	*St dev*	*SEM*	*Mean*	*St dev*	*SEM*
Survival (%)	42.55	7.13	4.12	25.80	10.91	6.30	11.92	7.50	4.33	89.78	0.77	0.44
Emergence (%)	40.30	5.38	3.11	24.88	9.94	5.74	11.44	7.51	4.34	89.33	0.00	0.00
IE (%)	54.89	6.02	3.48	72.15	11.12	6.42	87.19	8.41	4.86			

The probit model for the predicted probability of survival of juvenile *Ae. aegypti* in the presence of different concentrations of copper sulfate is illustrated in **Figure 2.9** and the predicted probability that a larva has emerged is illustrated in **Figure 2.10**. When compared to the control group on Day 16, the predicted survival and emergence of *Ae. aegypti* exposed to copper treatments was significantly lower at all concentrations tested ($p_{Cntrl\text{-}300Cu} = <0.001$, $p_{Cntrl\text{-}600Cu} = <0.001$, $p_{Cntrl\text{-}1200Cu} = <0.001$). As was observed with silver nitrate, a dose response is evident in emergence with copper sulfate ($p_{600Cu\text{-}300Cu} = <0.001$, $p_{1200Cu\text{-}600Cu} = <0.001$, $p_{1200Cu\text{-}300Cu} = <0.001$). **Table 2.8** presents day 16 of the model data.

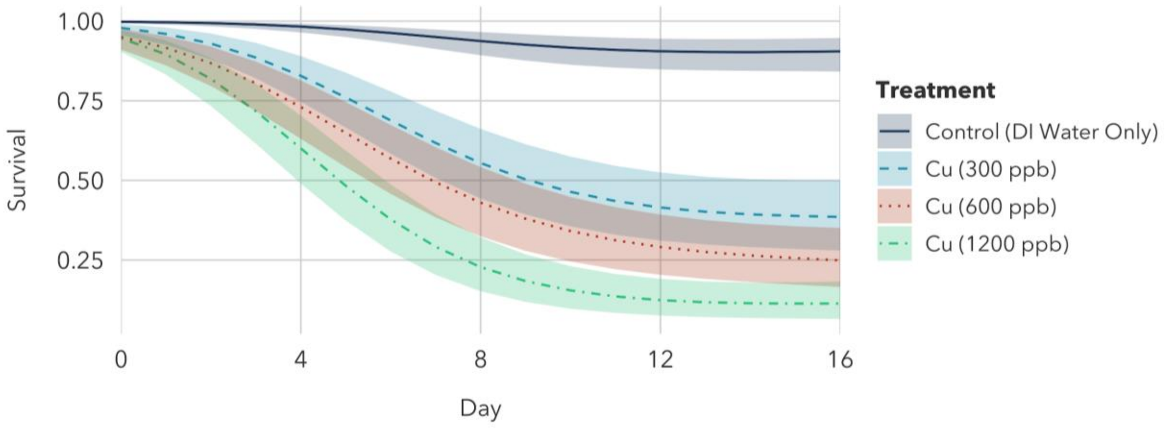


Figure 2.9 Predicted probabilities of survival of *Ae. aegypti* larvae in contact with copper sulfate at concentrations 300, 600 and 1200 ppb

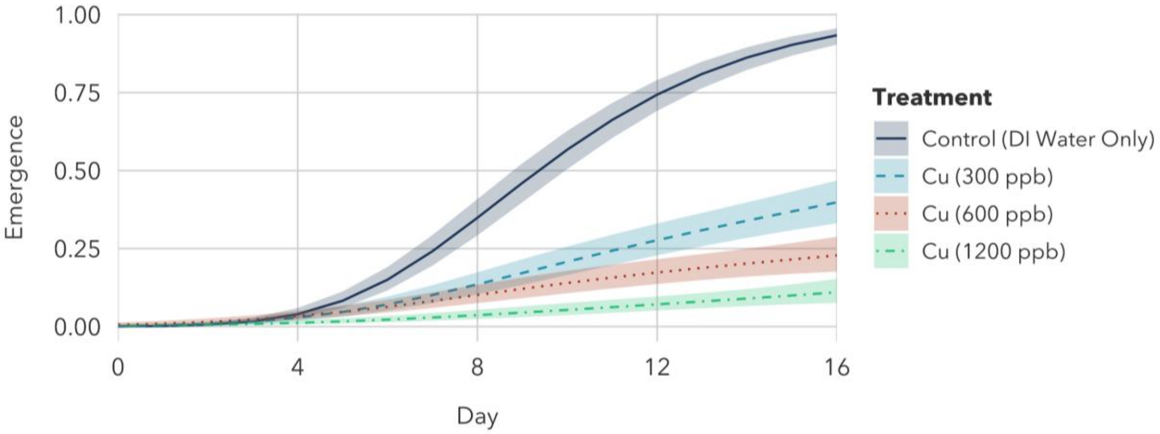


Figure 2.10 Predicted probabilities that *Ae. aegypti* larvae have emerged into adult mosquitoes after being exposed to copper sulfate at concentrations 300, 600 and 1200 ppb

Table 2.8 Predicted mean probability of survival and predicted probability of emergence of older instar *Aedes aegypti* on Day 16 after contact with copper sulfate treatments of 300 ppb, 600 ppb, and 1200 ppb. Modeled inhibition of emergence (IE) percentage produced with the probit regression model for emergence. Data corrected by Abbot's formula (1925).

Treatment	*Cu (300 ppb)*	*Cu (600 ppb)*	*Cu (1200 ppb)*	*Control*
Variable		*Predicted mean [95%CI]*		
Survival (%)	42.56 [34.02, 53.57]	27.50 [19.17, 37.88]	12.52 [7.05, 20.42]	90.52 [84.18, 94.76]
Emergence (%)	42.65 [36.46, 49.15]	24.43 [18.71, 30.17]	11.77 [7.96, 15.68]	93.36 [90.36, 95.58]
IE (%)	57.35 [49.48, 64.47]	75.57 [70.91, 82.82]	88.23 [84.47, 91.90]	

The probit regression model was applied to create a model for inhibition of emergence, depicted in **Figure 2.11**. At the conclusion of the experimental period, this model predicts that 1200 ppb concentration results in an 88.23% [*84.47*, *91.90*] inhibition of emergence, which is roughly 1.5x more effective than the 300 ppb treatment (IE%: 57.35% [*49.48*, *64.47]*); however, the concentration is four times greater.

From the linear regression model, provided in **Figure 2.11,** which establishes a relationship between concentration of copper and percentage inhibition of emergence, and through extrapolation, it is predicted that 100% inhibition of emergence could potentially occur at roughly 1500 ppb Cu. This concentration is over the drinking water quality standard set by the EPA, however, not surpassing the guideline set by the WHO. The linear regression line appears to underestimate the inhibition of emergence potential of the 600 ppb Cu treatment, which may indicate that there is a non-linear relationship between the concentration of the water disinfectant and percent inhibition of emergence at these low concentrations after a prolonged exposure to the treatment. It would be necessary to conduct a more comprehensive study testing a wider range of concentrations in order to verify the accuracy of this prediction.

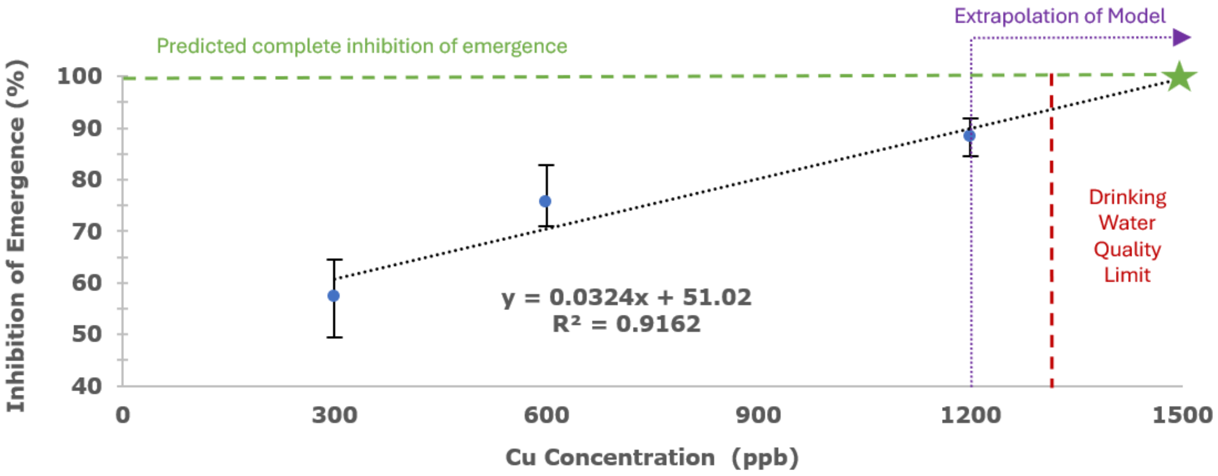


Figure 2.11 The modeled inhibition of emergence of *Ae. aegypti* larvae on day 16 of exposure to copper sulfate, with concentrations 300, 600, and 1200 ppb Cu. Observed error bars represent a 95% confidence interval

2.3.1.3. Sodium Hypochlorite

The study aimed to investigate the impact of exposing juvenile *Ae. aegypti* to varying concentrations of free chlorine on their survival and development. Sodium hypochlorite was used as the source of free chlorine, as when sodium hypochlorite is added to water, it dissociates to form hypochlorous acid and hypochlorite ions (see *Section 2.2.3*). The results showed that all concentrations of free chlorine tested (0.5, 1, and 2 ppm) adversely affected the development and survival of the *Ae. aegypti* when compared to the control group ($p_{Cntrl\text{-}0.5Cl} = <0.001$, $p_{Cntrl\text{-}600Cu} = <0.001$, $p_{Cntrl\text{-}1200Cu} = <0.001$), as reported in **Table 2.9**. The control group survival and emergence was again near 90% by day 16, while the treatment groups of 0.5, 1, and 2 ppm free chlorine caused survival to drop down to 58.40±*12.63*%, 24.75±*7.42*%, and 17.39±*8.49*% respectively. Inhibition of emergence for free chlorine concentrations on day 16 for 0.5, 1, and 2 ppm were 37.44±*12.32*%, 72.46±*7.95%*, and 80.65±*9.26* respectively.

Table 2.9 Observed data for sodium hypochlorite (NaOCl) treatments of 0.5, 1, and 2 ppm free chlorine (OCl^-/HOCl). Standard deviation (St. dev) and standard error of mean (SEM) is presented with the mean percentage survival, emergence percentage, and inhibition of emergence (IE) percentage of older instar *Ae. aegypti* larvae on day 16. Data is corrected with Abbot's formula (1925)

Treatment	*Free Cl (0.5 ppm)*			*Free Cl (1 ppm)*			*Free Cl (2 ppm)*			*Control*		
Variable	*Mean*	*St. dev*	*SEM*	*Mean*	*St. dev*	*SEM*	*Mean*	*St dev*	*SEM*	*Mean*	*St dev*	*SEM*
Survival (%)	58.40	21.88	12.63	24.75	12.85	7.42	17.39	14.70	8.49	90.67	2.67	1.54
Emergence (%)	56.53	20.75	11.98	24.98	12.99	7.50	17.59	14.95	8.63	89.78	2.78	1.60
IE (%)	37.44	21.35	12.32	72.46	13.77	7.95	80.65	16.04	9.26			

The probit model that predicts the survival of juvenile *Aedes aegypti* after they have encountered a single dose of free chlorine of concentration 0.5, 1, and 2 ppm is illustrated in **Figure 2.12**. **Table 2.10** presents the model data for Day 16. Similar to the response observed with the other two water disinfectants, a dose response was evident with free chlorine, with predicted survival probabilities of 57.53% [*8.14, 17.24*], 19.77% [*14.32, 26.56*], and 12.19% [*7.64, 18.30*] at the end of the observational period for 0.5, 1, and 2 ppm, respectively.

Table 2.10 Predicted mean probability of survival and predicted probability of emergence of older instar *Aedes aegypti* on Day 16 after contact with sodium hypochlorite treatments of 0.5 ppm, 1 ppm, and 2 ppm. Modeled inhibition of emergence (IE) percentage produced with the probit regression model for emergence. Data corrected by Abbot's formula (1925)

Treatment	*Free Cl (0.5 ppm)*	*Free Cl (1 ppm)*	*Free Cl (2 ppm)*	*Control*
Variable	*Predicted mean [95%CI]*			
Survival (%)	57.53 [8.14, 17.24]	19.77 [14.32, 26.56]	12.19 [7.64, 18.30]	91.63 [86.98, 94.90]
Emergence (%)	58.34 [45.76, 69.53]	21.57 [14.00, 34.51]	13.73 [7.55, 21.99]	92.85 [86.58, 96.58]
IE (%)	41.66 [31.12, 54.55]	78.43 [67.94, 86.38]	86.27 [79.95, 92.99]	

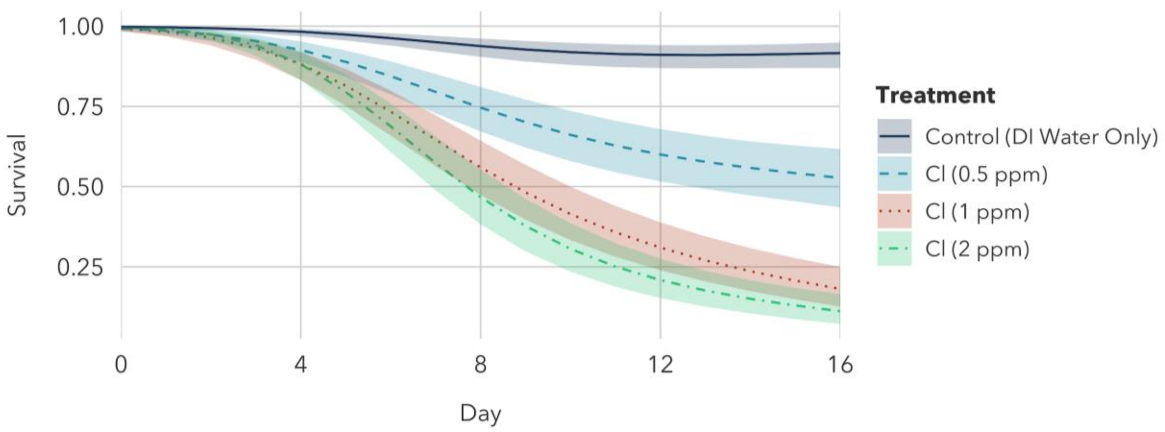


Figure 2.12 Predicted probabilities of survival of *Ae. aegypti* larvae in contact with chlorine at concentrations 0.5, 1, and 2 ppm of free chlorine

Similarly, the chlorine treatment decreased the larvae's ability to reach adulthood ($p_{Cntrl-0.5Cl} = <0.001$, $p_{Cntrl-600Cu} = <0.001$, $p_{Cntrl-1200Cu} = <0.001$). As the concentration of free chlorine increased, the predicted probabilities that a larva has emerged decreased: 58.34% [*45.76, 69.53*] for 0.5. ppm, 21.57% [*14.00, 34.51*] for 1 ppm, 13.73 [*7.55, 21.99*] for 2 ppm. These results suggest chlorine has a dose-dependent effect on the development of *Ae. aegypti* ($p_{0.5Cl-1Cl} = <0.001$, $p_{2Cl-1Cl} = 0.008$, $p_{2Cl--0.5Cl} = <0.001$). Depicted in **Figure 2.13**, the model also portrays that while a single dose of 0.5 ppm free chlorine reduced emergence , the treatments of 1 and 2 ppm were roughly twice as effective.

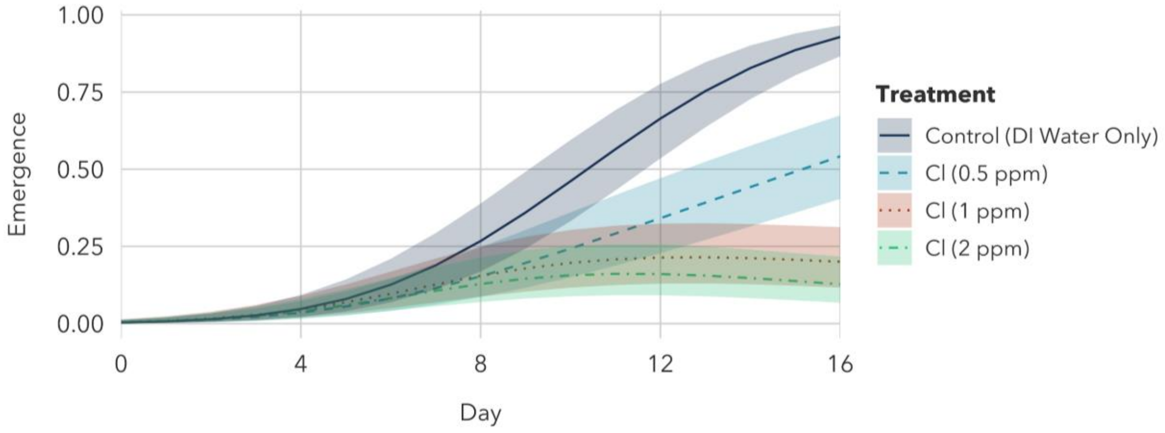


Figure 2.13 Predicted probabilities of emergence of *Ae. aegypti* larvae in contact with chlorine at concentrations 0.5, 1, and 2ppm of free chlorine

When considering the other treatments tested in this study (silver and copper), there is greater variation in the chlorine data for emergence as seen in the standard deviation reported for the observed data and captured within the model's larger confidence interval. Three possible explanations for this larger variation include:

1. the volatility of chlorine, which ultimately puts it at a disadvantage to silver and copper when comparing over a long exposure period, especially given the elevated temperature of the environment that can increase evaporation
2. the rate of consumption of the free chlorine concentrations were different within each beaker, caused by the larvae or other potential sources for contamination as well as any variations in tbe pH of the solution and temperature,
3. the ratio of hypochlorous acid (HOCl) to hypochlorite ion (OCl^-) dependent primarily on the pH of the solution (higher pH values corresponds with greater concentration of OCl^-),

The implications of both of these result in larvae having different contact times to the free chlorine, and the different specifications of the chlorine, depending on the environmental conditions of the individual beakers. HOCl is the more effective disinfectant of the two forms and thus may also be true for inducing larvicidal effects.

The relationship between the concentration of free chlorine and the inhibition of emergence of *Ae. aegpyti* is depicted in **Figure 2.14.** The linear model of the inhibition of emergence underpredicts the inhibition of emergence potential for the 1 ppm free chlorine treatment.

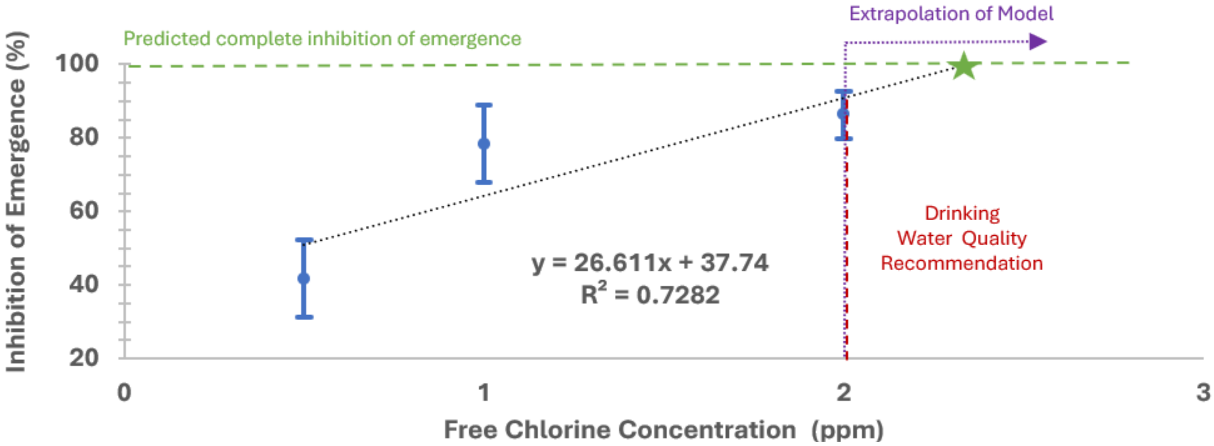


Figure 2.14 The modeled inhibition of emergence of *Ae. aegypti* larvae on day 16 of exposure to copper sulfate, with concentrations 0.5, 1, and 2 ppm free chlorine. Observed error bars represent a 95% confidence interval

2.3.1.4. Regression Analysis of Larvicidal Effects

For the three water disinfectants tested in this study, linear regression was used in the previous sections to explore the relationship between concentrations of disinfectants and inhibition of emergence and make predictions. The linear regression line applied to both the observed and modeled inhibition of emergence data for free chlorine treatments did not yield the best fit when compared to the fit obtained for silver nitrate (see **Figure 2.8**) and copper sulfate (see **Figure 2.11**) treatments. It is important to note that the dynamics of the free chlorine treatment are likely to be different from those of the silver and copper treatments because the larvae in the free chlorine treatment were no longer in contact with chlorine after 24 hours (due to reasons described in the previous section), while the larvae in the silver and copper treatments had more prolonged exposures.

Probit models assume that the relationship between the concentration of the chemical and the response follows a log-normal distribution. This relationship would assume a linear relationship on a log scale, which translates to an exponential relationship on a normal scale. It has been observed through the investigation of other insect growth regulators (IGR), such as methoprene, that the concentration-response relationship is based on a variety of factors including the specific mode of action of the IGR, the specific species it is acting against, as well as the concentration range being tested. For all the tested disinfectants, **Table 2.11** compares the linear regression with alternative exponential and logarithmic fits for the observed inhibition of emergence data. While logarithmic regression may provide a better fit for the data, it does not provide a detailed understanding into the underlying causes of the complexity or nonlinearity of the relationship between concentration and inhibition of emergence. Fundamentally, the relationship between concentration and inhibition of emergence cannot be fully understood with only three concentrations per water disinfectant.

Table 2.11 Linear, exponential and logarithmic regression models on the inhibition of emergence (%) values generated from the observed data for free chlorine, copper sulfate, and silver nitrate experimental data for day 16

Treatment	Free Chlorine		Copper Sulfate		Silver Nitrate	
Regression type	Equation	R^2	Equation	R^2	Equation	R^2
Linear	$y = 25.861x + 33.345$	0.741	$y = 0.0343x + 47.37$	0.948	$y = 0.2576x + 68.85$	0.932
Exponential	$y = 35.488e^{0.454x}$	0.669	$y = 49.932e^{0.0005x}$	0.923	$Y = 69.441e^{0.003x}$	0.920
Logarithmic	$y = 31.169\ln(x) + 63.517$	**0.886**	$y = 23.3\ln(x) - 77.635$	**0.998**	$y = 11.729\ln(x) + 37.606$	**0.995**

In **Table 2.12**, the predictions for reaching complete inhibition of emergence are given per the different regression models. The large variation seen between the different predicted concentrations for the different models emphasizes the importance of testing additional concentrations. This would allow for avoiding the limitations of extrapolation and obtaining a more accurate understanding of the complexities and nonlinearities involved.

Table 2.12 Predictions for reaching complete inhibition of emergence on day 16 given the extrapolation of different regression models

Treatment	Free Chlorine	Copper Sulfate	Silver Nitrate
Regression type		**Concentration**	
Linear	*2.575 ppm*	*1.533 ppm*	*120.99 ppb*
Exponential	*5.67 ppm*	*6.938 ppm*	*459.2 ppb*
Logarithmic	*3.228 ppm*	*1.151 x 10^9 ppm*	*202.12 ppb*

2.3.2. Younger Instar Experiments

This section reports the larvicidal results for the experiments conducted on late 1st instar *Aedes aegypti* utilizing silver nitrate, copper sulfate, and sodium hypochlorite treatments. Each of the following sections will start with observed data, which will serve as input for probit regression models. Significant differences will then be examined through analysis of the models. Observed data results are expressed in terms of *Percentage Mean±SEM* and the model data results are expressed in terms of *Predicted Probability Mean (UCI_95%, LCI_95%).*

2.3.2.1. Silver Nitrate

Silver nitrate was effective against young instar *Ae. aegpyti*, contributing to the observed 57.73±*7.20*%, 36.44±*11.23*%, and 7.95±*6.41*% survival of the larvae after 72 hours of exposure to 20, 40, and 80 ppb treatments, respectively. **Table 2.13** presents the observed survival of larvae after 24, 48, and 72 hr exposure to the silver nitrate treatments. The probit regression model was built using the observed data as the input. **Table 2.14** and **Figure 2.15** presents the predicted probability of survival model. By 72 hours, each of the treatments performed significantly different from each other ($p_{40Ag\text{-}20Ag} = <0.001$, $p_{80Ag\text{-}40Ag} = <0.001$, $p_{80Ag\text{-}20Ag} = <0.001$), with the highest concentration of silver nitrate (80 ppb) leading to the greatest mortality.

Table 2.13 Observed data for silver nitrate ($AgNO_3$) treatments of 20, 40, and 80 ppb. Standard deviation (St. dev) and standard error of mean (SEM) is presented with the mean percentage survival of younger instar *Ae. aegypti* larvae after 24, 48, and 72 hrs of exposure. Data is corrected with Abbot's formula (1925)

Treatment	*Ag (20 ppb)*			*Ag (40 ppb)*			*Ag (80 ppb)*			*Control*		
Time (hr)	*Mean*	*St. dev*	*SEM*	*Mean*	*St. dev*	*SEM*	*Mean*	*St dev*	*SEM*	*Mean*	*St dev*	*SEM*
24	92.78	4.47	2.58	89.70	1.92	1.11	86.06	7.46	4.31	99.11	1.54	0.89
48	81.13	11.95	6.90	65.55	2.36	1.36	54.11	15.69	9.06	94.22	2.04	1.18
72	57.73	12.30	7.10	36.44	19.45	11.23	7.95	11.10	6.41	82.22	2.04	1.18

Table 2.14 Predicted mean probability of survival (%) of younger instar *Aedes aegypti* after 24, 48, and 72 hrs of exposure to silver nitrate treatments of 20 ppb, 40 ppb, and 80 ppb. Data corrected by Abbot's formula (1925)

Treatment	*Ag (20 ppb)*	*Ag (40 ppb)*	*Ag (80 ppb)*	*Control*
Time (hr)	*Predicted mean [95%CI]*			
24	94.75 [91.50, 96.92]	91.51 [87.19, 94.63]	89.85 [85.30, 93.27]	99.24 [97.52, 99.81]
48	79.20 [72.29, 84.97]	63.89 [55.72, 71.47]	50.09 [41.91, 58.26]	94.24 [90.01, 96.91]
72	58.66 [49.92, 66.99]	36.87 [28.85, 45.52]	8.38 [4.90, 13.45]	82.43 [75.48, 87.98]

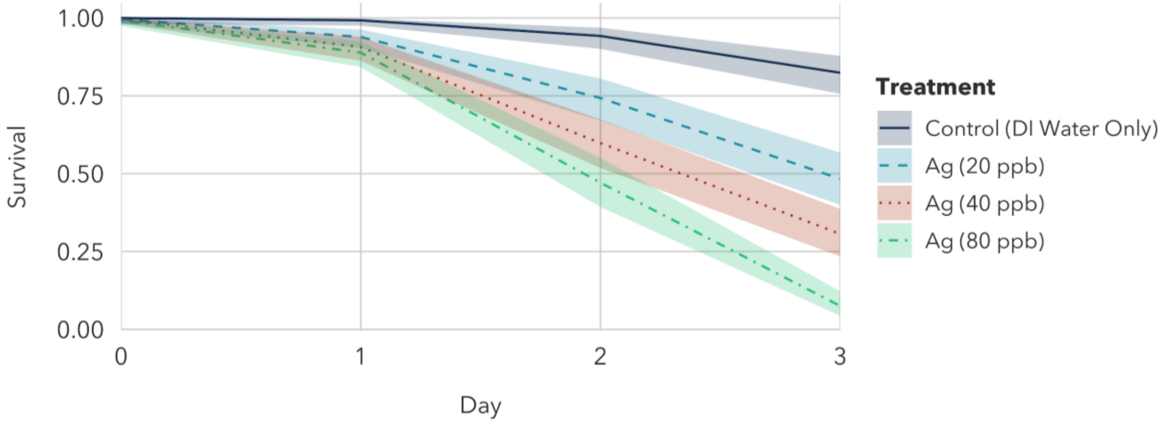


Figure 2.15 Predicted probabilities of survival of *Ae. aegypti* larvae in contact with silver nitrate concentrations 20, 40, and 80 ppb Ag

2.3.2.2. Copper Sulfate

The results suggest that copper sulfate was effective in controlling young instar *Ae. aegypti*. The larvae's survival rates after exposure to 300, 600, and 1200 ppb treatments for 72 hours were 21.85±*13.71%*, 21.85±*13.71%*, and 12.81±*8.79%*, respectively. **Table 2.15** displays the observed survival rates of larvae after 24, 48, and 72 hours of exposure to the copper sulfate treatments.

Table 2.15 Observed data for copper sulfate ($CuSO_4$) treatments of 300, 600, and 1200 ppb. Standard deviation (St. dev) and standard error of mean (SEM) is presented with the mean percentage survival of younger instar *Ae. aegypti* larvae after 24, 48, and 72 hrs of exposure. Data is corrected with Abbot's formula (1925).

Treatment	*Cu (300 ppb)*			*Cu (600 ppb)*			*Cu (1200 ppb)*			*Control*		
Time (hr)	*Mean*	*St. dev*	*SEM*	*Mean*	*St. dev*	*SEM*	*Mean*	*St dev*	*SEM*	*Mean*	*St dev*	*SEM*
24	82.59	14.96	8.64	82.59	14.96	8.64	68.31	24.64	14.23	99.11	1.54	0.89
48	57.24	25.06	14.47	57.24	25.06	14.47	32.41	33.08	19.10	94.22	2.04	1.18
72	21.85	23.74	13.71	21.85	23.74	13.71	12.81	15.23	8.79	82.22	2.04	1.18

The predicted probability of survival, which was generated through a probit regression model using the observed data, is presented in **Table 2.16** and **Figure 2.16**. The predicted probability of survival for first instar *Ae. aegypti* that were exposed to 300, 600, and 1200 ppb treatments for 72 hours were 20.67% [*8.66, 39.21*], 16.82% [*6.59, 33.90*], and 11.38% [*3.92, 25.69*], respectively. By the end of the experimental period, all treatments performed similarly in terms of producing larvicidal effects ($p_{CuTreatments\text{-}Control} = <0.001$; $p_{600Cu\text{-}300Cu} = 0.744$, $p_{1200Cu\text{-}600Cu} = 0.430$, $p_{1200Cu\text{-}300Cu} = 0.122$).

Table 2.16 Predicted mean probability of survival (%) of younger instar *Aedes aegypti* after 24, 48, and 72 hrs of exposure to copper sulfate treatments of 300 ppb, 600 ppb, and 1200 ppb. Data corrected by Abbot's formula (1925).

Treatment	*Cu (300 ppb)*	*Cu (600 ppb)*	*Cu (1200 ppb)*	*Control*
Time (hr)	*Predicted mean [95%CI]*			
24	88.71 [75.01, 95.97]	85.46 [69.89, 94.42]	74.39 [54.91, 88.25]	99.42 [96.88, 99.93]
48	53.40 [32.80, 73.11]	44.86 [25.47, 65.60]	26.94 [12.57, 46.72]	95.02 [85.93, 98.66]
72	20.67 [8.66, 39.21]	16.82 [6.59, 33.90]	11.38 [3.92, 25.69]	83.63 [66.76, 93.64]

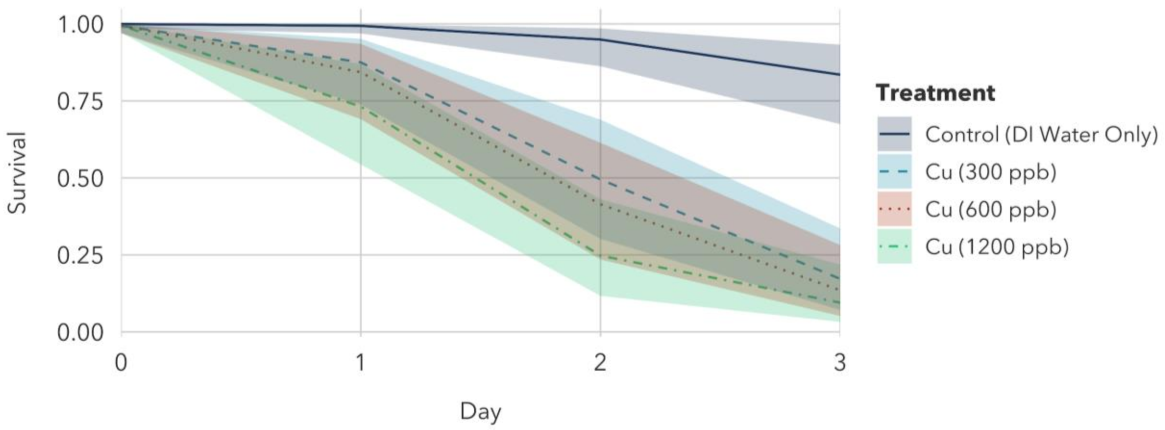


Figure 2.16 Predicted probabilities of survival of *Ae. aegypti* larvae in contact with copper sulfate concentrations 300, 600, and 1,200 ppb Cu

2.3.2.3. Sodium Hypochlorite

This study finds that sodium hypochlorite shows great efficacy in managing young *Ae. aegypti* larvae. After being subjected to treatments of 0.5, 1, and 2 ppm for 72 hours, the larvae exhibited survival rates of 15.40±*0.69%*, 8.60±*6.89%*, and 0±*0%*, respectively. The survival rates of the larvae following exposure to the copper sulfate treatments for 24, 48, and 72 hours are shown in **Table 2.17**.

Table 2.17 Observed data for sodium hypochlorite (NaOCl) treatments of 0.5, 1, and 2 ppm. Standard deviation (St. dev) and standard error of mean (SEM) is presented with the mean percentage survival of younger instar *Ae. aegypti* larvae after 24, 48, and 72 hrs of exposure. Data is corrected with Abbot's formula (1925)

Treatment	*Free Cl (0..5 ppm)*			*Free Cl (1 ppm)*			*Free Cl (2 ppm)*			*Control*		
Time (hr)	*Mean*	*St. dev*	*SEM*	*Mean*	*St. dev*	*SEM*	*Mean*	*St dev*	*SEM*	*Mean*	*St dev*	*SEM*
24	76.67	6.55	3.78	72.56	12.53	7.23	36.91	11.13	6.43	93.78	5.39	3.11
48	25.67	3.79	2.19	14.72	17.20	9.93	6.13	5.36	3.10	81.33	4.00	2.31
72	15.40	1.20	0.69	8.60	11.93	6.89	0.00	0.00	0.00	75.11	3.36	1.94

The generated probability of survival, illustrated in **Figure 2.17**, was built by using a probit regression model based on the observed data. For the first instar *Ae. aegypti* that were exposed to 0.5, 1, and 2 ppm free Cl treatments for 72 hours, the predicted probability of survival was 15.24% [*11.04*, *20.38*], 8.92% [*5.75*, *13.23*] , and 0.80% [*0.18*, *2.87*], respectively. **Table 2.18** provides the predicted probabilities of survival of the first instar larvae for the different chlorine treatment groups at 24, 48, and 72 hrs. While the 0.5 ppm and 1 ppm free chlorine treatments were not statistically significant on day 3 $p_{1Cl-0.5Cl} = 0.161$, both treatments were statistically different from the better performance of the 2 ppm treatment ($p_{(2Cl-0.5Cl)} = <0.001$; $p_{(2Cl-1Cl)} = <0.001$).

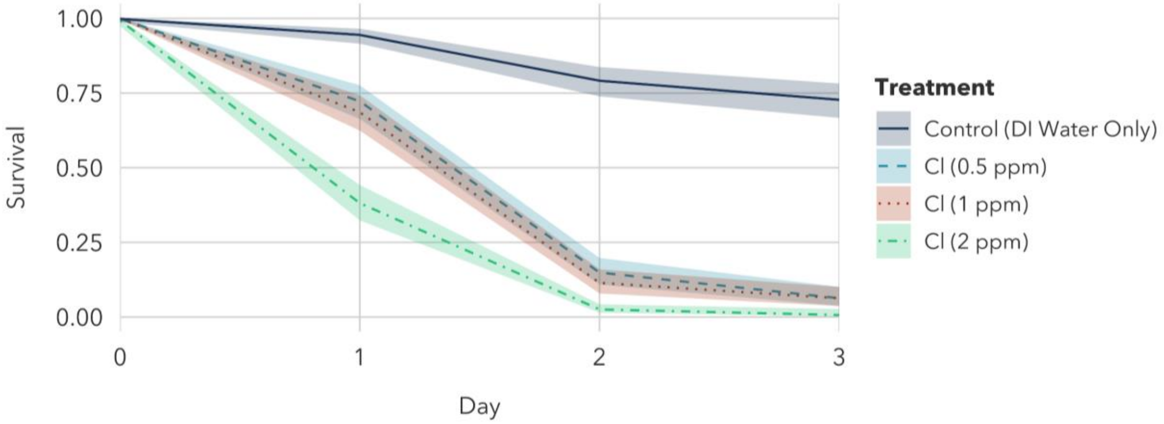


Figure 2.17 Predicted probabilities of survival of *Ae. aegypti* larvae in contact with chlorine at concentrations 0.5, 1, and 2ppm of free chlorine

Table 2.18 Predicted mean probability of survival (%) of younger instar Aedes aegypti after 24, 48, and 72 hrs of exposure to sodium hypochlorite treatments of 0.5 ppm, 1 ppm, and 2 ppm. Data corrected by Abbot's formula (1925)

Treatment	*Free Cl (0.5 ppm)*	*Free Cl (1 ppm)*	*Free Cl (2 ppm)*	*Control*
Time (hr)	*Predicted mean [95%CI]*			
24	77.29 [71.79, 82.13]	72.55 [66.53, 77.97]	41.02 [35.19, 47.05]	94.55 [91.65, 96.59]
48	25.37 [20.28, 31.05]	14.57 [10.53, 19.56]	3.34 [2.02, 5.29]	80.39 [75.26, 84.82]
72	15.24 [11.04, 20.38]	8.92 [5.75, 13.23]	0.80 [0.18, 2.87]	75.44 [69.54, 80.66]

2.3.2.4. Comparing the Disinfectants

With this study design, silver nitrate seemed to have the most potent effect on both the survival and development of the older instar larvae into an adult mosquito at the low dosing range (20 ppb Ag, 300 ppb Cu, 0.5 ppm free Cl) when compared to the two other disinfectants tested. At the mid-range dosing (40 ppb Ag, 600 ppb Cu, 2 ppm free Cl) and highest levels of dosing (i.e. 80 ppb Ag, 1200 ppb Cu, 2 ppm free Cl), there appeared to be nominal differences in efficacy between the water disinfectants ability to inhibit emergence by the end of the experimental duration.

To compare differences across water disinfectants, the probit regression model for emergence was used to build a model for inhibition of emergence. **Figure 2.18** provides the summary of the model generated IE% data for older instar on day 16. Based on the model output, it was determined that within the low dosage range, the treatment of 20 ppb Ag was notably more effective than both the 300 ppb Cu treatment and the 0.5 ppm free Cl treatment. The interpretation of the model data also demonstrated that as the concentrations of the water disinfectants increased into the mid-range and high range, the differences in efficacy between the various treatments became less pronounced.

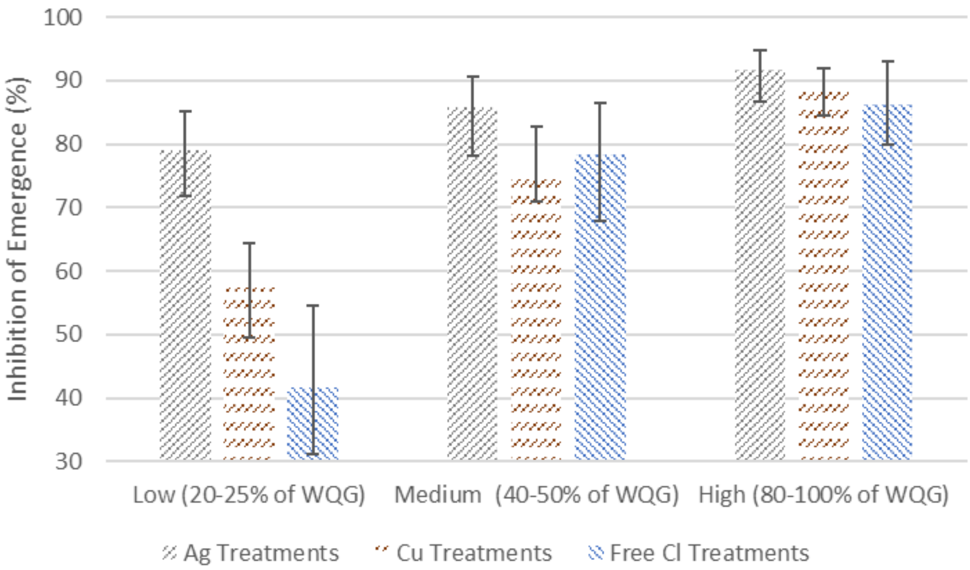


Figure 2.18 Summary of predicted probabilities of inhibition of emergence results regarding the efficacy of water disinfectants as larval control for older instar *Ae. aegypti.* IE% calculated from the model generated data from day 16. Error bars represent the 95% confidence interval

The analysis also considered the statistical significance across the treatments in order to determine if significant dose-responses were occurring. The key findings of this analysis are summarized below for each of the three disinfectants:

- *Silver nitrate treatments:* the 80 ppb Ag treatment was found to be significantly different from the 20 ppb treatment, but not significantly different from the 40 ppb treatment.
- *Copper sulfate treatments*: the model found that all treatments were statistically significant from each other.
- *Free chlorine treatments:* the 0.5 ppm free chlorine treatment was statistically significant from the 1 ppm and 2 ppm treatments; however the 1 ppm and 2 ppm treatments were not statistically different from each other.

The study design also revealed that the early instar larvae were highly vulnerable to the water disinfectants. The treatment that yielded the ideal scenario was 2 ppm Cl, with no larvae surviving the 72-hour exposure period. **Figure 2.19** displays the summary of the predicted probability of survival of the late first instar *Ae. aegypti* after 72 hrs of exposure to the water disinfectants.

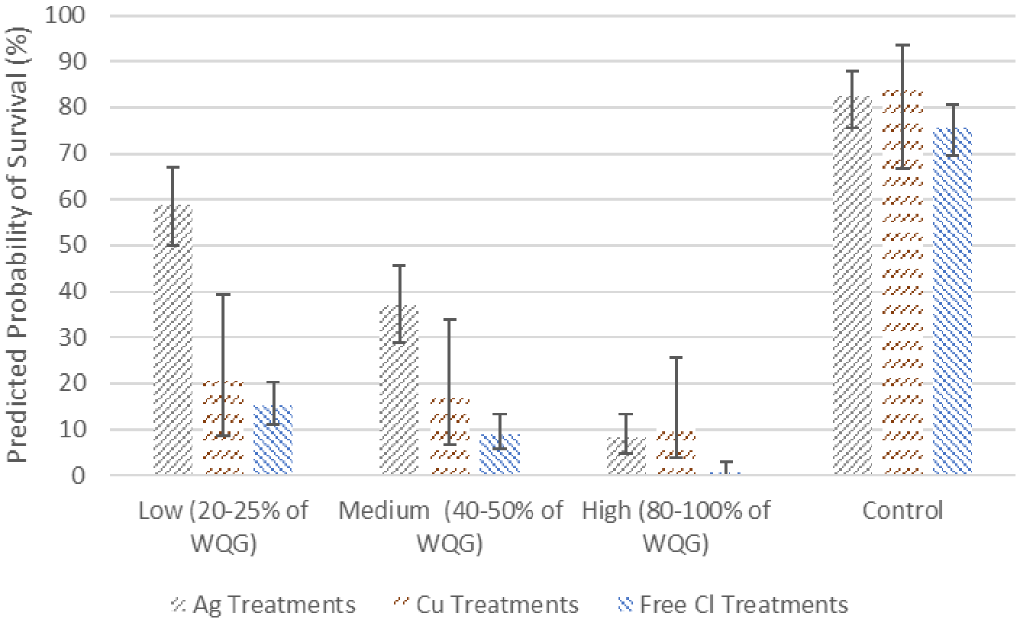


Figure 2.19 Summary of predicted probability of survival at 72 hrs regarding the efficacy of water disinfectants as larval control for younger instar *Ae. aegypti*. Error bars represent the 95% confidence interval

2.4. Discussion

This section analyzes the findings of the study and interprets them in the context of the existing literature. Higher prevalence of mosquito-borne diseases, such as dengue, has been correlated with unsafe HWS (Vannavong et al., 2017); therefore, larval source management in HWS is a core element of integrated vector control management programs (Pinchoff et al., 2021). *Aedes aegypti*, notorious vectors of Dengue, Zika, yellow fever, chikungunya, and other pathogens (Näslund et al., 2021), demonstrate resistance to common insecticides, such as temephos, currently applied in HWS (Dusfour et al, 2019); therefore, this investigation explored alternative chemicals that can effectively mitigate the emergence of mosquitoes transmitting disease, while simultaneously providing a safe household reservoir of water for human consumption.

The objective of this analysis was to enhance or guide the design of interventions for water storage containers that would lead to decreased proliferation of adult mosquitoes. Designing interventions with cost-effectiveness in mind is critical, which is why investigating the relationship between dosage and inhibition of emergence was a significant aspect of this research. The study did find that an elongated exposure to silver nitrate, copper sulfate and sodium hypochlorite at these low concentrations can negatively impact the growth and development of juvenile *Ae. aegpyti*. While the ultimate goal was to achieve 100% inhibition of emergence, the study was constrained by the drinking water regulations, which did not permit the use of chemicals at concentrations deemed unsafe for human consumption, thus causing all treatments to miss the target of full inhibition, as depicted in **Figure 2.18** (summary of results for older instar *Ae. aegypti* exposed to low, mid, and high range dosing of the various water disinfectants). Within the study presented in this paper, eggs were 1-2 months old before being exposed to the water disinfectants; thus, the different age of the eggs may have contributed to a variance in the susceptibility to the disinfectants. In the Perez & Noriega study, 1st instar larvae hatched from older eggs exhibited a significantly higher mean mortality in response to starvation. This information may explain why mortality rates in this study were generally higher than those previously reported in the literature metal toxicity to various mosquito species. *Section 5.1.2.* discusses various other factors that may have influenced the results obtained to deviate from the literature (e.g. rearing and feeding considerations).

Probit regression models were utilized to predict probabilities of survival and emergence over the experimental period. The predicted probability of emergence was then able to be used to generate a predicted probability that emergence would be inhibited. The IE% values generated by the model for the free chlorine, copper sulfate, and silver nitrate treatments in this study are susceptible to both bias and error, as is common for all models; therefore, caution should be exercised when making predictions based on these values. Ultimately, three concentrations per water disinfectant is not enough to fully understand the relationship between concentration and inhibition of emergence. When considering different types of regression for the model-generated IE% values, it was observed that both the linear and logarithmic fits provided better fit than the exponential fit. While logarithmic regression provided the best fit for the data, this investigation was not able to provide a detailed understanding into the underlying causes of the complexity or nonlinearity of the relationship between concentration and inhibition of emergence. The results presented in this study offer preliminary insights on the dynamics of the relationship between *Ae. aegpyti* development and low concentrations of three disinfectants but to gain a more comprehensive understanding of the factors that contribute to complexity (such as prolonged exposure), a wider range of concentrations must be investigated. This would lead to more accurate predictions and help develop effective strategies for controlling larvae in household water storage containers.

Overall, it is difficult to make direct comparisons between the results of the study presented within this chapter to the existing literature, as the objective in this investigation was to treat *drinking water* reservoirs with a desired goal to achieve complete inhibition of emergence, rather than determining a concentration that would kill a generic 50% of the population. Due to focusing on vector control for drinking water storage containers, the range of concentrations tested were small, essentially in the same magnitude, which is different from what is typically seen in the literature. This study highlights the need for further research to fully understand the relationship between concentration and inhibition of emergence, particularly when predicting within a smaller concentration range and for more prolonged exposures for the different disinfectants.

2.5. Conclusion

Understanding of the behaviors of *Aedes aegypti* has revealed that the presence of water storage containers provide environments for mosquitoes to breed in close proximity to where humans live, thus increasing the risk for the spread of vector borne diseases. The increase in cases and burden of mosquito borne diseases has been exacerbated by the emergence of resistance to common chemical interventions, such as temephos, currently utilized in large global mosquito mitigation efforts. As a result, there is a need for innovative and creative approaches to manage and control mosquito populations, including the treatment of water storage containers. In this study, alternative methods for treating water storage containers were examined, specifically the use of silver nitrate, copper sulfate, and sodium hypochlorite. The effectiveness of these treatments, which were tested at concentrations within the acceptable range for human consumption, were assessed by their ability to reduce the survival of juvenile *Ae. aegypti* and inhibit emergence of the larvae into an adult mosquito capable of transmitting diseases. The findings of this laboratory study suggest that using water disinfectants in water storage containers can serve two purposes simultaneously - making the water safe to drink and preventing the proliferation of disease-carrying mosquitoes.

Our study was unique in that it focused on the developmental effects of longer exposure to disinfectants over an extended observational period, which deviated from previous studies. Our findings demonstrate that lower concentrations of disinfectants can still have significant larvicidal effects, which provides a viable option for treating water storage containers. Although the ideal approach may be to eliminate larvae quickly and definitively, this method offers a practical solution specifically for the treatment of water storage containers.

By addressing two important issues simultaneously, public health projects that incorporate this approach may be more appealing to funding agencies and donors who prioritize a holistic approach to health and environmental concerns. Additionally, the use of water treatment chemicals for vector control can be seen as a cost-effective strategy for controlling disease transmission, as it targets the mosquito larvae at the source, which may be more efficient than other control methods. There is a strong need for sustained global efforts, backed by investments in alleviating poverty, to reduce the burden of VBDs. The findings from this research can provide valuable insights into one particular aspect of addressing the complex socio-technical issue we are facing globally.

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3. Finding Effective Chemical Combinations that Decrease Emergence

Abstract

Mosquitoes are the most notorious vector for spreading vector borne diseases (VBDs). Addressing underlying factors that lead to VBDs, such as universal access to safe drinking water, is crucial in preventing and controlling both VBDs and waterborne diseases. As we strive for global access to safe drinking water, countless individuals must turn to household water storage containers as a means of managing water insecurity. Household water storage containers, when not managed correctly, can harbor pathogens, becoming potential sources of waterborne diseases and mosquito breeding grounds. Appropriate household water treatment and safe storage practices can reduce the risk of both VBDs and waterborne diseases. In the previous chapter, the effectiveness of water disinfectants silver nitrate, copper sulfate, and sodium hypochlorite were evaluated in terms of their ability to control the *Aedes aegypti* vector for the application of treating household drinking water storage containers, particularly by reducing the survival of larvae and preventing their emergence as adult mosquitoes at concentrations of the disinfectant that are safe to consume for humans. None of the treatments were able to achieve the goal of completely inhibiting emergence, prompting this chapter to explore the effects of chemical combinations in an effort to reach the target. The treatments were combined at 40-50% of their recommended drinking water guidelines (e.g 80 ppb Ag + 600 ppb Cu; 600 ppb Cu + 1 ppm free chlorine) and tested against late first instar and late third instar *Ae. aegypti* larvae. The findings indicate that a combination of water disinfectants to be more efficacious in controlling the emergence of *Ae. aegypti*, compared to the use of individual chemicals in isolation. The silver plus copper combination treatment showed the greatest efficacy, achieving nearly almost complete inhibition of emergence of the older instar larvae (*98.52% [96.50, 99.47]*). The results of this study can provide direction for developing or executing a strategy for household water storage container management to reduce mosquito breeding. This study is situated within the wider framework of much needed integrated approaches to vector control, which is necessary for controlling neglected tropical diseases.

3.1. Introduction

While the modes of transmission of waterborne diseases and vector-borne diseases (VBD) are different, the underlying factors that contribute to their transmission, such as poor sanitation, poverty, and lack of access to clean water, are similar (Overgaard et al., 2021; Akanda et al., 2020). According to the WHO (2022), even with the persistent worldwide effort to expand the provision of safe drinking water, 2 billion people worldwide were still without access to safely managed drinking water services as of 2020. waterborne diseases, such as diarrhoea, cholera, typhoid fever, dysentery, polio, and cryptosporidiosis, are transmitted by microbiologically contaminated water (Cabral, 2010; WHO, 2000). An estimated 485,000 diarrhoeal deaths are caused by contaminated drinking water each year (WHO, 2022). VBDs, such as dengue fever, malaria, leishmaniases, hemorrhagic fever, Lyme disease, and Zika virus, are transmitted by arthropod vectors (e.g., mosquitoes, sandflies, ticks) that can carry viruses, bacteria, and parasites (Torto & Tchouassi, 2021; Swei, et al., 2020). Eighty percent of the world's human population is at risk of one or more VBDs (WHO, 2017a). Of the vectors responsible for spreading VBDs, mosquitoes are the most notorious (de Almeida et al., 2021), killing more people than any other creature in the world (CDC Center for Global Health, 2019). Chikungunya, dengue, Japanese encephalitis, lymphatic filariasis, malaria, yellow fever, Zika virus, Rift valley fever, and West Nile fever are some of the common VBDs associated with mosquitoes (Franklinos et al., 2019). While the number of people who contract mosquito-borne illnesses and die from them vary each year (based on various factors such as geographical region, climate, and public health measures), Qureshi (2018) estimates that almost 700 million people contract mosquito-borne illnesses every year, leading to more than one million deaths. Addressing underlying factors, such as universal access to safe drinking water, is crucial in preventing and controlling both types of diseases.

The United Nations (2013) has defined water security as "the capacity of a population to safeguard sustainable access to adequate quantities of and acceptable quality water for sustaining livelihoods, human well-being, and socio-economic development, for ensuring protection against water-borne pollution and water-related disasters, and for preserving ecosystems in a climate of peace and political stability." From a problem-oriented perspective, water insecurity is rooted in various factors that have led to a lack of access to safe and clean water (Hoekstra et al., 2018, Deshpande et al, 2020), including the following:

- environmental contamination (Delpla et al, 2009, Falkenmark, 2001),
- inadequate water supply infrastructure (UN-Water, 2021),
- climate change, resulting in increased extreme events such as drought and flooding (Stoler et al, 2021; Gosling & Arnell, 2016),
- conflict and political instability (Levy, 2019; Hidalgo, Boelens & Vos, 2017), and
- social inequity (Deshpande et al, 2020; Deitz & Meehan, 2019; Ezbakhe, Giné-Garriga & Pérez-Foguet, 2019; Anguelovski et al, 2016; Balazs & Ray, 2014; Elliott et al, 2003).

When water is perceived as scarce or unreliable, people may resort to storing water as a coping mechanism (Venkataramanan et al, 2020; Barrera, Ávila, & González-Téllez, 1993). This is especially true when centralized water supply systems are not in place or not functioning sufficiently, thus, making decentralized water treatment practices crucial in ensuring water stored is safe to drink (UN-Water, 2021).

When household water storage (HWS) containers, such as buckets, drums, or jars, are not properly managed, they can harbor pathogens, becoming a potential source of waterborne diseases (Wright, Gundry, & Conroy, 2004) and they can become potential breeding ground for mosquitoes (Padmanabha et al, 2010; Barrera, Ávila, & González-Téllez, 1993). The lack of household water connections for nearly half of the world's population (WHO & UNICEF 2015) highlights the need for appropriate measures to be implemented to reduce the risk of disease transmission. These measures include removing unused open containers, covering containers, and treating water containers (Quintero et al., 2014). According to WHO’s (2019) *International Scheme to Evaluate Household Water Treatment Technologies,* the implementations of household water treatment and safe storage (HWTS) practices by populations at risk could reduce the risk of diarrhoeal disease by as much as 61%. Furthermore, the studies that have linked unsafe water storage practices with mosquito proliferation (Overgaard, et al, 2021; Pinchoff et al, 2021; Vannavong et al, 2017) provide plausible evidence that implementing appropriate HWTS practices can not only reduce the risk of waterborne diseases but also contribute to the reduction of VBDs by reducing mosquito breeding sites.

Point-of-use water treatment (POUWT) technologies refers to the treatment of water at the place where it is being used (WHO, 2019). POUWT technologies are designed to treat HWS containers through the provision of an optimum dose of water disinfectant for the reduction of water pathogens originating from either the source water or conditions of storage (Pooi & Ng, 2018; Clasen et al., 2015). This technology is typically designed for the purpose of improving the quality of water for drinking or cooking purposes. Design considerations for POUWT technologies are typically based on:

- effectiveness of the technology to improve public health, involving reducing the potential of negative health effects from long-term exposure above the maximum contaminant level or other recommended drinking water quality standards of both the pathogen (Wu et al, 2021; Clasen, 2015; Fewtrell et al, 2005) and/or disinfectant (discussed in *Chapter 2*),
- the cost of technology and affordability to the end-user (Pooi & Ng, 2018),
- durability (Danwittayakul et al., 2017), and
- ease of use and access, including low maintenance requirements and energy requirements, contact time requirements, as well as locally available for end-user (Pooi & Ng, 2018), and
- the degree of social acceptability, considering the level of societal adoption and/or cultural assimilation to the technology, for example, controlling chlorine concentration to be

appealing by both smell and taste to enhance its acceptance (Mostafa et al., 2021; Bruchet & Duguet, 2004) or addressing distrust towards the addition of chemicals for cultural or historical reasons (Mostafa et al., 2021; Nagata et al., 2011)

Since POUWT technologies and systems treat the water in close proximity to the point of consumption, the risk of (re)contamination during storage and transport is reduced (Sobsey, 2002; Fewtrell et al., 2005). As described in **Table 3.1,** Pooi & Ng (2018) classifies common POUWT technologies into 3 main categories: flocculation and coagulation, filtration, and disinfection (refer to Clasen et al., 2015 for an assessment on the effectiveness of each of these general categories of POUWT interventions to prevent diarrhea). There are a variety of POUWT technologies with varying degrees of effectiveness in removing contaminants, which can be selected based on the specific requirements and circumstances.

Table 3.1 Classification of different POUWT from Pooi & Ng, 2018

Types	Description	Examples of POU Methods or POUWT Technologies
Flocculation & coagulation	Adding chemicals (coagulant) to the water that destabilize suspended particles, causing them to clump together into flocs. Mixing (flocculation) allows the flocs to grow and eventually settle. Results in the removal of turbidity in the water and reduces the supporting structure of microorganisms. *Direct household usage of coagulant usage rare	Natural coagulants (Moringa oleifera) Manufactured coagulants (P&G Purifier of Water, PolyGlu sachet)
Filtration	Separating solids from liquids by a physical process utilizing a filter medium. Targeting the removal of microorganisms by choosing filter medium pore size that does not let them pass through the medium.	Cloth filtration Ceramic water filters (PureMadi filter) Ultra/micro-filtration membranes (ROAMfilter Plus) Biosand filter
Disinfection	Inactivating and destroying microorganisms in the system	● Heat (e.g. boiling) ● Chemicals ○ Bleach (chlorine) ○ Tablets (MadiDrop+, Aquatab) ○ Nanotechnology (TiO_2, Ag nanoparticles) ● UV radiation/solar disinfection (polyethylene terephthalate or glass bottles, polyethylene bag)
Integrated Approach	Using two or more methods to increase treatment efficiency	Flocculation and coagulation followed by a filter membrane (Bioflocculant and portable membrane system) Flocculation and disinfection (P&G PUR sachet) Filter with activated carbon

When considering each chemical individually, we see that there are advantages, limitations, and drawbacks. **Table 3.2** compares the effectiveness and ease of use of silver, copper, and chlorine in water treatment applications. Chlorine is affordable, and highly effective against most waterborne pathogens; however, chlorine is notably not effective against *Cryptosporidium parvum* oocysts (Nakada et al, 2018; Rose et al., 2002; Korich et al., 1990) and *Mycobacteria* species (Sobsey, 1989). Additionally, the application can also result in an objectionable taste and odor to the water (Mostafa et al., 2021; Bruchet & Duguet, 2004; Sobsey, 2002) and can cause the production of harmful disinfection by-products (Li and Mitch, 2018; Wang et al., 2015). In contrast, silver and copper do not add taste, odor, or produce harmful disinfection by-products (Pathak & Gopal, 2012). On the other hand, when compared to chlorine, silver requires much higher contact times (WHO, 2017b). Studies have demonstrated silver's biocidal activity against a variety of microorganisms including bacteria, viruses, and protozoa (Singh et al, 2019; WHO, 2018; Pathak & Gopal, 2012; Swathy et al., 2014). For example, Cameron et al. (2016) observed silver nanoparticles and silver ions to significantly decrease *Cryptosporidium parvum* oocyst viability. Studies have demonstrated copper's effectiveness against a wide range of waterborne pathogens such as *E. coli*, *Legionella*, *Salmonella*, and *Cryptosporidium* (Sudha et al, 2012; Vincent, Hartemann, & Engels-Deutsch, 2016). While copper is less effective than silver at similar doses, its drinking water quality standard is lower, which permits higher concentrations to be employed for water disinfection. In comparison to silver nitrate, copper sulfate is a less expensive disinfectant option. In April 2023, ThermoFisher Scientific was selling a 500g bottle of $AgNO_3$ for roughly \$2.50/g and a 25,000 g bottle of $CuSO_4$ for roughly \$0.03/g, which translates to \$0.37/g Ag and \$0.01/g Cu. However, as just previously mentioned, higher dosage is required to achieve similar disinfection efficacy. As is the case for most metals, both the accumulation of copper and silver in the environment is a concern (Bowen, 1985).

Table 3.2 Summary of pros and cons for water disinfectants silver, copper, and chlorine

Water Disinfectant	Pros	Cons	Examples of POUWT Technologies
Silver	● Stronger than copper ● Good at disinfecting bacteria, some viruses (e.g. silver nanoparticles are effective against hepatitis B virus, murine norovirus, and syncital virus), and a protozoan species (e.g. silver nitrate can reduce infectivity of Cryptosporidium oocysts)	● Cost ● Not good at disinfecting most viruses and all other protozoans ● Requires longer contact times	MadiDrop+
Chlorine	● Can make on site ● Good at disinfecting parasites, viruses, and bacteria ● Highly effective - model for most disinfectants	● Availability ● Can alter taste and smell ● Not good at disinfecting protozoans (e.g. free chlorine is ineffective against protozoan cysts, especially Cryptosporidium) ● Form harmful byproducts	Aquatab
Copper	● Good at disinfecting viruses, bacteria, and some parasites (e.g. Cryptosporidium)	● Lower toxicity compared to silver	Copper Mesh

When using multiple chemicals together, they can provide greater disinfection efficiency and allow for the application of lower concentrations of chemicals, resulting in reduced effects on taste and odor, minimized formation of disinfection by-products when the disinfectants react with naturally present compounds in the water, avoidance of resistance forming by organisms/pathogens, shorter contact times, and cost savings (Patil et al., 2015; Lambert et al., 2003). Additionally, different chemical effects have been observed in the literature when water disinfectants have been used in combination. Some studies have observed synergistic effects, meaning that the interaction between two substances can produce a combined effect greater than the sum of their separate effects, for the utilization of water treatment chemicals for microbial disinfection efficiency (Estrella-You & Smith, 2022; Soliman et al, 2020; Patil et al., 2015; Landeen et al., 1989).

The research in *Chapters 2* and *3* are based on the premise of utilizing chemical disinfection for household water storage containers. *Chapter 2* delved into investigating alternative options for treating water storage containers for vector control using water disinfectants such as silver, copper, and chlorine. The selection of these chemicals was based on their established disinfection efficacies in water treatment processes and their common implementation in POUWT technologies and HWTS practices. The study presented in this chapter aims to investigate whether using these chemicals in combination can result in a higher level of mortality and inhibit the emergence of the disease vector *Aedes aegypti* more effectively than when used alone.

3.2. Materials and Methods

In this section, the techniques utilized for laboratory breeding and rearing of *Ae. aegypti* mosquitoes (*2.1*), the generation of different test concentrations (*2.2*), the assessment of larvae against various water treatment disinfectants (*2.3*), and the execution of data analysis (*2.4*) are described.

3.2.1. *Aedes aegypti* Culturing

The *Aedes aegypti* eggs used in the experiments were procured from Benzon Research, Inc. This colony, originating from the USDA "Gainesville" strain, has been maintained at Benzon Research since 1994. The eggs obtained from Benzon were between 2-3 weeks old. Unused eggs were discarded after a period of 1.5 months. In the Water Quality Laboratory at the University of Virginia, the mosquitoes were reared under a 12:12 hr light:dark cycle. The eggs and larvae of *Ae. aegypti* were cultured in Sterlite plastic trays (35.6×27.9×8.3 cm) filled with deionized water, kept at a temperature of 27.9 ± 0.2°C (82.2°F). The environmental conditions were monitored using an Extech RHT20 Humidity and Temperature Datalogger. The larvae were fed daily with a 3:1 mixture of liver powder and brewer's yeast (MP Biomedicals™). The larvae of the desired instar stage were collected for each experiment.

3.2.2. Water Treatment Disinfectants

The aim of this study was to determine the most effective water treatment (tested individually and in combination) for reducing the emergence of *Ae. aegypti* in water storage containers. The larvae were exposed to different concentrations of silver nitrate, copper sulfate, and sodium hypochlorite, all of which were within the safe drinking water guidelines established by public health agencies (see **Table 3.3**). To ensure safety and social acceptability, a range of concentrations was tested, none of which exceeded the upper limit of the drinking water quality recommendation. The concentration tested represent a middle range (40-50%) dosing considering drinking water quality guidelines.

Table 3.3 Concentrations tested in larvicidal bioassays with *Ae. aegypti*.

	Drinking Water Quality Guidelines	Survival Bioassay Chemical Treatments		
		Silver (Ag) AgNO3	**Copper** (Cu) CuSO4	**Chlorine** (HOCl/OCl-) NaOCl
Silver (Ag) AgNO3	**100 ppb** (WHO & EPA)	**40 ppb Ag**	**600 ppb Cu + 40 ppb Ag**	**1 ppm Cl + 40 ppb Ag**
Copper (Cu) CuSO4	**1.3 ppm** (EPA)		**600 ppb Cu**	**1 ppm Cl + 600 ppb Cu**
Chlorine (HOCl/OCl-) NaOCl	**<2 ppm Free Cl Residual @ 30 min** (CDC Safe Water System Program)			**1 ppm Cl**

The silver nitrate stock solution was prepared by dissolving 16.99 g of $AgNO_3$ powder (Artcraft Chemicals, CAS No. 7761-88-8) in 1000 mL of DI water. A 10 ppm silver solution was then made by adding 1 mL of the 100 mM $AgNO_3$ stock solution (10.79 g of silver in 1000 mL) to 1,077 mL of DI water. Concentrations were confirmed by ICP-MS analysis using the Agilent 7900 ICP-MS instrument (Agilent, Santa Clara, CA). Serial dilutions were performed to make test concentrations.

Similarly, the copper sulfate stock solution was prepared by dissolving 16.0 g of $CuSO_4$ powder (Alfa Aesar, CAS No. 7758-99-8) in 1000 mL of DI water. A 10 ppm copper solution was made by adding 1 mL of the 100 mM $CuSO_4$ stock solution (6.35 g of copper in 1000 mL) to 634 mL of DI water. Test concentrations were prepared by serial dilution. The copper level was confirmed using ICP-MS.

The stock solution of sodium hypochlorite (NaOCl) with 10-15% available chlorine was obtained from Sigma-Aldrich (CAS No. 7681-52-9). A stock solution of estimated concentration 25 ppm was prepared and further serial dilutions were performed to make free chlorine test concentrations. Free chlorine concentrations were measured using the USEPA DPD Method 8021 for low range free chlorine, measured using a digital colorimeter (HACH). Free chlorine is used as the metric for ensuring that the disinfectant concentration is high enough to effectively kill microorganisms and prevent the spread of waterborne diseases.

3.2.3. Survival Bioassays

In the laboratory study, the aim was to determine what water disinfectants (silver, copper, and chlorine), or combination of water disinfectants, were optimal for the control *Ae. aegypti* for the specific application of household water storage containers. The experimental methods were primarily based on the *WHO's Guidelines for laboratory and field testing of mosquito larvicides* (WHO, 2005).

The study examined the susceptibility of the *Ae. aegypti* larvae to disinfectants at two different stages: late third instar larvae and late 1st instar larvae. The study involved placing 25 mosquito larvae in 200 ml of solution containing varying concentrations of disinfectants, with control beakers containing only deionized water. The larvae were placed into test beakers using transfer pipettes. Larvae were fed a 3:1 LP:BY slurry. For the older instar experiments, the larvae were fed after observations on day 4, while for the younger instar experiments, the larvae were fed after observations on day 2. The larval activity was recorded every 24 hours until completion of adult emergence in all treatment groups or until the experiment reached Day 16. The efficacy of the water disinfectant on mosquito mortality was demonstrated by a few key indicators, such as: dead larvae, moribound larvae and pupae, and the presence of adult mosquitoes that did not fully separate from their pupal cases. To determine if a larva was dead or moribund, their response to disturbance in its aquatic environment was evaluated. If the larva did not move or respond, even after being probed, it was considered dead. Any larvae that completed their metamorphosis into adult mosquitoes during the experiment were considered alive for the purpose of calculating survival. Three replicates of each experiment were conducted, carried out on different days using fresh solutions and batches of larvae each time. The resulting data were analyzed statistically.

3.2.4. Data Analysis

Larvicidal activity was calculated by pooling Data from all replicates in an experiment.

To express the number of larvae that successfully developed into emerging adults, the emergence was calculated as a percentage:

$$Emergence\ (\%) = 100 - ((C - T) / C * 100) \qquad \textit{(Eq. 1)}$$

where C = percentage emergence in the untreated control and T = percentage emergence in the treated sample.

The inhibition of emergence (IE) was determined based on the number of exposed larvae. The IE% was calculated using the following formula:

$$Inhibition\ of\ Emergence\ (IE\%) = 100 - (T * 100)/C \qquad \textit{(Eq. 2)}$$

where T = percentage emergence in treated batches and C = percentage emergence in the control. Abbott's correction was applied when the emergence rate was between 80% and 95%.

To express the number of larvae that survived, the survival percentage was calculated using the following formula:

$$Survival\ (\%) = 100 - ((C - T) / C * 100) \qquad \textit{(Eq. 3)}$$

where C = percentage survival in the untreated control and T = percentage survival in the treated sample. Larvae that developed into successfully emerging adults was expressed in terms of emergence:

Probit analysis can be used to estimate the percentage of larvae survival based on the survival percentage data. In this case, survival percentage would be the binary outcome, with each larva either surviving or not surviving. Probit analysis involves fitting a probit regression model to the survival percentage data, where the predictor variable is the dose or treatment level, and the response variable is the probability of survival. The probit model assumes that the probability of survival follows a cumulative normal distribution, and the logarithm of the dose is a linear predictor of the probability of survival. The model estimates the dose or treatment level at which a certain proportion of larvae (e.g., 50%) are predicted to survive.

Statistical analysis was performed using R (*version 4.2.2*) and RStudio (*2022.07.2 Build 576*). To assess the dose response of silver, copper, and chlorine on the number of larvae, pupae, and emerged adult mosquitoes, the sample mean, standard deviation (SD), standard error of mean (SEM), was calculated on the observed data.

To build the predictive models presented in this paper, each experiment was considered a random effect. To account for non-linear changes in the rate per day, a natural spline with two degrees of freedom was used on time. Predictive models were created for each treatment using the *glmer* function from the *lme4* package. The *emmeans* function was used to make pairwise comparisons of the estimated means. For the predictive model of emergence inhibition, day 16 values for the control group were obtained from the emergence model. To generate a confidence interval, bootstrapping was performed using the upper and lower limits of the 95% confidence interval for emergence. The *bootMer* function was used to simulate 100 samples based on the model bounds.

3.3. Results and Discussion

The results portray data collected from testing silver nitrate, copper sulfate, and sodium hypochlorite on older juvenile *Ae. aegypti* (Section 3.1) and younger larvae (Section 3.2). For older instar experiments, survival and emergence data is presented over 16 days. For the younger larvae, results reflect survival over the course of 72 hours of exposure to the treatments.

3.3.1. Older Instar

In this section, the outcomes of experiments conducted on late 3rd instar *Ae. aegypti* using silver nitrate, copper sulfate, and sodium hypochlorite treatments will be presented. Each section will begin with observed data, which will be used to develop probit regression models to analyze significant differences. The observed data results are presented as *Percentage Mean* ± *SEM*, while the model data results are expressed as *Predicted Probability Mean (UCI_95%, LCI_95%).*

3.3.1.1. Silver + Copper

This section presents the results for the 40 ppb Ag + 600 ppb Cu combination treatment (Ag+Cu). The observed and model data for day 16 is presented in **Table 3.4. Figure 3.1** illustrates how the observed emergence data mapped on the probit regression model. The anticipated likelihood of larval survival over the experimental period is illustrated in **Figure 3.2** and the predicted probability that a larva has emerged is illustrated in **Figure 3.3**. From the model, the Ag+Cu combo performed significantly differently from each of the chemicals separately in terms of survival ($p_{(Ag+Cu)\text{-}Ag} = 0.002$, $p_{(Ag+Cu)\text{-}Cu} = <0.001$) and predicting how many larva had emerged ($p_{(Ag+Cu)\text{-}Ag} = 0.021$, $p_{(Ag+Cu)\text{-}Cu} = <0.001$) by the end of the observational period.

Table 3.4 Observed and model data (n=4) for silver nitrate + copper sulfate treatments on day 16. Data is corrected with Abbot's formula (1925).

Treatment	*Ag (40 ppb)*			*Cu (600 ppb)*			*Ag (40 ppb) + Cu (600 ppb)*			*Control*		
Observed												
Variable	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*
Survival (%)	11.23	7.74	3.87	29.19	14.78	7.39	3.25	4.11	2.06	91.33	1.72	0.86
Emerg (%)	9.94	8.80	4.40	22.00	13.90	6.95	2.52	4.10	2.05	88.33	5.03	2.52
IE (%)	89.01	9.47	4.74	74.47	17.08	8.54	97.29	4.38	2.19			
Model												
Variable	*Predicted mean [95% CI]*			*Predicted mean [95% CI]*			*Predicted mean [95% CI]*			*Predicted mean [95% CI]*		
Survival (%)	10.00 [6.31, 14.68]			29.53 [23.09, 37.71]			3.53 [1.87, 5.33]			92.07 [86.79, 95.58]		
Emerg (%)	7.15 [3.90, 1 4.09]			22.47 [13.50, 33.15]			1.48 [0.39, 2.94]			91.81[85.28,95.8]		
IE (%)	92.85 [88.24, 95.68]			77.53 [68.19, 85.34]			98.52 [96.50, 99.47]					

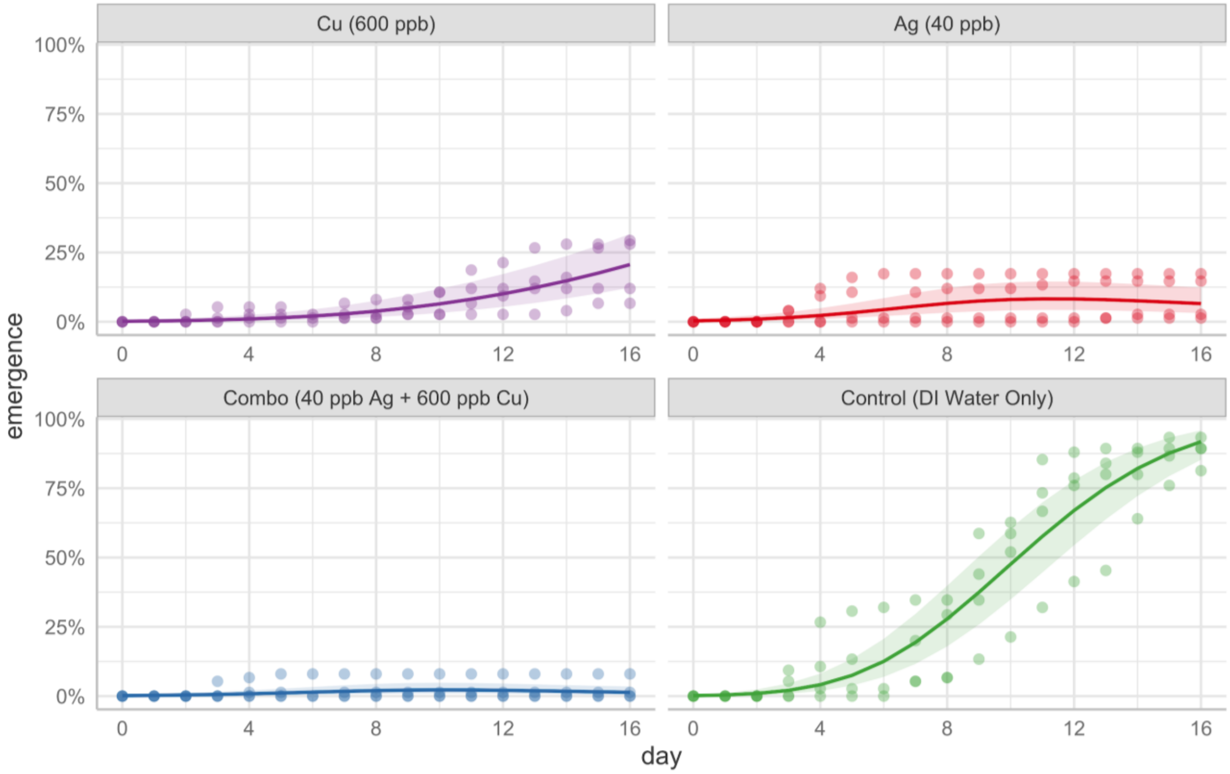

Figure 3.1 The predicted probabilities of emergence of older instar *Ae. aegypti* larvae during the experimental period for older larvae treated with copper sulfate (600 ppb), silver nitrate (40 ppb), and a combination of the two disinfectants (600 ppb Cu + 40 ppb Ag). The shaded areas represent the 95% confidence interval.

In **Figure 3.2,** it is shown that copper and silver may have varying rates of effectiveness as larvicides over time. For the predicted probability of survival, the initial part of the curve for both copper and silver have a similar decrease in survival rates of the *Ae. aegypti*, but about halfway into the experimental period, the survival rate for copper leveled out, while the survival rate for silver continued to decrease. This may suggest that the larvae become less susceptible to the effects of copper as they mature, while the toxicity of the silver treatment remains effective during the larvae's development.

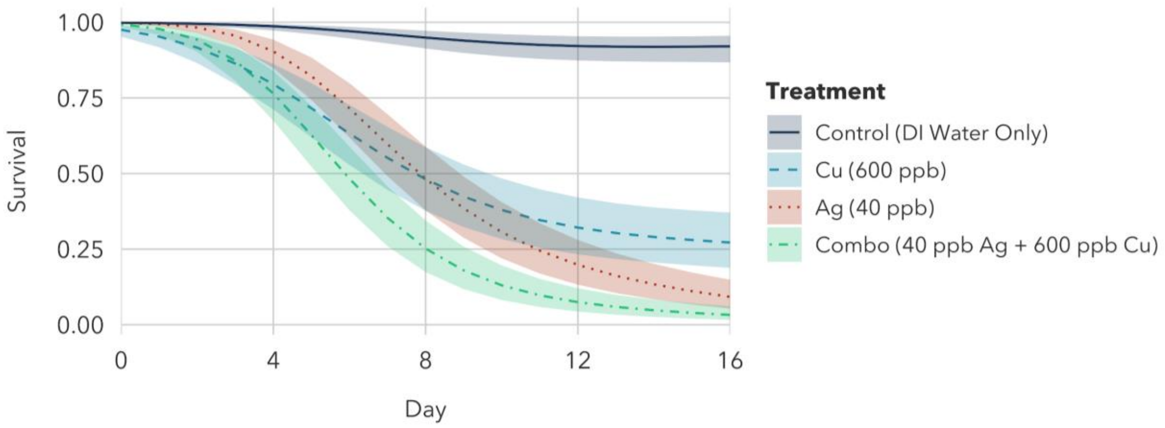

Figure 3.2 Predicted probabilities of survival of older instar *Ae. aegypti* larvae exposed to silver nitrate (40 ppb Ag), copper sulfate (600 ppb Cu), and Ag+Cu combination (40 ppb Ag + 600 ppb Cu) treatments. The shaded areas represent the 95% confidence interval

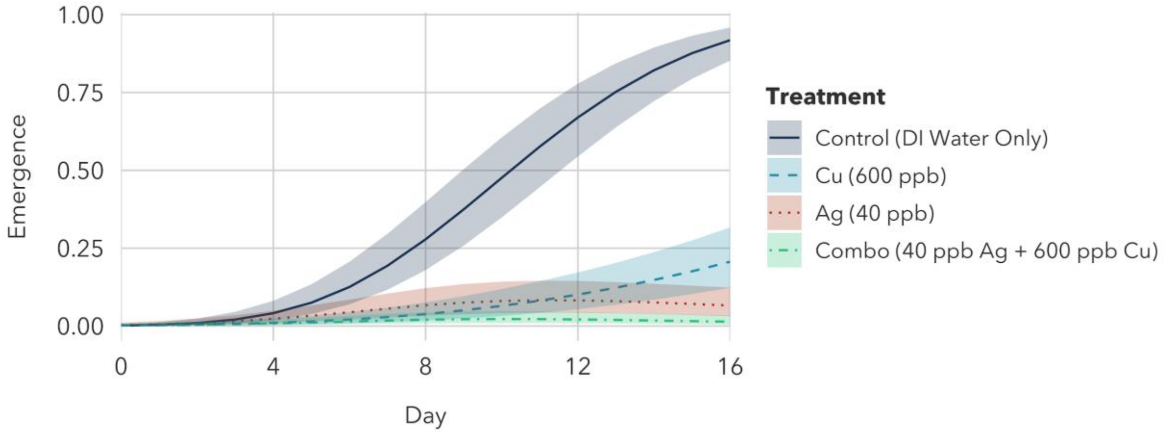

Figure 3.3 Predicted probabilities that older instar *Ae. aegypti* larvae have emerged into adult mosquitoes after being exposed to silver nitrate (40 ppb Ag), copper sulfate (600 ppb Cu), and Ag+Cu combination (40 ppb Ag + 600 ppb Cu) treatments. The shaded areas represent the 95% confidence interval

Figure 3.4 displays a side-by-side comparison of the model and observed data, depicting the inhibition of emergence on day 16. From this, we can see that the model overpredicts the average for IE% for all treatments; however, the model's average IE% consistently falls within the standard error of the mean (SEM) range of the observed data. This does provide indication that the model’s predictions are statistically consistent with the actual data. The combination of Ag+Cu was more effective in inhibiting the emergence compared to the individual chemicals used alone. Additionally, the use of the chemicals together (Ag+Cu) resulted in less variability in outcome.

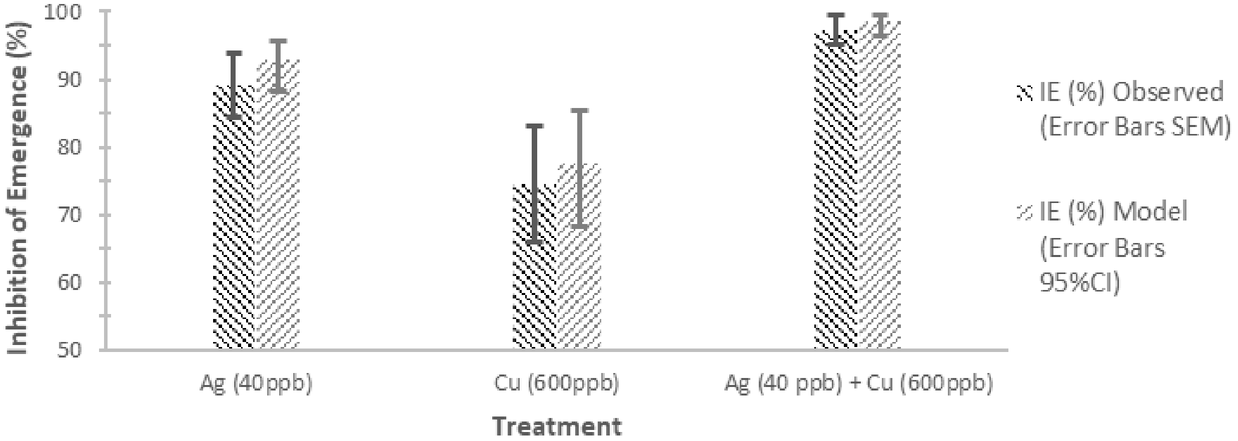

Figure 3.4 Comparing observed and model values for inhibition of emergence for silver nitrate (40 ppb Ag) and copper sulfate (600 ppb Cu) treated larvae in regards to untreated larvae on day 16.

3.3.1.2. Silver + Chlorine

In this section, the outcomes of the combined treatment using 40 ppb Ag and 1 ppm free chlorine (Ag+Cl) are presented. The observed and model data for day 16 is presented in **Table 3.5.**

Table 3.5 Observed and model data for silver nitrate + sodium hypochlorite treatments on day 16. Data is corrected with Abbot's formula (1925).

Treatment	*Ag (40 ppb)*			*Free Cl (1 ppm)*			*Ag (40 ppb) + Free Cl (1 ppm)*			*Control*		
Observed												
Variable	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*
Survival (%)	19.14	8.06	4.03	36.84	27.38	13.69	12.33	13.66	6.83	93.67	1.28	0.64
Emerg (%)	17.51	10.08	5.04	37.23	27.46	13.73	12.09	13.71	6.85	90.67	6.25	3.13
IE (%)	81.21	10.43	5.21	59.55	28.85	14.43	87.11	14.67	7.33			
Model												
Variable	*Predicted mean [95% CI]*			*Predicted mean [95% CI]*			*Predicted mean [95% CI]*			*Predicted mean [95% CI]*		
Survival (%)	17.30[12.03, 23.11]			29.26 [23.25, 36.70]			7.38 [4.49, 11.36]			94.42 [90.99, 96.72]		
Emerg (%)	11.69 [4.42, 24.67]			31.65 [21.95, 37.68]			6.25 [1.83, 15.35]			94.75 [85.62, 98.53]		
IE (%)	88.31 [78.05, 94.66]			68.35 [51.93, 82.01]			93.75 [84.65, 97.76]					

The probability of larval survival over the duration of the experiment displayed in **Figure 3.5** and the predicted probability that a larva has emerged is displayed in **Figure 3.6**. Analysis of the model indicates that Ag+Cl performed significantly better than either Ag or Cl separately in terms of predicted survival ($p_{(Ag+Cl)-Ag} = <0.001$, $p_{(Ag+Cl)-Cl} = <0.001$). For the predicted probability that a larva has emerged, the Ag+Cl treatment performed better than the chlorine alone ($p_{(Ag+Cl)-Cl} = 0.002$) , but was not significantly different from silver alone ($p_{(Ag+Cl)-Ag} = 0.154$, by the end of the observational period.

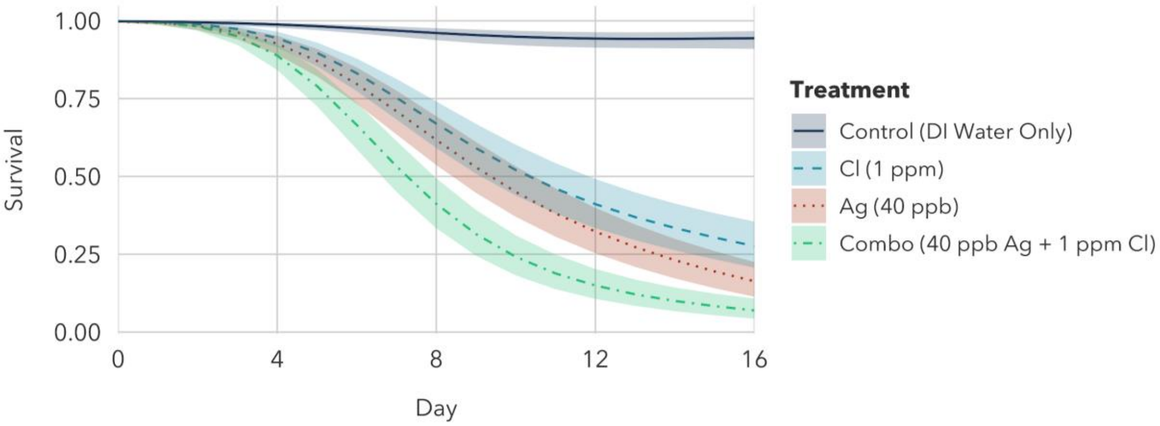

Figure 3.5 Predicted probabilities of survival of *Ae. aegypti* larvae in contact with silver nitrate (40 ppb Ag) and sodium hypochlorite (1 ppm free chlorine). The shaded areas represent the 95% confidence interval

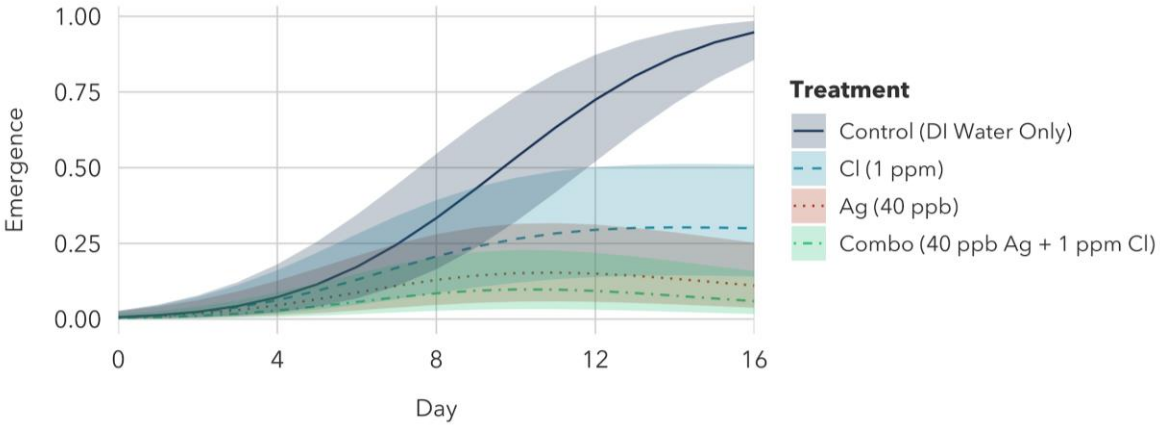

Figure 3.6 Predicted probabilities that *Ae. aegypti* larvae have emerged into adult mosquitoes after being exposed to silver nitrate (40 ppb Ag) and chlorine hypochlorite (1 ppm free chlorine) treatments. The shaded areas represent the 95% confidence interval.

A comparison of the observed data and model predictions for the IE% for day 16 are presented in **Figure 3.7**. The efficacy of the 40 ppb Ag treatment, $IE\%_{model} = 88.31$ *[78.05, 94.66]*, was similar to that of the Ag+Cl treatment, $IE\%_{model} = 93.75$ *[84.65, 97.76]*, $p_{(Ag+Cl)-Ag} = 0.154$)

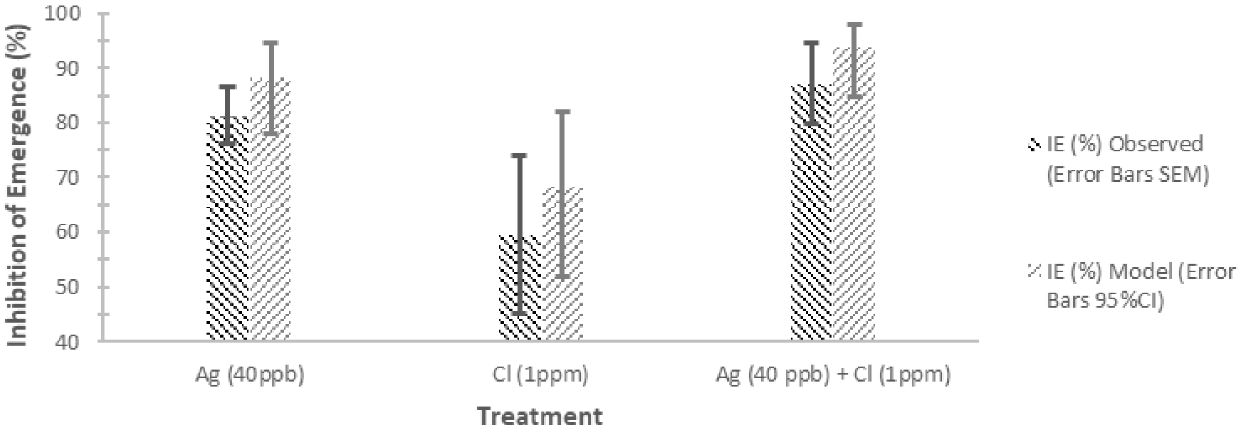

Figure 3.7 Comparing observed and model values for inhibition of emergence for silver nitrate (40 ppb Ag) and sodium hypochlorite (1 ppm free chlorine) treatments on older instar Ae. aegypti larvae on day 16

3.3.1.3. Chlorine + Copper

This section presents the results for the 600 ppb Cu + 1 ppm free chlorine combination treatment (Cu+Cl). **Table 3.6** presents the observed and model data for day 16. All treatments successfully decreased the larval population, resulting in the predicted probability of larval survival for 600 ppb Cu, 1 ppm Cl, and 600 ppb Cu + 1 ppm Cl was 19.87% (*16.51, 24.48*), 26.08% (*21.84, 31.84*), and 4.48% (*3.19*, *6.36*) as compared to the predicted emergence of the controls at 93.12% (*90.89*, *94.90*) on day 16. **Figure 3.8** shows the predicted probability of larval survival during the experimental timeframe, while **Figure 3.9** depicts the likelihood that a larva has emerged by day 16.

Table 3.6 Observed and model data for copper sulfate (600 ppb Cu) + sodium hypochlorite (1 ppm free chlorine) treatments on day 16. Data is corrected with Abbot's formula (1925)

Treatment	*Cu (600 ppb)*			*Free Cl (1 ppm)*			*Cu (600 ppb) + Free Cl (1 ppm)*			*Control*		
Observed												
Variable	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*
Survival (%)	25.08	7.31	3.66	24.46	10.01	5.01	5.86	1.06	0.53	90.67	2.18	1.09
Emerg (%)	17.87	8.07	4.04	23.77	9.47	4.74	5.51	1.27	0.63	90.33	2.75	1.37
IE (%)	80.04	9.37	4.68	73.90	9.87	4.93	93.92	1.25	0.62			
Model												
Variable	*Predicted mean [95% CI]*			*Predicted mean [95% CI]*			*Predicted mean [95% CI]*			*Predicted mean [95% CI]*		
Survival (%)	19.87 [16.51, 24.48]			26.08 [21.84, 31.84]			4.48 [3.19, 6.36]			91.59 [88.75, 93.85]		
Emerg (%)	22.67 [19.19, 27.77]			15.75 [12.87, 19.09]			4.29 [2.66, 6.06]			93.12 [90.89, 94.90]		
IE (%)	84.25 [81.08, 87.78]			77.33 [72.57, 81.12]			95.71 [94.25, 97.28]					

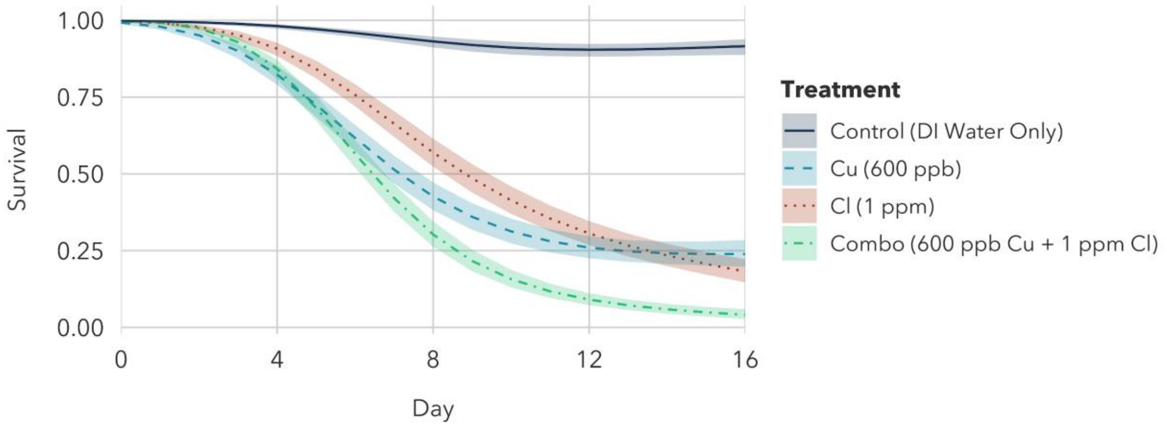

Figure 3.8 Predicted probabilities of survival of *Ae. aegypti* larvae in contact with copper sulfate (600 ppb Cu) and sodium hypochlorite (1 ppm free chlorine). The shaded areas represent the 95% confidence interval

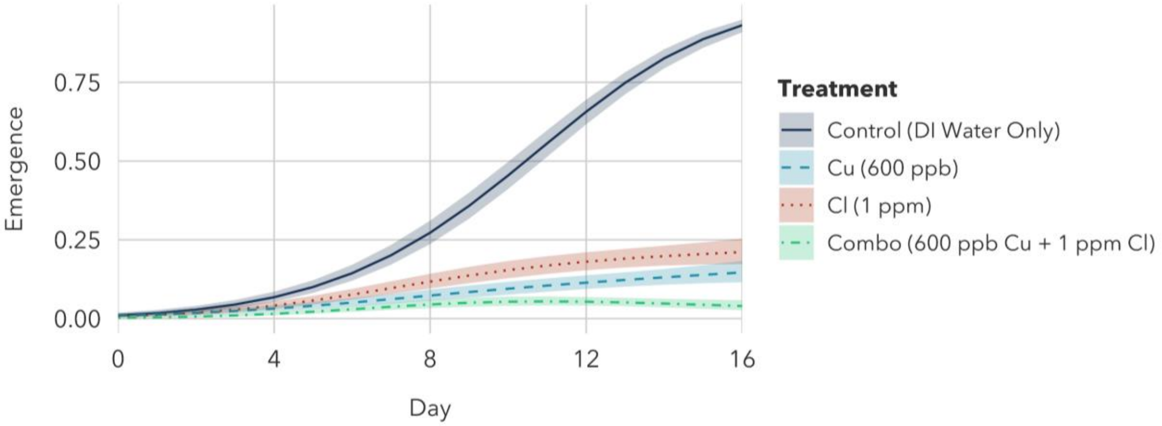

Figure 3.9 Predicted probabilities that *Ae. aegypti* larvae have emerged into adult mosquitoes after being exposed to copper sulfate (600 ppb Cu) and chlorine hypochlorite (1 ppm free chlorine) treatments. The shaded areas represent the 95% confidence interval

The results of the model indicate that the Cu+Cl combo performed significantly differently from each of the chemicals separately in terms of survival ($p_{(Cu+Cl)-Cu} = <0.001, p_{(Cu+Cl)-Cl} = <0.001$) and predicting how many larva had emerged ($p_{(Cu+Cl)-Cu} = <0.001, p_{(Cu+Cl)-Cl} = <0.001$) by the end of the observational period. This difference between treatments is also depicted in **Figure 3.10** in terms of IE%.

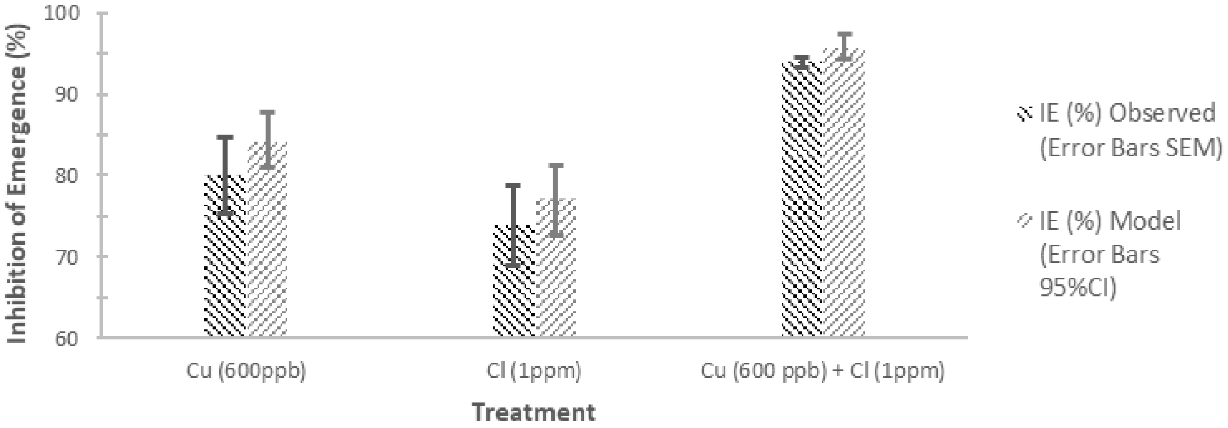

Figure 3.10 Comparing observed and model values for inhibition of emergence for copper sulfate (600 ppb Cu) and sodium hypochlorite (1 ppm free chlorine) treated larvae in regards to untreated larvae on day 16.

3.3.1.4. Silver + Copper + Chlorine

This section presents the results for the 40 ppb Ag + 600 ppb Cu + 1 ppm free chlorine combination treatment (Ag+Cu+Cl). The observed and model data for day 16 is presented in **Table 3.7**.

Table 3.7 Observed data for copper sulfate + sodium hypochlorite + silver nitrate treatments on day 16. Data is corrected with Abbot's (1925)

Treatment	*Ag (40 ppb)*			*Cu (600 ppb)*			*Cl (1 ppm)*			*Ag (40 ppb) + Cl (1 ppm)*			*Control*		
Observed															
Variable	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*
Survival (%)	13.43	4.49	2.59	21.69	6.90	3.98	25.95	7.90	4.56	3.82	5.40	3.12	92.44	1.54	0.89
Emerg (%)	13.05	5.00	2.89	14.64	8.28	4.78	24.19	8.36	4.83	3.82	5.40	3.12	91.56	1.54	0.89
IE (%)	85.80	5.19	3.00	83.93	9.24	5.33	73.67	8.69	5.02	95.89	5.77	3.33			
Model															
Variable	*Predicted mean [95% CI]*			*Predicted mean [95% CI]*			*Predicted mean [95% CI]*			*Predicted mean [95% CI]*			*Predicted mean [95% CI]*		
Survival (%)	10.00 [6.36, 16.62]			24.62 [18.17, 33.03]			24.33 [16.93, 32.35]			1.74 [0.62, 3.46]			93.4 3 [89.02, 96.32]		
Emerg(%)	10.39 [6.26, 15.33]			13.17 [8.51, 18.49]			23.61 [17.14, 32.60]			2.56 [1.27, 5.33]			94.12 [89.95, 96.79]		
IE (%)	89.61 [84.25, 94.24]			86.83 [81.06, 91.59]			76.39 [69.13, 82.91]			97.44 [94.77, 98.78]					

The anticipated likelihood of larval survival over the experimental period is visualized in **Figure 3.11** and the predicted probability that a larva has emerged is shown in **Figure 3.12**. According to the model, the combination of Ag+Cu+Cl demonstrated better performance compared to each chemical individually in terms of predicted survival ($p_{(Ag+Cu+Cl)-Ag} = 0.001$, $p_{(Ag+Cu+Cl)-Cu} = <0.001$, $p_{(Ag+Cu+Cl)-Cl} = <0.001$) and predicting how many larva had emerged ($p_{(Ag+Cu+Cl)-Ag} = 0.003$, $p_{(Ag+Cu+Cl)-Cu} = 0.001$, $p_{(Ag+Cu+Cl)-Cl} = <0.001$) by the end of the observational period.

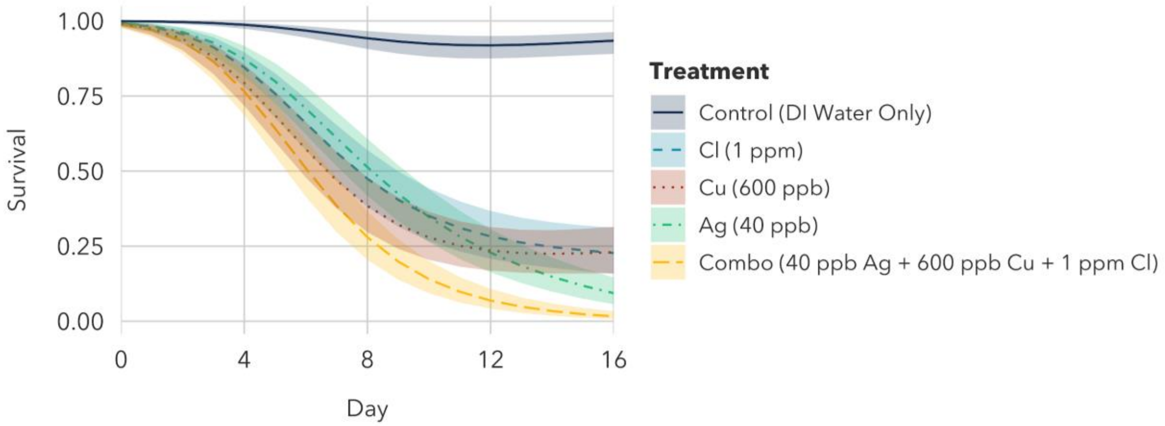

Figure 3.11 Predicted probabilities of survival of *Ae. aegypti* larvae in contact with copper sulfate (600 ppb Cu), sodium hypochlorite (1 ppm free chlorine), and silver nitrate (40 ppb). The shaded areas represent the 95% confidence interval

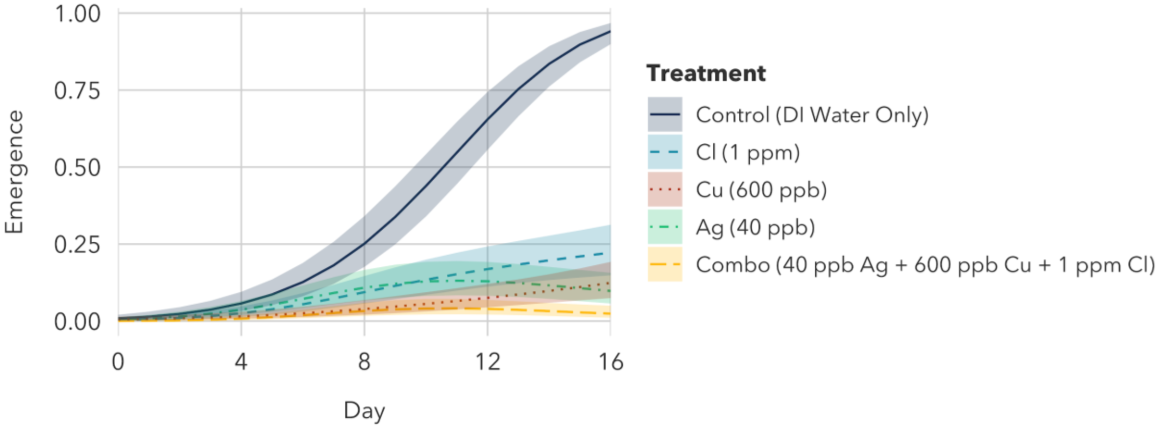

Figure 3.12 Predicted probabilities that *Ae. aegypti* larvae have emerged into adult mosquitoes after being exposed to copper sulfate (600 ppb Cu), silver nitrate (40 ppb Ag), chlorine hypochlorite (1 ppm free chlorine) treatments.

The difference between efficacy of treatments in terms of IE% (emergence inhibition percentage) is shown in **Figure 3.13**. The modeled IE% for treatments 40 ppb Ag, 600 ppb Cu and 1 ppm free chlorine were *89.61 [84.25, 94.24]*, *86.83 [81.06, 91.59]*, and *76.39 [69.13, 82.9]* respectively. The Ag+Cu+Cl combo exhibited the highest IE% at *97.44 [94.77, 98.78]*. **Figure 3.13** also provides a comparison of the observed data and model predictions for the IE% for day 16.. The IE%, for the combined use of Ag+Cu+Cl resulted in reduced variability in outcomes/predicted outcomes.

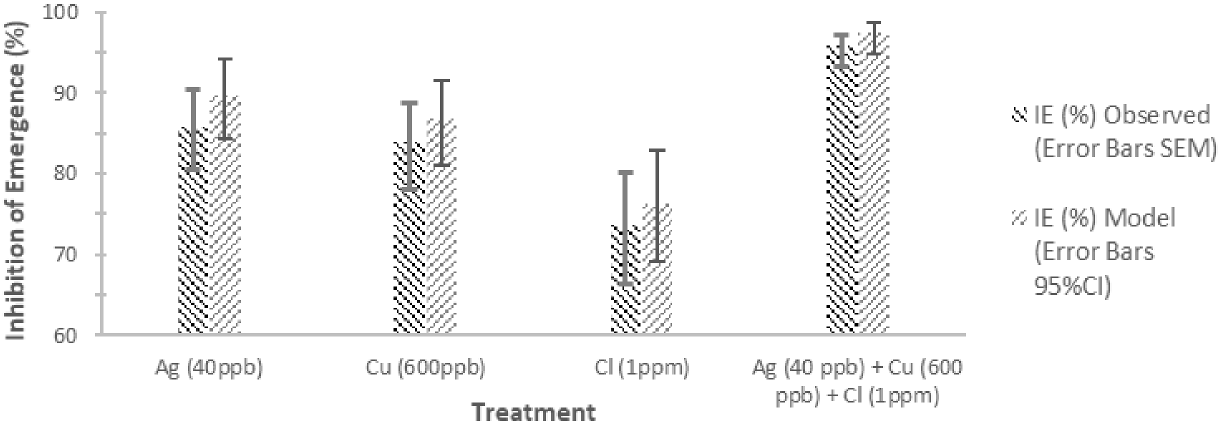

Figure 3.13 Comparing observed and model values for inhibition of emergence for silver nitrate (40 ppb Ag), copper sulfate (600 ppb Cu) and sodium hypochlorite (1 ppm free chlorine) treated older *Ae. aegypti* larvae in regards to untreated larvae on day 16.

3.3.1.5. Comparing Efficacy of Water Disinfectants in Combination

In this section, we compare the modeled outcomes of the combined treatment. **Figure 3.14** shows the modeled inhibition of emergence values for the various treatment combinations on day 16 of silver nitrate (40 ppb Ag), copper sulfate (600 ppb Cu) and sodium hypochlorite (1 ppm free chlorine). It is important to note that these experimental groups were not tested simultaneously. Overall, all treatments demonstrated a high level of efficacy in inhibiting the emergence of the juvenile *Ae. aegypti* larvae and pupae into adult mosquitoes, and they outperformed the individual chemical treatments. However, the differences between the various combinations are minimal. All the combination models predicted viability for emergence, as the 95% confidence intervals did not ever reach 100% inhibition of emergence. That being said, the Ag+Cu treatment was the closest of the treatment combinations to reach complete inhibition of emergence.

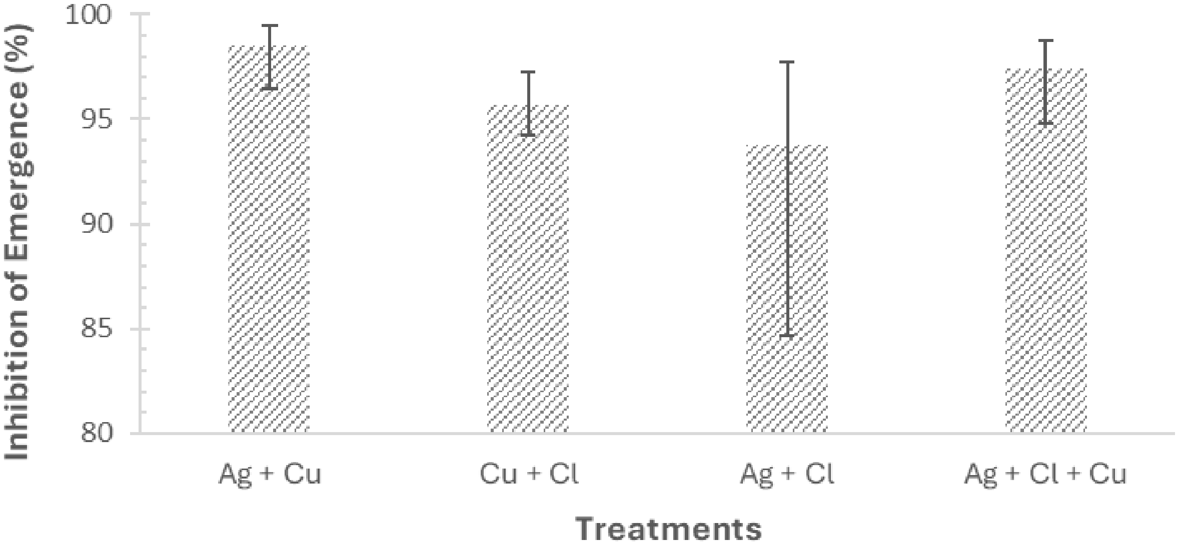

Figure 3.14 Comparing modeled inhibition of emergence (IE) values for combinations of silver nitrate (40 ppb Ag), copper sulfate (600 ppb Cu) and sodium hypochlorite (1 ppm free chlorine) treatments on older instar *Ae. aegypti* larvae in regards to untreated larvae on day 16. Error bars represent the 95% confidence interval.

3.3.1.6. Comparing Efficacy of Water Disinfectants Individually

This section presents a comparison of the modeled outcomes of individual treatments. It is important to note that the experimental groups being compared in **Figure 3.15** and **Figure 3.16** are not tested simultaneously and therefore did not have shared controls.

The data used to create **Figure 3.15**, a comparison of IE% on day 16, is an aggregation of the repetitions conducted from the combination experiments described in this chapter and the individual experiments presented in Chapter 2. A total of 750 mosquito larvae were subjected to treatment with 40 ppb silver nitrate, while 675 larvae were treated with 600 ppb copper sulfate, and another 675 larvae were treated with 1 ppm free chlorine. When tested individually at a dose equivalent to 40-50% of the water quality guideline for the chemical, silver nitrate was found to be the most effective larvicide for inhibiting the emergence of the disease vector.

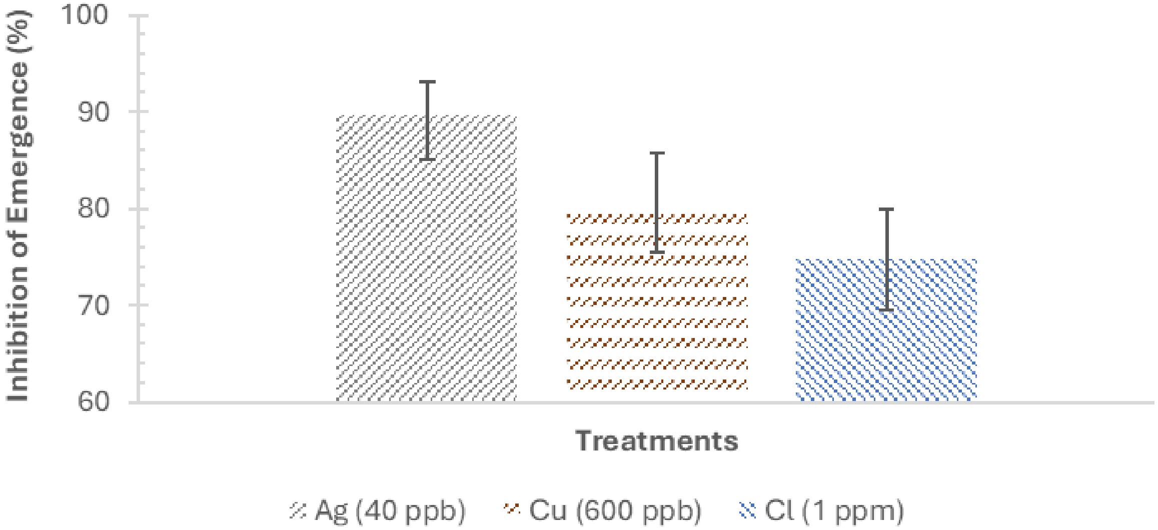

Figure 3.15 Comparing the modeled IE% for day 16 of late third instar *Ae. aegypti* larvae in contact with copper sulfate (600 ppb Cu), sodium hypochlorite (1 ppm free chlorine), and silver nitrate (40 ppb Ag) with a 95% confidence interval. Based on the data collected from all experiments that tested 40 ppb silver nitrate (750 larvae), 600 ppb copper sulfate (675 larvae), and 1 ppm sodium hypochlorite (675 larvae)

A comparison of the predicted probabilities of survival on day 16 was generated using data that is an aggregation of the repetitions conducted from the combination experiments described in this chapter and the individual experiments presented in *Chapter 2*. As previously mentioned, a total of 750 mosquito larvae were treated with 40 ppb silver nitrate, while 675 larvae were treated with 600 ppb copper sulfate, and another 675 larvae were treated with 1 ppm free chlorine. Notably, when tested individually at a dose equivalent to 40-50% of the water quality guideline for the chemical, silver nitrate was also found to be the most effective larvicide in terms of mortality.

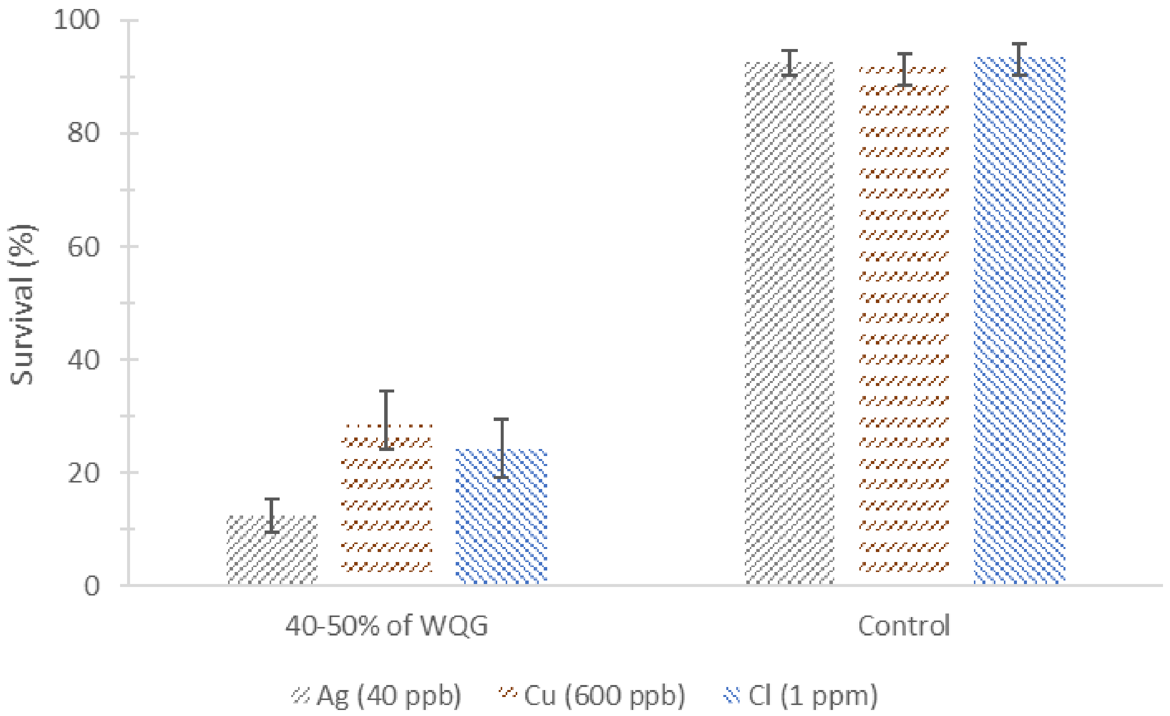

Figure 3.16 Comparing the predicted probabilities of survival for day 16 of late third instar *Ae. aegypti* larvae in contact with copper sulfate (600 ppb Cu), sodium hypochlorite (1 ppm free chlorine), and silver nitrate (40 ppb Ag) with a 95% confidence interval. Based on the data collected from all experiments that tested 40 ppb silver nitrate (750 larvae), 600 ppb copper sulfate (675 larvae), and 1 ppm sodium hypochlorite (675 larvae)

3.3.2. Younger Instar

This section presents the findings of larvicidal experiments conducted on late 1st instar *Aedes aegypti* using silver nitrate, copper sulfate, and sodium hypochlorite treatments. The subsequent sections will begin with the observed data, which will be used as input for probit regression models. The models will be analyzed to determine significant differences between the treatments. The results from the observed data are expressed as *Percentage Mean* ± *SEM*, while the results from the model data are expressed as *Predicted Probability Mean (UCI_95%, LCI_95%).*

3.3.2.1. Silver + Copper

In Chapter 2, it was observed that the utilization of silver nitrate and copper sulfate treatments shows great efficacy in managing young *Ae. aegypti* larvae. In this chapter, we compared individual performance of the chemicals (40 ppb Ag, 600 ppb Cu) to the combination of the chemicals together (40 ppb Ag + 600 ppb Cu). After being subjected to treatments of 40 ppb Ag, 600 ppb Cu, and Ag+Cu for 72 hours, only 22.67±*5.05%*, 17.33±*4.29%*, and 3.56±*1.18%*, respectively, survived up to that point. The survival observed and model data following exposure to the treatments for 24, 48, and 72 hours are shown in **Table 3.8**. The predicted probability of survival over the term of the experiment is illustrated in **Figure 3.17**. The results of the model indicate that the Ag+Cu combo performed significantly differently from each of the chemicals separately in terms of survival ($p_{(Ag+Cu)-Ag} = <0.001$, $p_{(Ag+Cu)-Cu} = <0.001$). The difference between the Ag and Cu treatments were not significantly different ($p_{Cu-Ag} = 0.315$).

Table 3.8 Observed survival (%) and modeled predicted probability of survival for copper sulfate (600 ppb Cu) and silver nitrate (40 ppb Ag) treatments for younger instar *Ae. aegypti*. Data is corrected with Abbot's formula (1925).

	Ag (40 ppb)			*Cu (600 ppb)*			*Ag (40 ppb) + Cu (600 ppb)*			*Control (DI Water Only)*		
Observed												
time (hr)	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*
24	85.78	6.30	3.64	83.11	14.13	8.16	79.11	14.38	8.30	96.44	5.05	2.91
48	56.00	4.81	2.78	35.11	11.18	6.46	14.22	5.55	3.20	91.11	5.39	3.11
72	22.67	8.74	5.05	17.33	7.42	4.29	3.56	2.04	1.18	82.22	6.30	3.64
Modeled												
time (hr)	*predicted mean [95% CI]*			*predicted mean [95% CI]*			*predicted mean [95% CI]*			*predicted mean [95% CI]*		
24	88.50 [82.32, 92.97]			84.20 [76.42, 90.07]			79.73 [70.86, 86.75]			97.58 [94.92, 98.96]		
48	53.34 [43.08, 63.37]			34.49 [25.27, 44.74]			13.74 [8.28, 21.25]			90.60 [84.61, 94.66]		
72	23.28 [15.69, 32.57]			17.14 [10.83, 25.41]			3.33 [1.35, 7.26]			82.97 [74.73, 89.25]		

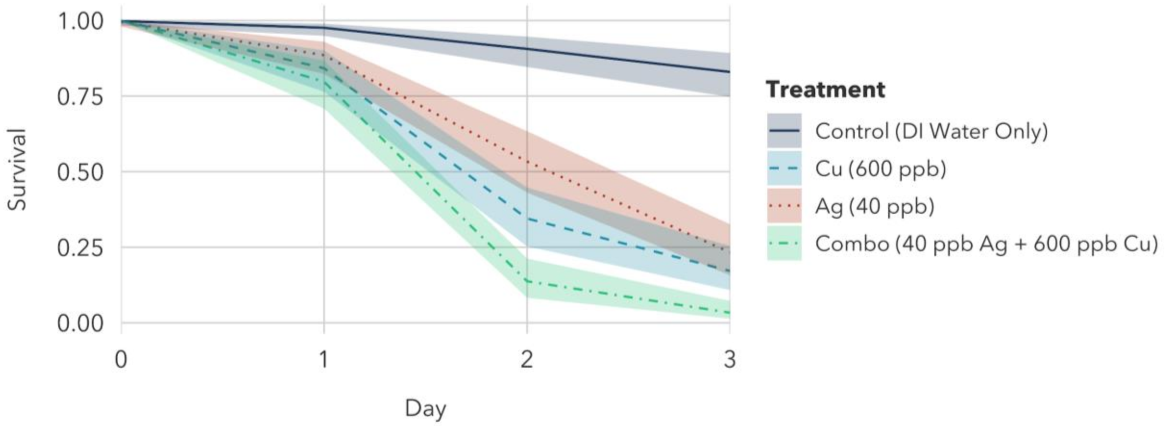

Figure 3.17 Predicted probabilities of survival of *Ae. aegypti* larvae in contact with silver nitrate (40 ppb Ag), copper sulfate (600 ppb Cu), and a combination of the two disinfectants. Shaded area represents the 95% confidence interval of the probit regression model.

In **Figure 3.18**, the observed values for survival are compared with the model predicted probability of survival at 72 hours of exposure. The model appears to overpredict mortality for the silver treatment but underpredict the efficacy of the copper treatment after 72 hours of exposure.

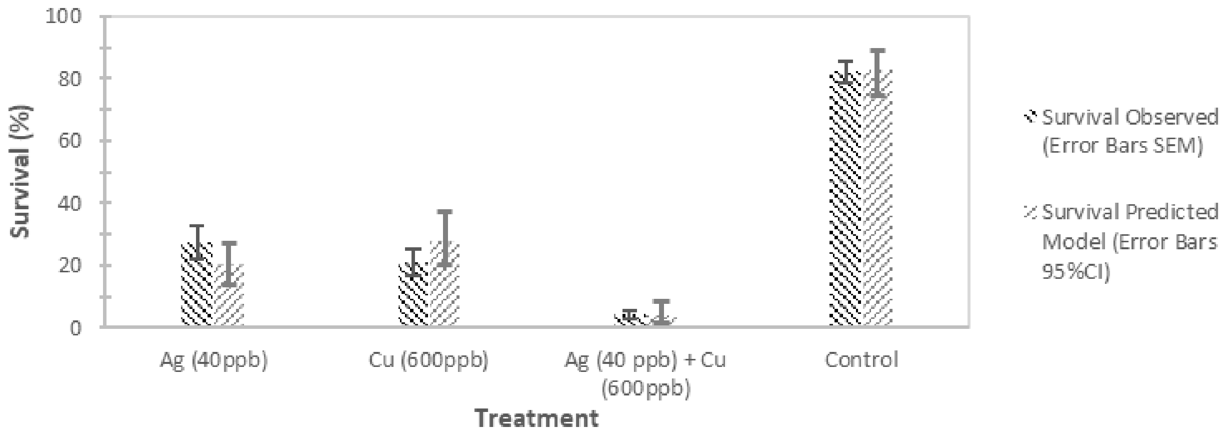

Figure 3.18 Comparing observed and model values for survival for copper sulfate (600 ppb Cu) and silver nitrate (40 ppb Ag) treated larvae in regards to untreated larvae at 72 hrs. Data is corrected with Abbot's formula (1925).

3.3.2.2. Silver + Chlorine

Within this set of experiments, it was observed that the performance of silver individually in decreasing survival (40 ppb Ag: 30.22±*8.30%*) was not as good as that of chlorine individually (1 ppm Cl: 8.44±*4.64%*) or the combination of both chemicals together (40 ppb Ag + 1 ppm Cl: 5.78±*2.35%*) after 72 hours of exposure. **Table 3.9** shows the observed survival and model predicted probability of survival data after exposure to the treatments for 24, 48, and 72 hours. **Figure 3.19** illustrates the predicted probability of survival throughout the duration of the experiment and **Figure 3.20** presents the side-by-side comparison of the observed data versus the modeled predicted probability of survival data. Results of the model indicate that the Cl and Ag+Cl treatments perform better than the Ag alone treatment in terms of survival ($p_{(Ag+Cl)-Ag} = <0.001$, $p_{Ag-Cl} =<0.001$); however, the combination treatment was not significantly different from the Cl alone treatment ($p_{(Ag+Cl)-Cl} =0.446$).

Table 3.9 Observed survival (%) and modeled predicted probability of survival for silver nitrate (40 ppb Ag) + sodium hypochlorite (1 ppm free chlorine) treatments for younger instar *Ae. aegypti*. Data is corrected with Abbot's formula (1925).

	Ag (40 ppb)			*Cl (1 ppbm)*			*Ag (40 ppb) + Cl (1 ppb)*			*Control (DI Water Only)*		
Observed												
time (hr)	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*
24	80.89	7.58	4.38	65.33	10.67	6.16	60.44	16.29	9.41	92.89	3.85	2.22
48	48.89	12.10	6.98	25.78	22.68	13.09	21.33	18.33	10.58	85.78	8.68	5.01
72	30.22	14.38	8.30	8.44	8.04	4.64	5.78	4.07	2.35	77.78	6.01	3.47
Modeled												
time (hr)	*predicted mean [95% CI]*			*predicted mean [95% CI]*			*predicted mean [95% CI]*			*predicted mean [95% CI]*		
24	83.81 [73.88, 90.89]			69.37 [56.61, 80.13]			64.79 [51.61, 76.40]			95.17 [90.17, 97.89]		
48	46.51 [33.46, 59.95]			21.53 [12.91, 32.80]			16.64 [9.45, 26.65]			84.74 [74.89, 91.61]		
72	30.31 [19.30, 43.48]			8.99 [4.26, 16.82]			6.22 [2.67, 12.68]			79.17 [67.55, 87.89]		

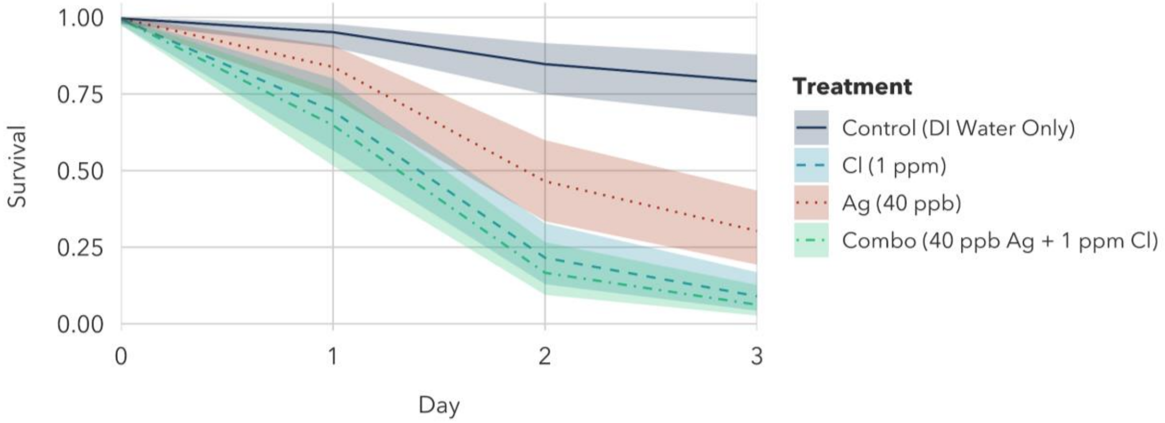

Figure 3.19 Predicted probabilities of survival of *Ae. aegypti* larvae in contact with silver nitrate (40 ppb Ag), sodium hypochlorite (1 ppm free chlorine), and a combination of the two disinfectants. Shaded area represents the 95% confidence interval of the probit regression model.

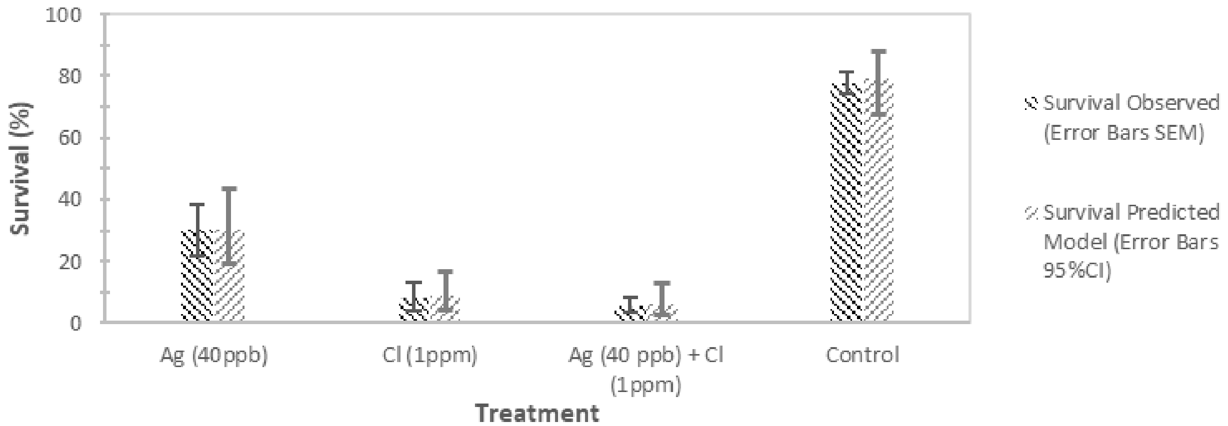

Figure 3.20 Comparing observed and model values for survival for silver nitrate (40 ppb Ag) and sodium hypochlorite (1 ppm Cl) treated larvae in regards to untreated larvae at 72 hrs. Data is corrected with Abbot's formula (1925).

3.3.2.3. Chlorine + Copper

Within this set of experiments, it was observed that all of the treatments were extremely effective in decreasing survival (600 ppb Cu: 10.67±*2.78%*, 1 ppm Cl: 6.22±*4.95%,* 600 ppb Cu + 1 ppm Cl: 1.33±*0.77%*) after 72 hours of exposure. **Table 3.10** shows the observed survival and model predicted probability of survival data after exposure to the treatments for 24, 48, and 72 hours. The predicted probability of survival over the course of the experiment is depicted in **Figure 3.21**, while **Figure 3.22** presents a comparison of the observed data versus the modeled predicted probability of survival data. Besides all treatments being significantly different controls, the only other statistical difference between treatments was found between the Cu+Cl combo and Cu alone treatments ($p_{(Cu+Cl)-Cu} = <0.001$).

Table 3.10 Observed survival (%) and model predicted probability of survival for copper sulfate (600 ppb Cu) + sodium hypochlorite (1 ppm free chlorine) treatments for younger instar *Ae. aegypti*. Data is corrected with Abbot's formula (1925).

	Cu (600 ppb)			*Cl (1 ppbm)*			*Cu (600 ppb) + Cl (1 ppb)*			*Control (DI Water Only)*		
Observed												
time (hr)	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*	*mean*	*sd*	*sem*
24	63.56	4.07	2.35	68.44	15.57	8.99	29.78	22.32	12.89	93.78	5.39	3.11
48	27.56	3.08	1.78	11.56	13.15	7.59	7.11	6.30	3.64	81.33	4.00	2.31
72	10.67	4.81	2.78	6.22	8.57	4.95	1.33	1.33	0.77	74.67	2.67	1.54
Modeled												
time (hr)	*predicted mean [95% CI]*			*predicted mean [95% CI]*			*predicted mean [95% CI]*			*predicted mean [95% CI]*		
24	67.88 [61.89, 73.44]			68.53 [61.74, 74.74]			34.71 [28.60, 41.25]			94.54 [91.47, 96.67]		
48	23.38 [18.62, 28.75]			11.32 [7.61, 16.19]			3.41 [2.08, 5.36]			80.37 [74.74, 85.17]		
72	11.83 [7.98, 16.84]			6.18 [3.53, 10.18]			2.44 [1.01, 5.31]			75.04 [68.55, 80.73]		

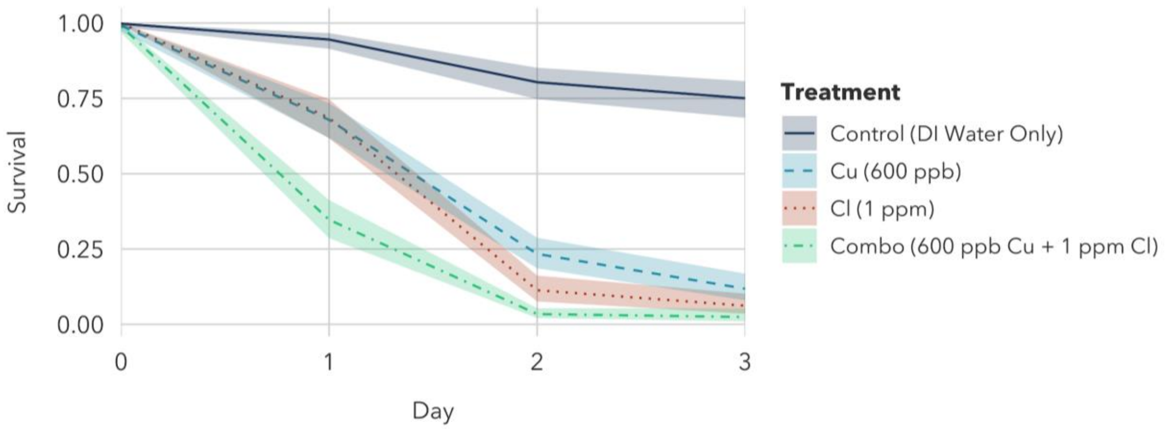

Figure 3.21 Predicted probabilities of survival of *Ae. aegypti* larvae in contact with copper sulfate (600 ppb Cu), sodium hypochlorite (1 ppm free chlorine), and a combination of the two disinfectants. Shaded area represents the 95% confidence interval of the probit regression model.

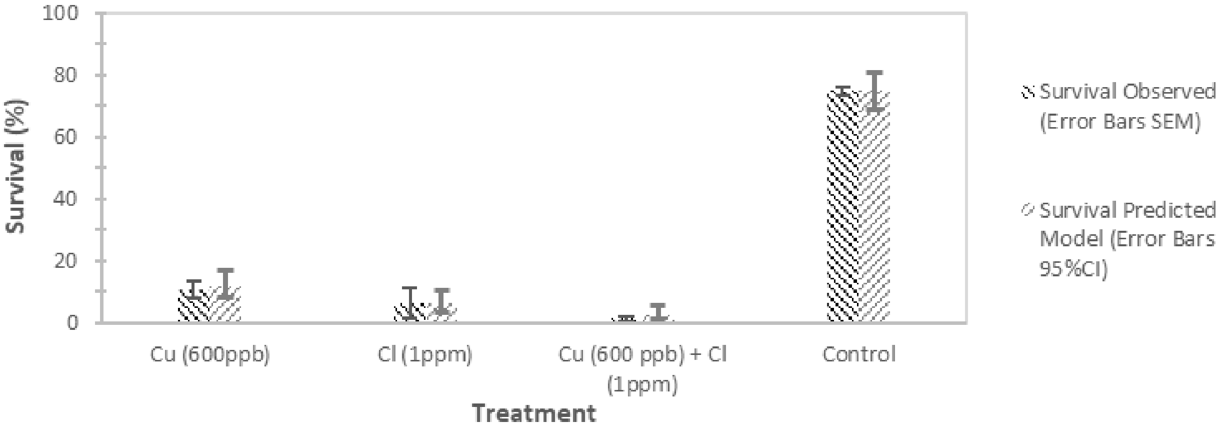

Figure 3.22 Comparing observed and model values for survival for copper sulfate (600 ppb Cu) and sodium hypochlorite (1 ppm free chlorine) treated larvae in regards to untreated larvae at 72 hrs. Data is corrected with Abbot's formula (1925).

3.3.2.4. Comparing Efficacy of Water Disinfectants in Combination

In this section, the modeled outcomes of the combined treatment are compared. **Figure 3.23** shows the predicted probability of survival for the various treatment combinations on day 16 of silver nitrate (40 ppb Ag), copper sulfate (600 ppb Cu) and sodium hypochlorite (1 ppm free chlorine). These experimental groups were not tested simultaneously. All treatments demonstrated a high level of efficacy in killing young *Ae. aegypti* larvae. Unlike the older instar larvae experiments presented in *Section 3.3.1*, the combination treatments against younger instar larvae did not always outperform the individual chemical treatments; however, this is because all treatments were highly effective. Of the combinations, Cu+Cl appears to have had the most toxic effect on the younger instar larvae.

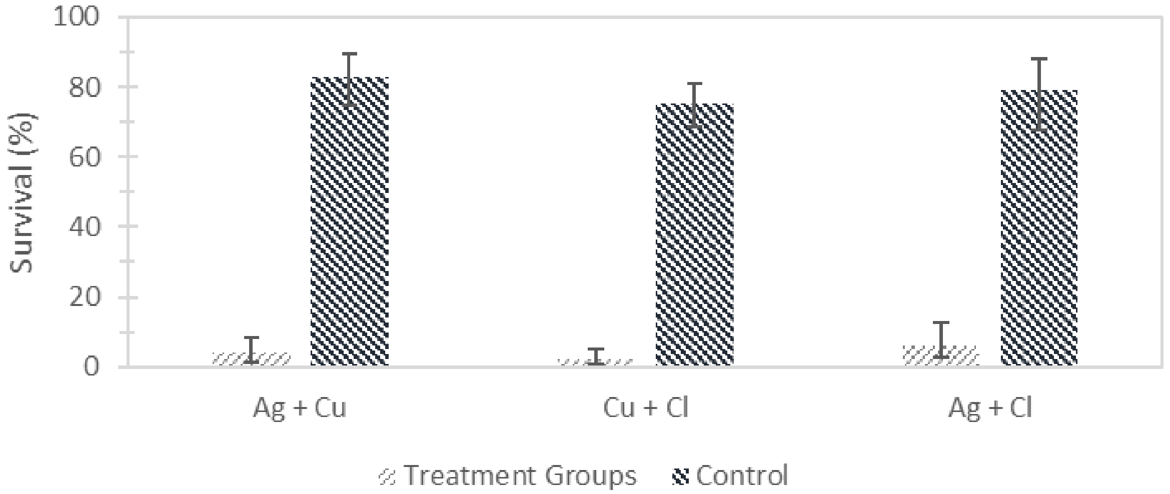

Figure 3.23 Comparing model values for inhibition of emergence for combinations of silver nitrate (40 ppb Ag), copper sulfate (600 ppb Cu) and sodium hypochlorite (1 ppm free chlorine) treated late 1st instar larvae after 72 hrs of exposure.

3.3.3. Efficacy of Water Disinfectants Individually: Comparing Data from Chapter 2 to All Data Collected in Aggregate (Chapters 2 & 3)

In *Chapter 2*, individual experiments were conducted to test the effectiveness of each larvicide on mosquito larvae survival. In *Chapter 3*, combination experiments were conducted to test the effectiveness of combinations of the same larvicides on mosquito larvae survival. A more robust model (introduced in **Figure 3.15)** for the IE% of the individual treatments was developed using the aggregated data, based on the larger sample size for 40 ppb Ag (750 larvae), 600 ppb Cu (675 larvae), and 1 ppm Cl (675 larvae). The results in Chapter 2 were based on a subset of this larger sample of 225 larvae for each of the treatments. **Figure 3.24** depicts the comparison between the results presented in *Chapter 2* for the predicted probability of survival on day 16 and the aggregate data (data collected in Chapter 2 and in Chapter 3 on the water disinfectants at a dose equivalent to 40-50% of the water quality guideline for the chemical tested individually). The comparison shows that increasing the sample size did not significantly alter the model, indicating that the results were reproducible, thus providing confidence in the reliability and robustness of the study's results.

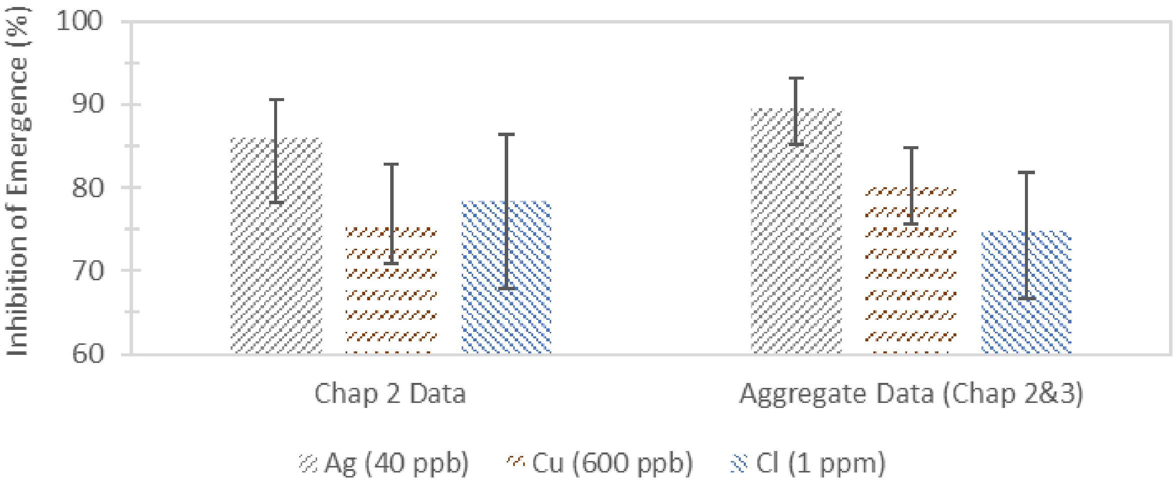

Figure 3.24 Comparing the predicted probabilities of survival for day 16 of late third instar *Ae. aegypti* larvae in contact with copper sulfate (600 ppb Cu), sodium hypochlorite (1 ppm free chlorine), and silver nitrate (40 ppb Ag) with a 95% confidence interval. Based on the data from the experiments portrayed in Chapter 2 for 40 ppb silver nitrate (225 larvae), 600 ppb copper sulfate (225 larvae), and 1 ppm sodium hypochlorite (225 larvae) versus the aggregate of data collected from all experiments from Chapters 2 and 3 that tested 40 ppb silver nitrate (750 larvae), 600 ppb copper sulfate (675 larvae), and 1 ppm sodium hypochlorite (675 larvae).

3.4. Conclusion

Household water storage containers, when not properly managed, can serve as host for disease-causing pathogens and become potential breeding grounds for mosquitoes, leading to the transmission of waterborne diseases and vector borne diseases. Implementing appropriate household water treatment and safe storage practices can reduce the risk of both types of diseases. This study investigated the effectiveness of water disinfectants silver nitrate, copper sulfate, and sodium hypochlorite as larvicides against juvenile *Aedes aegypti*. For the older late third instar larvae, when comparing the efficacy of the water disinfectants, silver performed the best inhibiting emergence. The results also suggest that combining water disinfectants is more effective than using individual chemicals alone in controlling emergence of *Ae. aegypti*. This research can be used to design interventions aimed to improve access to safe drinking water, promote proper household water management, and reduce the risk of disease transmission.

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4. Social Justice in Engineering Design: Applying in the Classroom

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Constructing a More Restorative, Inclusive, Engineering Practice: A Case Study of Engineering Social Justice in an Introductory Civil Engineering Course

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Abstract

Within a core undergraduate civil engineering course, students engaged with an online learning module and participated in an in-class workshop that emphasized the intersection of social justice and the construction design process. The results presented in this paper analyze how engineering undergraduate students felt about interacting with engineering concepts contextualized through a social justice perspective. Students who participated in the study took both a pre and post survey, which allowed for comparative data on the student's preconceptions of social justice as well as if the intervention improved their perceptions of the importance of social justice in engineering. The results show, even before the intervention, students perceived value in engineering social justice issues. That said, engaging in the intervention still had significant impacts on students' perceptions of social justice. After their experience with the online module and in-class discussion, students were significantly more likely to: think they will encounter social justice issues; have an opportunity to address social justice issues; see social justice as relevant to engineering; and feel they knew more about social justice than before the module. The results from this work supports other findings in the engineering education literature that suggests students are interested in and benefit from contextualizing the societal implications of engineering work throughout their engineering education. The student feedback presented in this paper contributes important insights on the development of future modules and workshops on engineering and social justice.

4.1. Introduction

Engineering doesn't occur in a vacuum, devoid of inequalities, social hierarchy, and politics, thus, neither should students' exposure to engineering concepts in the classroom. Without unpacking racial and cultural supremacy values held in society, engineers will further perpetuate, rather than disrupt, the normative standards of marginalization, oppression, exploitation, and dehumanization of groups (Semmes, 1995; Karwat, 2019). One of the ways engineering educators are seeking to address sustained injustice is through changes to engineering practice. For example, "engineering for good" seeks to have the practice of engineering be explicitly dedicated to benefit underserved populations over other traditional goals and priorities, such as financial profit and technological efficiency (Kleine & Lucena, 2021). Yet, the cultural transformation to engineering practice that "engineering for good" is attempting to make is likely to clash with the existing state of engineering curriculum that is built on cultural pillars of meritocracy and depoliticization as identified by Cech (2013). Generating traction for socially just engineering practices requires changes in how engineering students are taught. This paper explores an approach that seeks to develop students' critical thinking skills through the inclusion of diverse perspectives and engagement of students' positionality while exploring what it means to design justly.

To date, in both curriculum and pedagogy, many scholars are arguing for a new approach to engineering education that supports building a profession that serves humanity in pursuit of social justice (Reynante, 2021; Roscoe et al., 2019; Chen et al., 2020; Leydens & Lucena, 2017; Rulifson & Bielefeldt, 2017; Winberg & Winberg, 2017; Karwat, 2015; Leydens et al., 2014; Campbell, 2013; Cumming-Potvin & Currie, 2013; Baillie & Pawley, 2012; Riley, 2008; Baillie, 2006) and incorporates transformational pedagogical approaches that often integrate concepts by Paulo Freire (2000) and bell hooks (1994). Building on this foundational work, our research team critically considered the undergraduate engineering curriculum–from both our personal experiences engaging with it and our perception of its current state at our affiliated university–and raised the following question: "What roles in society are engineers truly being prepared to undertake?" We feel that in countless ways, the current state of engineering curriculum has helped to preserve a culture that does not place equity or justice at the heart of engineering practice. As a result, the positive and negative impacts of work done by engineers are not shared equitably in society, which furthers the pervasive systematic oppression of historically marginalized groups.

In an effort to work towards addressing this gap, our team of engineering graduate students founded Social Justice in Engineering Design (SJ-ED) to advocate for engineering for good and the transformation of engineering practices through the integration of social justice in engineering education. Our work was influenced by efforts at various institutions by instructors who incorporated engineering social justice (ESJ) into the classroom (e.g. Armanios et al., 2021; Johnson, Leydens, Moskal, Silva, & Fantasky, 2015; Leydens, Johnson, & Moskal, 2021; Chen, Chapman, & Mejia, 2020; Mejia, Chen, Dalrymple, & Lord, 2018; Hendricks, & Flores, 2021; and Riley, 2015). Thus, through SJ-ED.org, an open-access educational platform, we provide content that illuminates the intersection of social justice in engineering. The learning modules and supplemental resources found on the website seek to engage engineering students, faculty, as well as practitioners.

In order to assess the effectiveness of SJ-ED educational material created to date, this paper shares the findings on how student perceptions and attitudes were impacted after engaging with an SJ-ED learning module on ESJ concepts in a civil engineering classroom. With our findings, we offer actionable methodologies, strategies, and recommendations for disrupting the status quo of engineering education. This study contributes important insights on effectively developing learning materials on ESJ. We hope this work serves to build the capacity of institutions and individuals to incorporate social justice in their engineering education curriculums.

4.2. Methods

4.2.1. The Intervention

In this study the "intervention" consisted of an SJ-ED learning module and worksheet along with a corresponding in-class discussion based on the material. The "Building Equity in the Design Process" (BEDP) module follows a team of engineers (see **Figure 4.1**) working on a transportation revitalization project. The module walks the learner through each of the stages of the design process while highlighting ways to create just outcomes. In utilizing a fictional case study inspired by true events, the module sought to provide the learner with insights into how they might effectively develop an inclusive, equitable, and just practice of design.

Figure 4.1 Team of engineers in SJ-ED BEDP module illustrated by Charleen Lopes (2021)

Within the module, the student learned some historical context of highway construction in the United States. For example, highway projects such as the interstate system have had a disproportionate impact on marginalized communities through displacement (Bullard, 2004). In addition to the history of highway construction, the student also learned about socially just design principles that, if followed, can enhance outcomes. This includes the vital work required to collaboratively involve communities as primary stakeholders and universal, inclusive, and barrier-free design principles that elevate design work. Due to the content it covers, this module may be particularly well-suited for and resonate with engineering students learning about the design process and practitioners who work with the built environment (engineering, architecture, urban planning, construction, etc.).

To measure the effectiveness of the module on student learning, it was incorporated into an undergraduate civil engineering course with an enrollment of fifty-nine students. The primary instructor for the course agreed to include the activity and workshop within the course content; therefore, while participation in the study was voluntary for students in the class, all of the students would complete the learning module and worksheet as well as engage in the in-class workshop. Students who consented to the study would take a pre-survey before engaging with the learning module and worksheet and complete a post-survey after the in-class workshop. Groups of fifteen students were assigned to read various segments of the BEDP learning module. In addition, they were asked to complete the corresponding worksheet to the sections they were assigned. The students had five days to complete these tasks outside of class before the workshop.

The in-class workshop reviewed the content from the module and engaged the students in a conversation about the ways in which engineering practice could be transformed in order to prioritize socially just and equitable outcomes. On the day of the workshop, students were placed in six teams, each with roughly 8-10 students, ensuring that at least two members of every team were “experts” on each segment of the module. The workshop provided a space for students to grapple with the major themes from the module and learn from others’ personal lived experiences and perspectives. **Table 4.1** provides the breakdown of the different segments within the BEDP module and examples of the discussion questions students were asked to consider in the workshop. The teams had approximately ten minutes to discuss the questions in their small groups after which the entire class discussed each team's responses and extracted recurring themes.

During the workshop, an online polling site (Polling Everywhere) was utilized for student teams to submit responses to questions directed to them by the facilitators. For individuals who felt more comfortable sharing thoughts anonymously, the polling platform allowed any students to interact in the activity at their own desired comfort level. It was imperative to the facilitators that the students felt safe when: adding their perspective, posing questions back at facilitators, and challenging responses from their peers. The workshop ended with a discussion on the major takeaways from the module and workshop. Students were provided with a link to a follow-up survey where they could share their perceptions of ESJ and feedback regarding the activity.

Table 4.1 The segments of the BEDP module coupled with an example discussion question used within the in-class workshop

Segments of Module	**Example Discussion Questions from Workshop**
(0) Introduction	*How might engineers build trust with non-traditional designers?*
(1) Address Community Needs and Amplify Voices of Color	*How can we responsibly develop an understanding of what communities' needs are?*
(2) Project Planning	*Is there a difference between the design parameters or design details prioritized by the engineer and community members in Hoo City?*
(3) Design Development	*As a trained engineer, what roles/tasks could we perform on the job or "extracurricular activities" that would work toward facilitating environmental justice and equitable development?*
(4) Documentation and Transparency	*Give examples of metrics that could be used to evaluate the success of the project and/or the health of the engineering design process?*
(5) Implementation **(6)** Operation	*What might be some ways that an engineer could change the dynamics of the job site or workplace to create a socially just environment?*
(7) Evaluating Impact	*Give examples of evaluation criteria you would recommend for the Hoo City project?*

4.2.2. Research Questions

The SJ-ED team had the following three research questions that guided the study of the intervention:

- ➢ **RQ1:** What are undergraduate engineering students' perception of social justice and its relation to engineering, engineering education, and their future careers?
- ➢ **RQ2**: What are the students' primary motivations to study engineering?
- ➢ **RQ3**: Did the intervention change perceptions that the students may have of social justice and its relation to engineering, their engineering education, and future careers?

This paper concentrates on the third research question. Data was gathered by analyzing students' descriptions and facilitators' reflections of their experiences with the intervention. These results provided insights into how connected students felt to the experience.

4.2.3. Recruitment and Data Collection

All procedures were reviewed and approved by the University of Virginia Institutional Review Board. Students did not receive compensation for participation in the surveys. Responses from the pre-survey were collected from September 21 and September 27, 2021. The in-class workshop occurred on September 28. Post-survey data collection occurred from September 30 to October 21. **Table 4.2** depicts participation within the pre-survey and post-survey. Participation in the survey refers to students who (1) consented to participate in the research study and confirmed they were at least 18 years old, (2) confirmed they were affiliated with the discipline of engineering, and (3) answered at least one additional question in the survey. The dropout rate indicates the percentage of students who did not complete the entirety of the survey. As made evident by **Table 4.2**, not all students took both surveys.

Table 4.2 Participation in surveys

Class Enrollment: 59 students	**Participation**	**Survey Dropout Rate (%)**
Pre-Survey	47 students (79.66%)	14.89%
Post-Survey	32 students (54.23%)	31.25%

In the study, participants completed questions before and after working through an ESJ module and workshop via the online research platform Qualtrics. In the pre-survey, participants were asked to create and retain an identification code to connect their pre- and post-survey responses. This allowed student responses to remain anonymous. The questions that students answered before and after the intervention pertained to their perceptions of social justice in engineering to discern the efficacy of the module to elucidate the intersection of social justice and engineering. The post-survey included additional questions to collect student feedback on the module and workshop experiences. Within both the pre- and post-surveys, the investigators utilized the Attitudes Towards Social Justice subscale (SJA) and the Social Justice Behavioral Intentions (SJBI) subscale from the Torres-Harding et al. (2011) Social Justice Scale. The full Social Justice Scale has four subscales based on a four-factor conception of Ajzen's theory (1991, Organizational Behavior and Human Decision Processes 50:179–211) designed to measure social justice-related values, attitudes, perceived behavioral control, subjective norms, and intentions.

4.2.4. Study Population

Undergraduate engineering students were the target population for the activity. During the Fall of 2021, students in the study were enrolled at the University of Virginia within a specific introductory civil engineering course. The study occurred at a predominately-white institution in the South. This is elucidated in order to highlight the potential effects this may have had on the study population, e.g. considering implications of classroom/campus climate, the researchers, and the research process (Secules, 2021). To get a snapshot of the students enrolled in the course, the pre-survey collected basic demographic information. This information is presented within **Table 4.3**, representing roughly 70% of the students in the total class enrollment. There was roughly an even split between students who identified as male and female. The majority of the students within the class identified as white, female or male, and described themselves or their family as middle to upper income.

Table 4.3 Key demographics from the study population

Pre-Survey Responses for Demographics	
Age *(n = 41)*	100% of the respondents indicated that they fell within the age range of 18-24
Gender *(n = 41)*	● 46.3% identified as female, ● 46.3% identified as male, ● 4.9% identified as genderqueer, and ● 2.4% preferred not to disclose their gender
Race/ Ethnicity *(n = 39)*	● 59.0% identified as White only ● 7.7% identified as Asian only ● 7.7% identified as Black or African-American only ● 7.7% identified as Hispanic, Latino, or Spanish origin only ● 2.6% identified as Asian AND Black or African-American ● 5.1% identified as Asian AND White ● 2.6% identified as Middle Eastern or North African AND White ● 7.7% preferred not to disclose race/ethnicity
Income *(n = 40)*	● 5% High Income ● 35% Upper Income ● 35% Middle Income ● 15% Low-Middle Income ● 10% preferred not to disclose income information

4.2.5. Data Analysis

The data collected via Qualtrics was downloaded into Microsoft Excel. Data analysis was conducted using RStudio Software (Version 3.5.1; 2018). Descriptive statistics (i.e. average, median, mode) were used to analyze pre-survey and post-survey data. Within the pre-survey, independent sample t-tests were used to ascertain whether groups of students had significant differences ($p < 0.05$) in their perspectives.

Using paired sample t-tests, results from students who completed both the pre- and post-surveys were compared. The null hypothesis (H_0) is that there was no difference in mean for pre- and post-responses, and the alternative hypothesis (H_1) was that there was a difference in mean for pre- and post-responses.

4.3. Positionality of the Researchers

In the literature, many scholars are advocating for statements of positionality and intentional reflexivity from scholars in engineering education (Hampton et al., 2021; Secules, 2021; Secules & Groen-McCall, 2019; Mejia et al., 2018; Sochacka et al., 2009, 2018). At the time of data collection, all five members of the SJ-ED team and co-authors of this paper are engineering graduate students in the department in which the study occurred. Three members identify and present as Black women, one member as a white female, and one member as a neurodivergent white male. Two of the students also received their undergraduate engineering degrees from this same institution where the study took place. During the research process, researchers participated in collective reflexivity activities including weekly meetings and writing reflexivity statements at different intervals of the project. This included the SJ-ED team preparing a manuscript for Engineering Social Justice and Peace that documents their individual and collective reflections on their work to center social justice in engineering pedagogy (Carroll, Gordon, Hancock, Stenger & Turner, 2022).

4.4. Results

4.4.1. Pre-Survey Results

The students within the class already had favorable impressions of social justice values, goals, and behaviors before being introduced to the intervention. **Figure 4.2** clearly illustrates the students' positive preconception toward social justice, with all students agreeing to some degree that "it is important to try to change larger social conditions that cause individual suffering and impede well-being" and that "it is important to allow others to have meaningful input into decisions affecting their lives."

Linking social justice-related attitudes with behaviors, the responses from the SBJI scale are depicted in **Figure 4.3**. From their aggregated responses to the SBJI scale, we can observe that most students have expressed intentions to act for social justice in some capacity. Only two students disagreed to varying degrees of planning "to engage in activities that will promote social justice" in the future.

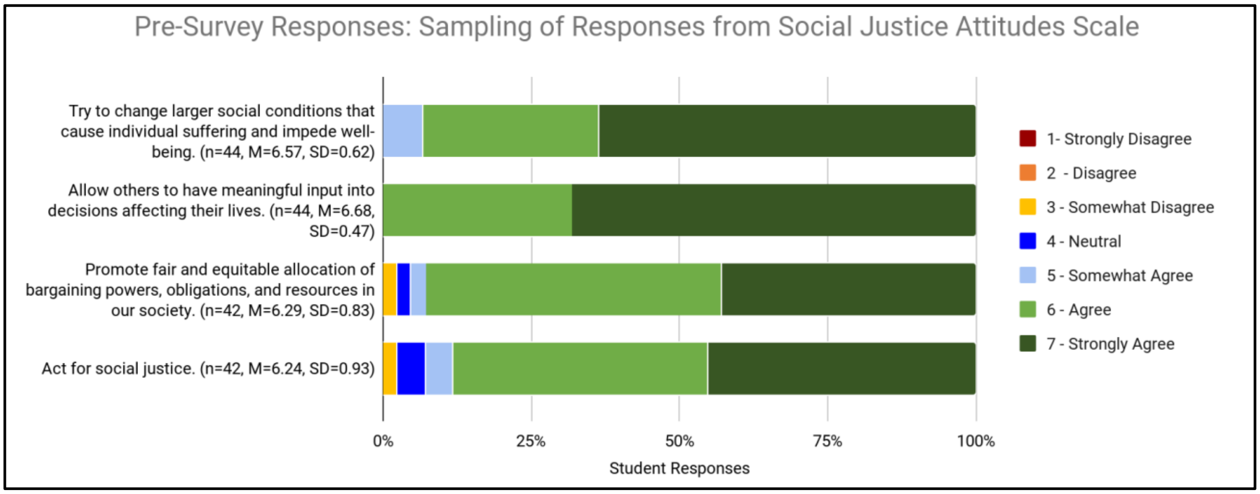


Figure 4.2 Pre-Survey responses from some of the questions within the SJA Scale

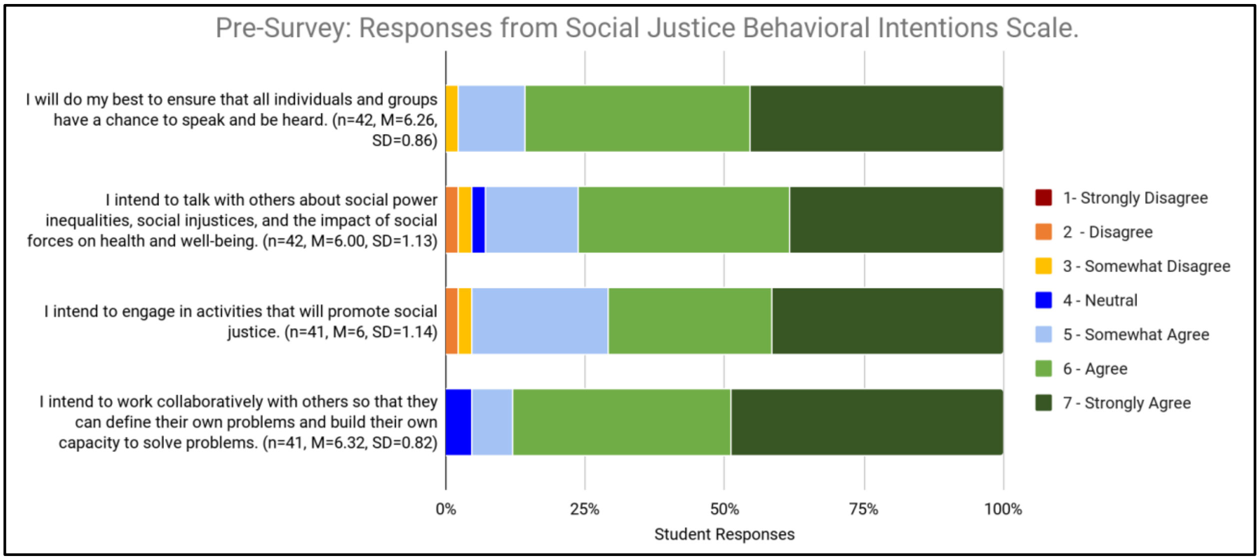


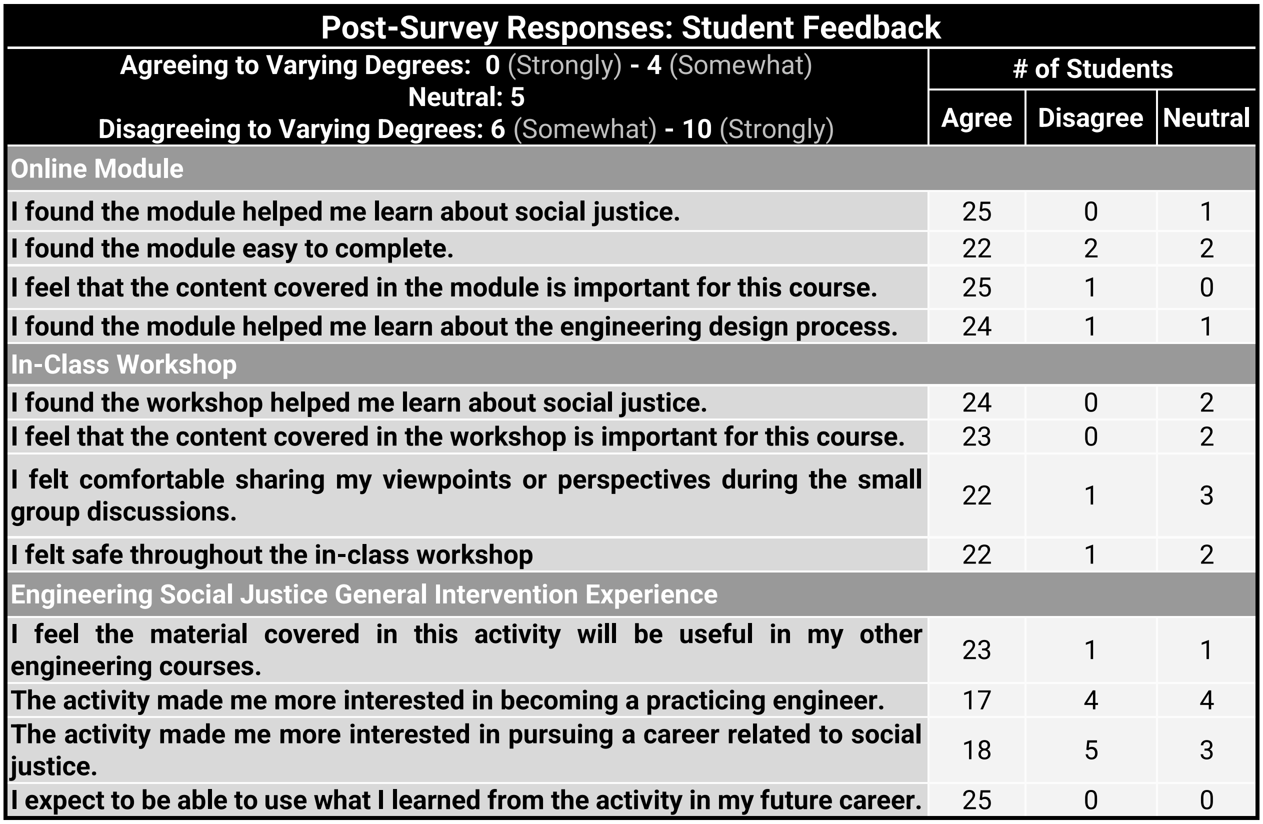
Figure 4.3 Pre-Survey responses from the SJBI Scale

4.4.2. Post-Survey Feedback

The post-survey allowed for the efficacy of the intervention to be assessed and provided the students an opportunity to share feedback on their experience with it. The efficacy was measured from pre- and post-surveys matched to student ID codes. After their experience with the SJ-ED module and in-class discussion, students were significantly more likely to think they will encounter social justice issues (*MD = 1.05, t[16] = -2.4, p = 0.03*) and have an opportunity to address social justice issues (*MD = 1.12, t[15] = 2.47, p = 0.03*). Students were also more significantly likely to see social justice as relevant to engineering (*MD = 1.47, t[16] = 3.36, p = 0.004*) and felt they knew more about social justice than before the module (*MD = 1.71, t = 3.12, df = 16, p = 0.007*).

Students were asked to provide feedback for (1) the BEDP online module, (2) the in-class workshop, and (3) the general experience of the ESJ intervention as a whole. **Table 4.4** provides a summary of student responses to the feedback questions. Ninety-six percent of students who responded to the post-survey found the module to be helpful in learning about social justice and felt that the content presented in the module was important material for the undergraduate civil engineering course. Ninety-two percent of the students found the module helped them learn about the engineering design process. **Figure 4.4** depicts the distribution of responses for the online module feedback questions.

Table 4.4 BEDP intervention feedback



Post-Survey Responses: Student Feedback			
Agreeing to Varying Degrees: **0** (Strongly) **- 4** (Somewhat) Neutral: 5 Disagreeing to Varying Degrees: **6** (Somewhat) **- 10** (Strongly)	# of Students		
	Agree	Disagree	Neutral
Online Module			
I found the module helped me learn about social justice.	25	0	1
I found the module easy to complete.	22	2	2
I feel that the content covered in the module is important for this course.	25	1	0
I found the module helped me learn about the engineering design process.	24	1	1
In-Class Workshop			
I found the workshop helped me learn about social justice.	24	0	2
I feel that the content covered in the workshop is important for this course.	23	0	2
I felt comfortable sharing my viewpoints or perspectives during the small group discussions.	22	1	3
I felt safe throughout the in-class workshop	22	1	2
Engineering Social Justice General Intervention Experience			
I feel the material covered in this activity will be useful in my other engineering courses.	23	1	1
The activity made me more interested in becoming a practicing engineer.	17	4	4
The activity made me more interested in pursuing a career related to social justice.	18	5	3
I expect to be able to use what I learned from the activity in my future career.	25	0	0

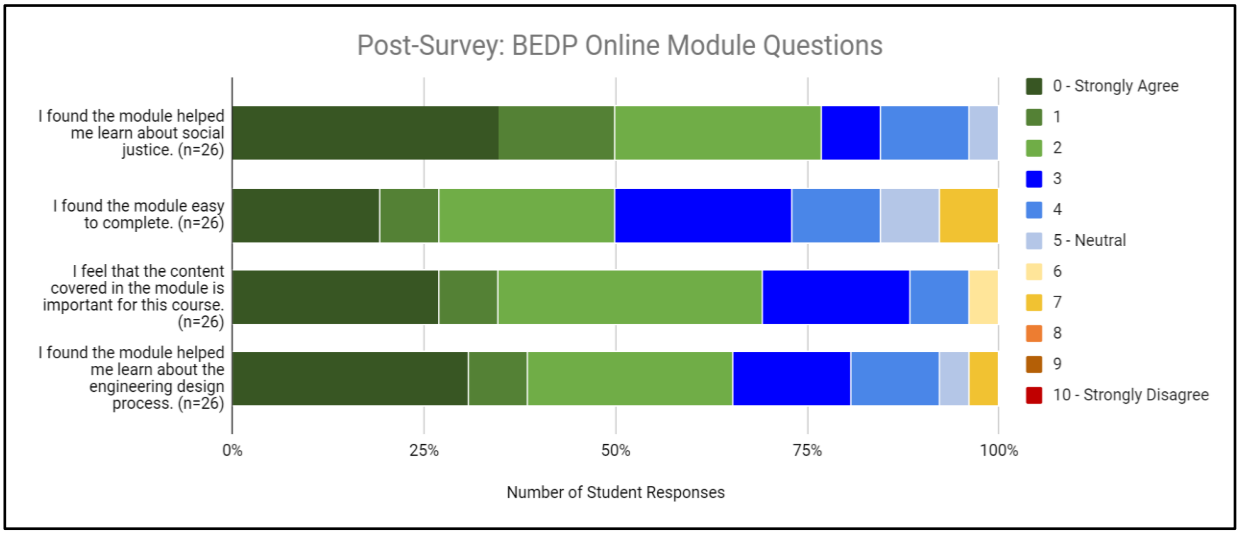


Figure 4.4 Post-Survey student feedback for the BEDP online module

Most students also found the workshop to be useful, with 92% of students indicating the workshop helped them learn about social justice. The feedback questions also considered how the students felt discussing ESJ in an engineering classroom environment. The vast majority of students expressed feeling comfortable and safe in their environment; however, one student indicated that they felt uncomfortable sharing their viewpoints or perspectives during the small group discussions, and a different student denoted feeling unsafe at least at some point during the in-class workshop. **Figure 4.5** depicts the responses for the in-class workshop feedback questions.

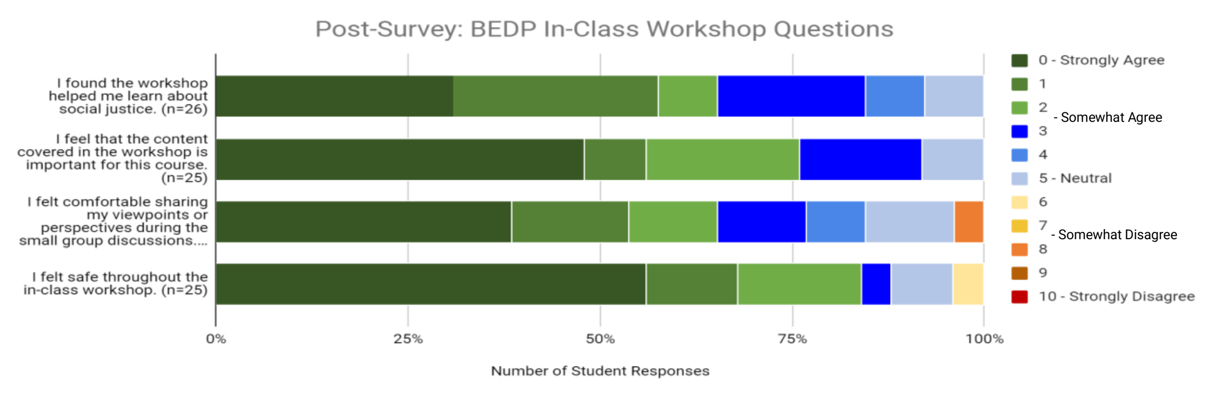


Figure 4.5 Post-Survey student feedback for the BEDP in-class workshop

The students were given an opportunity to answer questions relating to their full experience engaging with the social justice engineering content in terms of both the assigned learning module and in-class workshop. Ninety-two percent of the students who responded to the post-survey expressed that they felt the content covered in the activity would be useful in other engineering courses. Seventeen students responded that the activity made them more interested in becoming a practicing engineer, and eighteen students declared that it made them more interested in pursuing a career related to social justice. The twenty-five students who responded to the question about whether they expect to use what they learned in the activity in their future career all agreed they did in fact believe they would. **Figure 4.6** depicts the distribution of responses for the feedback on the overall experience of the engineering social justice activity.

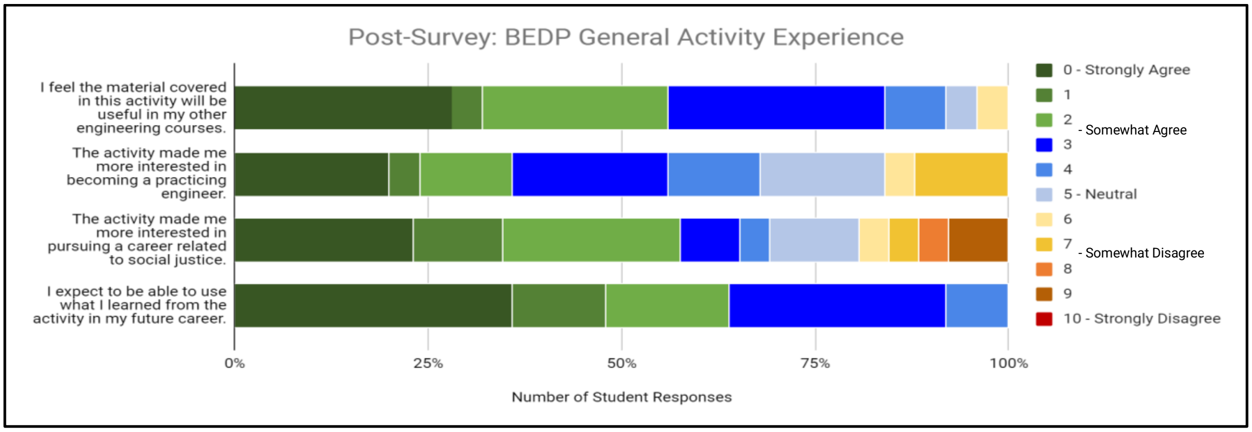


Figure 4.6 Post-Survey student feedback for the general engineering social justice intervention

The last portion of the survey gave students the opportunity to respond to three free-response questions regarding their general impression of the ESJ intervention. Students were asked to identify: (1) their favorite part of the activity, (2) what they would change about the activity, and

(3) what would make learning about social justice easier for them. From the feedback we gathered, we were able to identify some consequential themes that will aid in the future facilitation and creation of ESJ learning materials. These themes are presented in **Table 4.5** with an example of a student's short response.

Table 4.5 Themes and samples of the open-response feedback from students related to their experience with the intervention

Feedback Questions	Major Themes	Examples of a Student Response
What was your favorite part of the activity?	● The engineering social justice content	*"In the online module, I found the articles and podcasts about specific examples of social justice in engineering the most impactful."*
	● The format of the workshop to be discussion-based	*"Having the class discussions and being able to hear other people's answers to the questions."*
	● Ability to share their experiences with peers	*"I enjoyed getting to have discussions with my classmates and expand my thinking."*
What would you change about the activity?	● Shortening the length of the online module	*"I would change the length of the online module. A shorter, more concise version would increase engagement and understanding across the board."*
	● Decreasing the size of the discussion groups	*"Maybe make the groups smaller so more people get to talk."*
	● Making content more relatable to their experiences as engineering students	*"Since many of us have never worked in industry before, sometimes it was difficult for us to relate to the questions about practices in the engineering workplace. One thing that I would change would be to include more questions/examples related to student's experiences in class/at school/in their extracurriculars."*
What would make learning about social justice in the classroom easier for you?	● Integrating social justice concepts throughout the course and further layering social justice within different courses	*"It would be easier to learn if it was integrated as part of the civil engineering course because then you would be taught the principles of social justice as they apply to what we're learning in class."*
	● Providing more opportunities for students to have conversations on engineering social justice	*"If open discussions were promoted more."*
	● Making safe, intentional spaces where students can explore these topics	*"If there was a sort of standard of ethics in the classroom. Not everyone necessarily believes the same thing and it's hard to share ideas without the fear of being judged."*

4.5. Discussion

4.5.1. Efficacy of Results

Participation in the study varied between students who opted into taking the pre-survey and post-survey. Only 17 paired samples, where students took both the pre- and post-survey, were identified. This presented challenges in evaluating the efficacy of the intervention to change attitudes and perceptions of social justice in engineering. Due to the smaller sample size of these particular results, we understand the larger impact selection bias played, more specifically bias resulting from loss to follow-up and general volunteer bias. Another study design challenge we faced was being able to track students taking both pre- and post-surveys while not collecting information that permits the identity of an individual to be reasonably inferred by either direct or indirect means.

Although we were able to collect important, influential feedback from the students, we believe conducting interviews would have been extremely beneficial to collect even more in-depth feedback. As mentioned in Canney & Bielefeldt, (2016), focused interviews provide strong and clear qualitative support for student views. Especially after analyzing the short responses from students, it became evident to the researchers that the survey’s short response format was insufficient for providing a clear understanding of students’ perceptions and beliefs that build their perspectives, opinions, and attitudes on the intersection of engineering and social justice.

4.5.2. Measured and Perceived Student Benefit from the Perspective of ESJ Facilitators

Our surveys measured the efficacy of our ESJ module in improving students' perceptions of social justice in relation to engineering practice. An analysis of pre- and post-survey paired samples showed that, after the short assignment and single class session, students were significantly more likely to: think they will encounter social justice issues; have an opportunity to address social justice issues; see social justice as relevant to engineering; and feel they knew more about social justice than before the module. These results suggest that simple activities integrated into an engineering classroom can have significant impacts on how undergraduate engineering students view their responsibility as engineers in society.

Not all significant findings from our study were measured through the surveys. As the facilitators of the in-class workshop, we observed the group processes occurring in the classroom. Accordingly, as facilitators, we witnessed and experienced:

- *a “warm-up” period:* when the students were quieter at the beginning of class, perhaps uncomfortable engaging with the new material and apprehensive about de-neutralizing the space. With each discussion segment, the volume of the classroom would get noticeably louder, indicating more engagement and students’ mounting comfort level in their environment. This was accompanied by more voluntary responses from an increased proportion of students within both the small and large group discussions. This was also evident by the increased robustness and depth of responses from students as the session progressed.

- *an information overload:* when the students either gave the impression of or voiced being overwhelmed by the amount of new, complex concepts presented during a single class during one sitting. From discussions within the workshop, it was evident to the researchers that the students did not have much experience talking about concepts pertaining to social justice, including how their positionality shapes their engineering perspectives and design practices.
- *the power of collaborative learning:* when the students were empowered to share their knowledge, perspectives, experiences, and ideas with their peers, the learning experience was strengthened among the participants. We believe the most valuable information was shared within the small group discussions, where students were able to learn from their peers. It seemed that it was through students supporting each other throughout the activity that helped the class exit the warm-up period and enter into an interactive, transformative learning experience.
- *enthusiasm by the students to engage in the material:* when the students started adapting to the new classroom environment and gaining traction within the discussion segments, the vigor of conversations magnified. Energetic exchanges of differing perspectives within the group discussions were observed by the facilitators who noted that the students seemed both highly inquisitive and the discussion appeared to be very positive and respectful. At the end of the workshop and outside of the classroom, many of the students have expressed gratitude for the unique learning opportunity on ESJ.

As an additional consideration, this course occurred during the Fall of 2021. This was the first semester students at this particular university had in-class instruction after enduring a year of school predominantly, if not completely, virtual. During and after the workshop, many students commented on how this was the first interactive classroom experience they had had since returning to in-person classroom facilitation. Due to this, many expressed an increased appreciation for the opportunity to have discussions with classmates and learn more about the group of students in their class.

4.5.3. Techniques and Recommendations for ESJ Facilitation

Based on our observations and student feedback, we developed a series of techniques and recommendations to aid in the facilitation of ESJ. The key areas on which we will focus our recommendations are:

- ➢ Creation of safe spaces and collaborative learning experiences
- ➢ Integration and layering of ESJ content
- ➢ Exploration of positionality of self and students
- ➢ The art of choosing your battles

- ➢ Building networks of solidarity at the institution and beyond.

Focusing on these key areas will set up a facilitator or engineering department for success when incorporating engineering social justice into the curriculum.

Creating safe spaces and encouraging collaborative learning experiences. The number one priority for an ESJ facilitator is to effectively support students to engage in challenging conversations around engineering social justice through the co-creation of safe learning environments with students. Whether for a workshop or semester-long course, building a strong classroom community based on mutual trust and respect is important. The facilitator must focus on what they can control–shaping a welcoming atmosphere that allows students to feel engaged, connected, supported, and accountable to the community.

One strategy for building a classroom community is to collaboratively create fundamental ground rules and community culture norms for the classroom that ensure the safe navigation of uncomfortable or difficult discussions in class. Another way to ensure students feel safe is for the facilitator to be transparent with concepts that will be discussed in class as well as giving autonomy to the students to choose their level of participation in discussion. For example, for topics that require personal reflection for added value, students should only be expected to share voluntarily or anonymously. In effect, a safe classroom seeks to remove barriers to student engagement.

Students should be given every opportunity to share their voice and have a sense of investment in each other's learning. This helps break down the often intimidating sense of hierarchy between teacher and student in higher education and encourages the development of robust, collaborative learning spaces. As per our aforementioned observations, we can personally attest to the deep learning that can occur from peer to peer when students are able to bring in their own knowledge and personal lived experiences to contextualize content in the classroom. In this scheme, the role of the facilitator is to be affirming and prompt reflection and inquiry, making it clear that there are no right or wrong answers, but rather opportunities to bring in a variety of perspectives that may culminate into the creation of more equitable and just design processes and outcomes. This philosophy is necessary for the addition of ESJ in the classroom because social justice is contextual and non-static.

Importantly, as the facilitator's understanding of social justice concepts and students' perceptions of ESJ evolves, so must the classroom. In our work, we used student feedback to improve future workshops. In this study, students shared to what extent they believed the space is optimal for their learning and growing. For example, many students commented that they enjoyed the small group discussions. Two students even commented on reducing the size of the small group discussions, with one student citing that a group of 3-4 students would give more time for each student to share. From this, we have structured our following ESJ workshops to prioritize these more intimate discussions, which in turn has led to more fruitful small and large group discussions and engagement with the material.

Integration and layering of engineering social justice content. Another important takeaway from our DEIJ work, including this paper's focus on the explorations of ESJ education, is that years of social conditioning cannot be broken down through a simple prescriptive procedure. It requires a layering of learning and engagement with educational and training opportunities for students, faculty, and staff that dismantle cultural barriers in education and engineering. These cultural barriers will look different at every institution and with every new generation of students who enter our classrooms.

Building a foundation for new knowledge and to utilize new lenses to critique our present knowledge base will require a level of intentionality that spans beyond the current scope of siloed, disconnected actions, including the intervention we have described in this study. We have shown that while one ESJ workshop may raise curiosity, at least temporarily, to question the status quo and change attitudes toward the relevance of engineering and social justice, a week-long exposure to justice concepts is likely not enough to create a lasting and substantial change in their understanding of its deep interconnectedness to themselves and their practice of engineering. Just as we do not expect students to come into a thermodynamics course without first completing prerequisites in chemistry, calculus, and physics, we should not expect students to fully appreciate the relevance of social justice in engineering if it is not integrated throughout their undergraduate engineering curriculum.

Layering content throughout the curriculum can help to avoid the information overload we observed in the undergraduate students. When done well, layering content can build confidence in the students by easing them into new material, stacking concepts, and adding depth at each new level. Through our various experiences teaching ESJ, we also witnessed the significance and impact of giving students time to process and reflect on the material. For the facilitation of ESJ, this requires revisiting and further developing concepts, as well as providing alternative perspectives and additional scenarios. Unfortunately, within our study design, students were not given sufficient time to process the dense content. In hindsight, we would have planned an additional workshop to circle back and reinforce concepts.

Though the traditional engineering curriculum is often perceived to be static, new concepts do arise and become prominent. One recent and now omnipresent example can be seen with sustainability. The culture shift towards a sustainable future arose by a greater public awareness of the depletion of natural resources and the dangers of environmental degradation and climate change. In the United States, following the murder of George Floyd, the civil unrest that characterizes the summer of 2020 sparked another culture shift, a racial reckoning (Chang et al., 2020) and an urgency for social justice. There is a wealth of literature on best practices for the integration of sustainability into engineering curricula and pedagogical approaches that can help provide a roadmap for how to adapt social justice into engineering curricula. Even so, the integration of the principles of sustainability at each institution and within each classroom looks different, including how sustainability is defined or co-opted, as well as what pillars of sustainability are prioritized over others. Thus, by applying the lessons from the inclusion of new topics, like sustainability, we can apply best practices while avoiding pitfalls as we seek to integrate social justice into the engineering curriculum.

Positionality of self and students. As our team relies on utilizing critical, liberatory pedagogies and integral approaches to ESJ education, we are of the opinion that "learner's subjectivity and social positionings play an essential role in the practice of inquiry and knowledge production (Acevedo, 2015)." In our experience, when introducing social justice concepts in an engineering classroom, our group has had the most success decreasing the previously mentioned "warm-up" period by initiating exercises that allow students to (1) explore their individual and social identities and (2) reflect on how their positionality is related to the course content. We recognized that the depth of student discussion in this study differed compared to other ESJ workshops our team has facilitated where we had introduced the concept of positionality. We recommend referencing Acevedo et al (2015) for a more detailed overview on the application of positioning (biographical, discursive, somatic, spatial) via critical pedagogy and integral education.

From our experience, there are a number of actions ESJ facilitators can take to incorporate positionality into their pedagogy. First, the facilitator should practice reflexivity at regular intervals to evaluate their positioning in the classroom through the consideration of the implications of power dynamics and minority and majority relationships in the classroom. The facilitator should expect that their students come from different backgrounds and have unique lived experiences, and thus, the space needs to be able to adapt to meeting the needs of all students in the class. This includes being cognizant and respectful of the fact that historically marginalized groups could experience class content differently (e.g. added burdens, trauma or victimization) and face more barriers than other students within the classroom. Thus, facilitators should take proactive steps to develop a universally inclusive learning environment that can support historically underrepresented students. To initiate this pursuit in your classroom, reference Arif et al.'s (2021) *Ten simple rules for supporting historically underrepresented students in science.*

Lastly, exploration of positionality can help facilitators pick content that is relevant to the students. In the case of our intervention, the undergraduate students were primarily in their second year of college. Due to their current experience level as engineers, we found that most students felt the questions posed in the workshop pertaining to being a practicing engineer on a construction site were unrelatable. After the workshop, students suggested content would be more engaging if it drew from relatable lived experiences rather than primarily from the outlook of a potential future career. Thus, for future learning materials, we would recommend that opportunities for student feedback be interspersed throughout the development process to ensure the materials are relevant to and fulfill the student's interests and needs around ESJ.

The art of choosing your battles. As ESJ scholars, we acknowledge that we have blind spots and regularly feel underqualified to talk about certain types of oppression; however, we do not use this as an excuse to avoid these topics in the classroom. When considering whether social justice in the classroom is necessary or even appropriate, Hinshaw (2007) stated:

> *"To believe otherwise would be to dismiss our potential impact as teachers on the beliefs and actions of our students, to disregard the extent to which we are already participating in the formation of docile student bodies accepting of the status quo, and to shrug off our responsibility to issues of social justice."*

Due to there always being a surplus of content over time we have in the classroom, picking content becomes an art. We recommend that facilitators find ways to open up rather than close down discussions around social justice and engineering. To meet these recommendations, facilitators should seek to cultivate trust with the students while building a safe learning environment to explore these topics. We have found that collecting and implementing student feedback and evaluation as well as encouraging and supporting student selected projects can all help such a classroom environment develop.

The facilitator should seek to open up the conversation around ESJ. One way to start the conversation is by encouraging students to take on multiple perspectives on class content. Using the ESJ module as an example, students could learn a traditional "technical" engineering skill alongside the development of an understanding for the social justice implications that have resulted in its application by engaging with non-traditional information sources (e.g. podcasts, readings, speakers, etc.). To make sure demographics of the class do not dictate the discussion, effectively limiting perspectives that are shared in the classroom, these nontraditional information sources can also provide insight from those perspectives absent or marginalized within the classroom.

In some cases, this approach will result in rich classroom discussion, and in others it could result in resistance. We strongly believe it is important to appreciate the differences in opinion that can arise in the classroom and create learning opportunities that meet students where they are. As a consideration, facilitators should be aware that the positions and beliefs students hold may not be well thought out (Hinshaw, 2007); therefore, a classroom becomes the *ideal* environment for the intersections of knowledge and experiences to lead to collective, critical thinking, further resulting in transformational learning experiences. Facilitators should be prepared to deal with the probability that providing students with space in the classroom to critically examine their positions and beliefs might lead to differing opinions and resistance. It is important to not constrain the possible disagreements that could result from these differences, but rather facilitate respectful conversations that address them. By demonstrating and teaching the skills that are necessary to facilitate and engage in these respectful conversations, instructors are cultivating within students interpersonal skills useful both in and out of the classroom.

We recognize that there will never be enough time, in a course, let alone a single workshop, to unravel the ways in which injustice presents itself in engineering, within the lived experiences of our students, and within the classroom. As such, we suggest that the role of the facilitator is not to have the most knowledge in the room on social justice, but rather to have expertise in creating spaces that allow for collective learning and inquiry at the intersection of social justice and engineering. In our experience as facilitators, we have had numerous instances where we ran out of time to engage with all of the comments and perspectives that students added to the discussion. In these cases, we found that providing opportunities for students to reflect on those discussions allowed for us to revisit them in the future in a deeper and richer way. In summary, we found that picking your battles on ESJ is less about adhering to strict lesson plans and more about making the most impact in the time that you have.

Building networks of solidarity at the institution. Just as there was a lag period before sustainability was holistically blended into the engineering curriculum, it might be that the *institutional* integration of social justice into the engineering curriculum is slow to develop. Yet, individual educators can integrate social justice content through assignments, classes, and courses. In addition, instructors can take steps to reduce their knowledge gaps regarding the application of social justice to engineering in order to enhance their capabilities to provide instruction about it in the classroom. Yet, these and other possible steps don't have to be solitary efforts but rather can be used as opportunities to start or strengthen social justice solidarity networks at your institution.

The creation or expansion of networks of collective action for social justice, however small they are to start, may well be the catalyst for the large-scale changes needed to incorporate social justice into engineering education and practice. *Engineering, Social Justice and Peace* serves as an example of just such a network. It is composed of academics, practitioners, and students in a range of disciplines related to researching, documenting, and furthering efforts at the nexus of engineering, social justice, and peace. We must continue to build and support the networks, both within and outside of our own institutions.

4.6. Concluding Remarks

The engineering curriculum seeks to educate and inspire engineering students to resolve some of the world's greatest challenges. Yet, when we look critically at engineering curriculum, for what role in society are engineers truly being prepared? In many ways, the current state of engineering curriculum has helped to preserve a culture that does not place justice at the heart of engineering practice. Through engineering social justice education, the role of the engineer can encompass bridging the gap between innovation, sustainability, and social justice. Not only does an ESJ curriculum shape well-rounded practicing engineers to be ready to tackle complex challenges, it also has the potential of addressing the STEM gap of greater diversity in the field of engineering by creating a sense of purpose, social agency, and belonging as a student, as a practitioner, and as an educator.

As engineering educators, we need to take steps to avoid performative actions or no action at all and move toward incorporating and embodying the ideals of social justice. As we engage in these actions or through our own lived experiences, we must expand the collective understanding and knowledge around social justice. With this expanded knowledge, we must continually strive to co-define and co-develop the best practices for integrating social justice into the engineering classroom. In so doing, we can seek to create an engineering practice that serves humanity through the creation of socially just outcomes.

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5. Strengths and Limitations, Key Findings and Broader Impacts

In this chapter, the strengths and limitations of the studies conducted in this dissertation are analyzed and discussed. Evaluations of the strengths and limitations help contribute to a clearer understanding of the significance and generalizability of the findings. Additionally, future research recommendations are provided, especially where limitations have been identified within this study. The key findings of the research are summarized, and the broader implications of the work are explored, emphasizing how the research can be applied in practice to address real world contexts. Through summarizing the strengths and limitations of the studies and highlighting their key findings and broader implications, this chapter serves as a comprehensive overview of the research explored within this dissertation.

5.1. Reflections on the Efficiency of Water Disinfectants to Produce Larvicidal Effects

5.1.1. Summary of the Key Findings and Broader Impacts

The main contribution of this work was expanding the range of options available for vector control efforts through an investigation into the effectiveness of using water disinfectants for controlling *Aedes aegypti* mosquito populations in water storage containers. This work could have significant implications for public health efforts to prevent the spread of mosquito-borne diseases by providing alternatives for treating household water storage containers within a broader integrated vector control plan. This study helps to identify these optimal water disinfects or combinations of disinfectants, providing guidance to communities, public health officials, and vector control experts on matching solutions to different contextualized situations. This can be especially important in areas where water insecurity is prevalent, resistance to certain chemicals has developed, or where the use of one disinfectant alone is not sufficient to control mosquito populations or the types of waterborne pathogens (bacteria, viruses, protozoa) living in drinking water storage containers.

There were also methodological contributions to this work. The study design was unique in that it used lower chemical concentrations than typical larvicidal studies and a longer exposure time to assess the impact of the disinfectants on mosquito development, specifically observing the survival and emergence. The study also highlights the importance of considering real-world conditions and factors that may affect the results of laboratory-based experiments. The work also expands on the growing literature on the efficacy of using water disinfectants in tandem. Depending on the waterborne pathogens or mosquito species present in a given area, different combinations of disinfectants may be more effective than others. Ultimately, the results obtained suggest that this approach may be a viable alternative for vector control and thus future research will help expand the generalizability of these results to different contexts (e.g. species of mosquitoes, types of source water, etc.).

Key findings from this study include:

1. At concentrations 40-50% of the drinking water quality guidelines (WQD), silver was the most effective water disinfectant when compared to alternative disinfectants copper and chlorine, in inhibiting the emergence (IE) of older instar *Aedes aegypti* larvae ($IE_{Day=16}$ = *89.61% [85.12, 93.15%]*). See Table 5.1 for comparison of water disinfectants silver nitrate, copper sulfate, sodium hypochlorite at low, middle, and high concentrations within drinking water quality guidelines).
2. Younger instar larvae were more susceptible to the larvicides than the older instar larvae. At concentrations 20-25% of the drinking water guidelines, silver showed the greatest larvicidal efficacy against younger instar *Aedes aegypti* larvae when compared to alternative water disinfectants copper and chlorine ($Survival_{Day=3}$ = *58.66% [49.92, 66.99]*); however, at the 80-100% range of the WQG, chlorine was observed to be the most effective ($Survival_{Day=3}$ *=0.8% [0.18, 2.87]*).
3. To further reduce the emergence of *Ae. aegypti*, the water disinfectants can be used in tandem. The combination of disinfectants when tested at roughly 40-50% of the drinking water quality guideline with the strongest efficacy to inhibit the emergence of older *Ae. aegypti* was a close call between Ag+Cu ($IE_{Day=16}$ *= 98.52% [96.50, 99.47%])* and Ag+Cu+Cl ($IE_{Day=16}$ *= 97.44% [94.77, 98.78%])*. Thus, the recommendation, based solely on this set of environmental conditions and mosquito strain, would be to choose Ag+Cu for cost effectiveness and simplicity. For younger instar, all combinations achieved almost complete mortality after 72 hours of exposure.

Table 5.1 Summary of inhibition of emergence results regarding the efficacy of water disinfectants as larval control for older instar *Ae. aegypti*. IE% calculated from the observed data reported on day 16

Dosing	**Low**		**Middle**		**High**	
Treatments	**mean**	**sd**	**mean**	**sd**	**mean**	**sd**
Ag	*Ag (20 ppb)*		*Ag (40 ppb)*		*Ag (80 ppb)*	
IE (%)	72.4	7.16	81.56	5.86	88.66	9.87
Cu	*Cu (300 ppb)*		*Cu (600 ppb)*		*Cu (1200 ppb)*	
IE (%)	54.89	6.02	72.15	11.12	87.19	8.41
Free Cl	*Free Cl (0.5 ppm)*		*Free Cl (1 ppm)*		*Free Cl (2 ppm)*	
IE (%)	37.44	21.35	72.46	13.77	80.65	16.04

The design of this study was centered around the broader impact of improving public health, specifically in underserved communities that lack access to a household water connection that provides access to a reliable, continuous clean supply of water and thus rely on storing water around the home. Public health projects that incorporate a holistic approach to health and environmental concerns have the potential to address multiple issues simultaneously, making them a more attractive investment for funding agencies and donors. By addressing two important issues simultaneously, the use of water treatment chemicals for vector control can be seen as a cost-effective strategy for controlling disease transmission.

5.1.2. Strengths, Limitations, Future Research Opportunities

One of the study design's greatest strengths was that it accounted for factors relevant to the application of the chemicals in a HWS container. For example, all water disinfectants were tested at concentrations within the range safe for human consumption. The chemicals chosen for this particular study have been observed in the field in numerous other studies for their efficacy in reducing waterborne pathogens (Estrella-You,Harris, Singh,& Smith, 2022; Pooi & Ng, 2018; WHO, 2018; Vincent, Hartemann & Engels-Deutsch, 2016; Arnold, & Colford, 2007). Treating HWS containers with the most effective chemicals identified in the study could potentially reduce the emergence of disease transmitting vectors, as well as improve the quality of drinking water. The results of these studies in aggregate provide valuable information for the development of POUWT technologies and strategies to design public health initiatives. It provides the opportunity for water disinfectants or POUWT technology to be chosen based on relevant public health concerns e.g. type of waterborne pathogens in source water and/or prevalence of mosquito-transmitted disease.

As with all laboratory studies, there are many study limitations and opportunities to expand on the research. First, many other mosquito species transmit disease, thus there is an opportunity to understand if other species will be as susceptible, if not more, to the water disinfectants at these low concentrations. This study evaluated the susceptibility of the *Aedes aegypti* species, more specifically, a strain that has been propagated in a lab since 1994. The controlled conditions in which the mosquitoes have been reared may limit their range of genetic diversity (Gloria-Soria, Soghigian, Kellner, & Powell, 2019), as well as potentially decreasing their ability to adapt to environmental stressors (Aguilar et al., 2010). Since it is possible for mosquitoes of the same species in different regions to develop differences in resistance to insecticides and other chemicals (Ryan et al., 2019), it is important to explore whether the local strains may be less susceptible to the water disinfectants. Testing the water disinfectants against local species and strains of mosquitoes will provide more accurate representation for how efficient these treatments will be in any given region. Furthermore, studying both lab-reared and field-collected mosquito strains will help to develop a better understanding of the factors that influence the evolution and the development of resistance to chemicals. Another opportunity not explored within this particular study was if the water disinfectants affected the life span of the adult mosquito. All mosquitoes that emerged were classified as "survived" for the remainder of the experimental period instead of collecting data on if contact with the water disinfectants at a juvenile stage shortened their life span in the adult stage, thus shortening the time frame in which they can transmit disease.

In this study, the juvenile *Ae. aegypti* were not immediately killed by the chemicals in most cases. However, the study revealed a pattern in which larvae in the treatment groups had delayed growth in comparison to those in the control groups. Moreover, many larvae in the treatment groups did not survive long enough to reach adulthood as the days in the experiment progressed. It is unclear how much of the reduced survival and delayed growth were directly caused by the disinfectants or other factors (e.g. food regimen, environmental conditions, age of eggs). Further research is necessary to determine the precise mechanisms that account for these findings. These limitations and future research opportunities will be expanded upon below.

In this study, the same environmental conditions were used in order to compare across the experiments. Thus, these results reflect what would happen in these very specific conditions. The following are examples of study design considerations that, if changed, would alter the results of the study:

- Mosquito species and strain,
- Larvae rearing, e.g., age of eggs, feeding, population density
- Environmental conditions, e.g. light:dark cycle, temperature, container type/geometry, size and depth of aquatic environment
- Larvae age at time of contact with disinfectant,
- Using different formulations of the water disinfectants
- Dosing and contact time with disinfectant, e,g, rate of dosing or application (continuous dosing, single application, reapplication of treatment), fate and transport of chemical over time
- Water source/type, e.g., surface water, groundwater, rainwater, piped supply, treated vs. untreated
- Water quality parameters that may alter disinfect consumption and/or mode of action, e.g., dissolved oxygen, pH (especially in the context of the speciation of chemical, i.e., OCl-/HOCl ratio), conductivity, nutrients, salinity, turbidity, other interfering microbes/microorganisms,

While it is unrealistic to test all of these iterations within a laboratory setting, altering some of these design considerations will be extremely beneficial to understanding the efficiency of the water disinfectants under other conditions.

Pertinent to this study, the rearing of the mosquito larvae and feeding regiment extended the length of time that the mosquito larvae developed. In the Anoopkumar et al. (2017) study, *Ae. aegypti* larvae spent approximately 8-9 days in the aquatic juvenile stages (see **Figure 5.1**). The *Aedes* *aegypti* USDA "Gainesville" strain procured from Benzon Research, when fed *daily*, emerged predominantly between days 9 through 11 when tested within the laboratory/environmental conditions utilized for the experiments. However, for the experiments with late 3rd instar larvae, food was withheld during the first 3 days of the experimental period. Lack of sustenance in their aquatic environment extended their time of development. This trend is evident, as most emergence that occurred in the control group from the later instar experiments did not occur until well after day 4 of the experiment, with some larvae taking up to 16 days to reach the adult stage. This extended life cycle due to food stress conditions is well documented in *Aedes aegypti* (Souza et al., 2019; Zeller & Koella, 2016; Perez & Noriega, 2012; Mitchell-Foster et al., 2012; Beserra, Fernandes, & Ribeiro, 2009; Arrivillaga & Barrera, 2004). Although Chapter 2 explains the rationale behind selecting this particular food regimen, it is crucial to acknowledge that the experimental design induced stress on the larvae, which negatively affected its development, potentially influencing both its fitness, survival, and emergence. This stress might have resulted in a stronger response to the water disinfectants for the treatment groups, especially since larvae experienced longer duration in contact with the disinfectant due to a longer juvenile developmental phase. Furthermore, the age of the eggs used may have had an additional impact on development time, as the ones used in the experiments varied between 2 weeks to 2 months old. A study by Perez & Noriega (2012) observes how the duration of quiescence and extent of nutritional depletion may affect the survival of larvae, especially those hatching in a suboptimal habitat. Lastly, high larval density during the rearing period may have also contributed to an extended life cycle (Mitchell-Foster et al., 2012; Beserra, Fernandes, & Ribeiro, 2009).

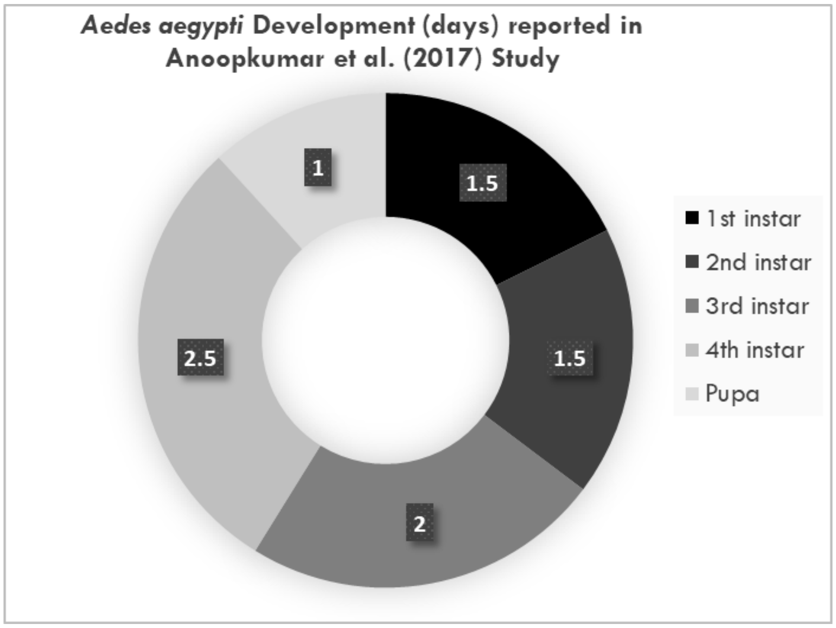

Figure 5.1 *Ae. aegypti* development in days reported in Anoopkumar et al. (2017)

In a lab, mimicking household water use is also extremely difficult, especially when behavior related to HWS is so varied from household to household, even within similar cultures, climates, and geographic regions. After laboratory testing that evaluates the susceptibility of mosquitoes obtained in the field and testing a larger range of environmental conditions, the next potential step to expand this research would be to conduct a field study to see how the treatment of water disinfectants performs in a less-controlled environment. Field studies involve observing and collecting data on the actual behaviors of individuals, rather than relying on simulations or hypothetical scenarios presented within a laboratory study design. Within conducting an experiment in a real-world context, there is an opportunity to glean important information regarding:

1. the interaction between people and their HWS,
2. the prevalence of mosquitoes or other vectors within the home and the relevance of HWS containers to breeding mosquitoes in that context,
3. social acceptability of the water treatment disinfectants and ease of use,
4. and the disinfectants efficacy in treating the source water for both water pathogens and the local strains of juvenile mosquitoes.

Collecting this data in a field study can inform the development of more effective and relevant water management and vector control strategies. Field studies also create an opportunity to build knowledge communities through collaboration and engagement and to invest and stand in solidarity with communities affected by adverse public health effects of mosquito proliferation.

5.2. Reflections on Engineering Social Justice

5.2.2. Summary of the Key Findings and Broader Impacts

This work demonstrates a viable pathway to integrate social justice into engineering practice and engineering education. One of the main contributions of this work was the co-design of accessible instructive materials that serve to educate engineers on how to contextualize engineering work with a social justice lens. ESJ education allows undergraduate students to think critically about the role an engineer can play in bridging the gap between innovation, society, sustainability, and justice. A curriculum that centers social justice has a strong potential to shape well-rounded practicing engineers, ready to tackle complex challenges, by expanding their toolbox through training on how to implement critical lenses, multidisciplinary approaches, inclusive design practices, and reflexivity into their engineering practice. This training prepares engineers to deconstruct systems of oppression and build an engineering culture that serves humanity and the health of our environment.

Another core contribution of this work was to understand how engineering social justice (ESJ) learning materials affect the perceptions, attitudes, and intentions of engineering undergraduate students, specifically considering their relationship to engineering, social justice, and their future careers. The study conducted in an undergraduate civil engineering course measured the learning outcomes that resulted from one of the co-designed SJ-ED modules. Key findings from the study include that the module significantly increased students' perceived relevance and knowledge of SJ as well as their belief they will encounter and have an opportunity to address SJ issues. Based on observations and student feedback during the study, the following techniques and recommendations were provided to aid in the facilitation of ESJ: creation of safe spaces and collaborative learning experiences, integration and layering of ESJ content, exploration of positionality of self and students, finessing the art of choosing your battles, and building networks of solidarity at the institution and beyond.

The SJ-ED team developed a F.O.R.M. model that has undergone refinement throughout the life cycle of their study. This model provides guidance for individuals interested in creating and analyzing engineering social justice activities, and is informed by the expertise, experiences, and reflexive practice of the SJ-ED members. **Table 5.1** portrays the key components of the F.O.R.M. model and how it can be used to achieve social justice goals in engineering.

Table 5.2 SJ-ED's F.O.R.M. Model for contextualizing, creating, and analyzing engineering social justice activities. Sourced from Hancock, Turner, Gordon, Stenger, Carroll, & Louis (2023)

Model		Description
F	**F**ACULTY	Recognize the faculty's **ability and desire** to establish transformative **learning environments** that allow for a more diverse student base to both cultivate an engineering identity and authentically pursue and practice engineering according to values beyond basic ethics.
O	**O**RIENTATION	Address the **attitudes** students, faculty, and education institutions have regarding how engineering can be applied. Examine how changing priorities within the curriculum can help enhance student's **intentions** to implement equitable design practices as well as remain in the discipline.
R	**R**ELEVANCE	Gauge the faculty's and student's perception of the **relative significance and applicability** of social justice to engineering. Contextualize the positionality of the education institution. Highlight relevant examples of how to transform unjust social policies and practices in their (student's, faculty's, institution's, etc.) present world/communities.
M	**M**OTIVATION	Understand what **influences and encourages** engineering students to enter into the profession. Data gathered can inform on the needs and aspirations of students that can be translated into tailoring learning activities.

Expanding ESJ education also has the potential of addressing the STEM diversity and inclusion gap through its central themes of:

- engineering with a sense of purpose, thus empowering students to build and be motivated by their social agency
- authentic inclusion of new ways of thinking, learning, and practicing engineering, thus building genuine sense of belonging in the field for students from diverse backgrounds
- creation of knowledge communities that build collective understandings of *just* outcomes through co-defining and co-developing best design practices, specifically with those who have been historically excluded from engineering design processes and disenfranchised within the society
- taking direct action toward incorporating and embodying the ideals of social justice to intentionally address the gap.

Personally, ESJ has been the key to my retention in engineering, and thus I do this work in solidarity with others who have faced exclusion and marginalization in this field. I hope this work encourages others to explore the profession and increases their appreciation for how their unique perspective and understanding of engineering is extremely ***valuable*** and ***needed*** to change the current status quo of engineering culture. We hope this work serves to build the capacity of institutions and individuals to incorporate social justice in their engineering education curriculums.

5.2.2. Strengths, Limitations, Future Research Opportunities

Engineering social justice (ESJ) is considered by many to be a relatively new field of study; however, it is firmly rooted in philosophy and activism that has occurred over hundreds of years. The devastating loss of Black lives and protests during the Summer of 2020 reignited the urgency in need for action to address systemic racism and inequality in all areas, including within engineering. The main strength of this study is that it was a response to this call to action and provided empirical evidence on the importance of providing ESJ learning opportunities in the classroom. The study builds on addressing important systemic issues of injustice in our society, and specifically within the practice and education of engineering. By doing this study, we were able to spread awareness of how the engineering profession contributes to fostering societal marginalization and oppression and engage in building critical consciousness in students, researchers, and practitioners.

Another strength of this study was that it was the product of relationships formed through solidarity that evolved into a dynamic knowledge community (SJ-ED). Early on in SJ-ED’s formation, the members within the collective shared in common being graduate students at the same university. In order to expand upon the representation of experiences, collaborative partnerships were formed with other researchers, educators, industry professionals, and students with a wide variety of backgrounds, including individuals with educational backgrounds in other forms of knowledges. The shared, collective, and collaborative nature of this work created depth, or a more comprehensive understanding of the issues and gaps in addressing the underlying social implications of engineering. These partnerships are what facilitate the translation of research findings into practice.

Within Leydens et al. (2021), the authors remark on the need for more publications in the area of "actual integration of social justice in the curriculum or student perspectives on such integration," thus a strength of this study was that it brought to the forefront student perspectives. In doing so, a limitation of the study design was the small scope of qualitative methods used to expand on the quantitative survey data. While the surveys provided valuable insights into participants' experiences and perspectives, the inclusion of interviews in the study design would have provided a more detailed and nuanced understanding of participants' experiences, opinions, and attitudes. Despite these limitations, the study's findings can still provide valuable insights into the experiences and perspectives of the sample. However, future research with larger and more diverse samples and a more comprehensive mixed methods approach, including interviews, can provide a more robust understanding of the topic.

The surveys were the main tool for data collection in this study. A challenge we faced within our study design was being able to track students taking both pre- and post-surveys while protecting the identity of the participants. There was also a large loss in follow-up post intervention; thus, the results reflecting the impact of the intervention, which is based only on the data from participants that completed both the pre and post survey that could be matched, reflect a much smaller sample size then some of the other results reported in the study that were based on participation in solely the pre or post survey. This is important to acknowledge because it reflects a higher potential for selection bias to impact the results, more specifically bias resulting from loss to follow-up and general volunteer bias. The implications of this and commentary on how to address these concerns in future research is discussed in great detail within Hancock, Turner, Gordon, Stenger, Carroll, & Louis (2023).

Other limitations of the study included the small sample size and the sample's homogeneity. The results from the study reflect the unique perspectives of individuals within the class. The results that portray the perceptions and attitudes of the students should also be contextualized within the time period in which the study took place since there was still a heightened awareness of social injustices at this time within the general U.S. population. The homogeneity of the sample can primarily be attributed to the study being conducted at a predominately white institution and within an institution and discipline that has a well-documented gap in diversity. Thus, the small sample size and homogeneity of the sample limits the external validity of the study, suggesting that these findings should not be used to make generalizations about the broader population. This also affected the ability of the research to examine how intersectionality in identities played a role in forming attitudes, perceptions and intentions related to ESJ. While this study provides a useful snapshot, especially for the institution in which the study took place, this small sample size does not accurately represent the diversity of perspectives and experiences within the larger population of students studying engineering. Furthermore, the homogenous sample may not capture the range of experiences and perspectives that exist within society. Therefore, it's important to consider these limitations when interpreting the findings of the study.

While there has been some progress in incorporating social justice into engineering education, there is still a significant gap in creating and facilitating effective ESJ learning activities that equitably engage people from both dominating and minoritized cultural groups (Hancock, Turner, Gordon, Stenger, Carroll, & Louis, 2023). Future research in ESJ is needed to identify the most effective strategies for creating inclusive and culturally responsive ESJ learning environments, as well as to evaluate the impact of such efforts on student learning outcomes and attitudes towards social justice issues. Many engineering faculty members lack the knowledge and skills necessary to effectively engage students in ESJ education. Thus, future research regarding faculty participation and perspectives can help to define and address this gap, as well as build strategies that ensure faculty are equipped to create inclusive and culturally responsive learning environments.

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