MODELING RISKS ASSOCIATED WITH WATER REUSE IN AGRICULTURE

BY

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DISSERTATION

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Abstract

According to the World Resources Institute, as of 2019, there are 68 countries experiencing at least medium-high water stress, with 17 experiencing extremely high water stress (Hofste, Reig & Schleifer, 2019). This is dire news, and any solution must balance the rate of human water consumption with the rate of water replenishment, with the overall reduction in freshwater usage being key for all scenarios. Two main ways to accomplish this balance are increasing agricultural efficiency and recycling wastewater (Hofste, Reig & Schleifer, 2019).

Agricultural efficiency can be increased through the adoption of more advanced agricultural methods that use less water, such as aquaponics, which is the practice of growing fish and plants together in a soilless system. Aquaponics has the potential to be a reliable source of healthy food for places lacking the land required for traditional agriculture, such as urban areas and islands. However, little research has been done on factors that determine the well-being of an aquaponics system. Chapter 1 of this dissertation dealt with pathogens in aquaponics and revealed a complete lack of research on this subject. However, conclusions could be inferred from studies in the related fields of aquaculture and hydroponics. It was found that studies conducted in these systems tested ultraviolet (UV) irradiation, blue light-emitting diodes, media filtration, membrane filtration, heat, sonication, and three miscellaneous methods as pathogen control strategies. Across the pathogen control methods, water quality and flow rate were key factors determining the efficiency of disinfection. Chapter 2 explored how to model changes in pH for the purpose of prediction and decision-making. The linear regression model developed was able to provide short term predictions for pH with an accuracy of 0.74 using the pH values of the prior two days, but improvements could be made through incorporation of data that is collected continually, rather than daily or weekly.

Though aquaponics is promising for small scale agriculture, it cannot replace traditional agriculture. Thus, adjustments need to be made to make traditional agriculture more sustainable, such as irrigating with a combination of recycled wastewater and freshwater. One concern with this option is that wastewater can be contaminated with harmful pathogens or chemicals that can be inhaled by individuals downwind of the sprayed water. Chapter 3 modeled the risks from ammonia toxicity, as well as infection with non-tuberculosis Mycobacteria (NTM) and Legionella pneumophila, with the intention of determining whether it is safer to anaerobically
digest and disinfect wastewater prior to irrigation or to avoid increased ammonia levels by not disinfecting. The results of this study indicated that NTMs pose a negligible risk, ammonia has minimal risk, and *L. pneumophila* can be a risk to individuals within 500 m of the active irrigator. It is therefore recommended that wastewater be anaerobically digested, or disinfected in an equivalent way, before spraying. Chapter 4 examined a similar question of the risk of respiratory infection with *L. pneumophila* to individuals living up to 7 km away from the irrigator. The model output found that low pressure irrigation yields a risk of infection in excess of $10^{-6}$ infections/exposure event up to 1 km from the source, while high pressure irrigators can cross this threshold up to 2 km from the source. The main conclusions of these studies were to utilize low pressure irrigators, avoid irrigation on windy days, use personal protective equipment if work is required downwind of the active source, and irrigate on days where the weather conditions are conducive to bacterial inactivation.

These chapters, when combined, serve to improve the functionality of water-reusing systems and quantify the risks associated with water-conserving measures.
Acknowledgements

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Chapter 1

Transmission of waterborne fish and plant pathogens in aquaponics and their control with physical disinfection and filtration: a systematized review

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1.1. Abstract

The objective of this systematized review was to collect and analyze information about the waterborne spread of fish and plant pathogens through aquaponics systems, as well as investigate physical disinfection or filtration methods used to control transmission. Information gathered from comparable aquaculture and hydroponics systems was also considered for its applicability to aquaponics, and a bias assessment of accepted literature was conducted. One-hundred and forty sources were included in the review, with 85 from aquaculture systems, 55 from hydroponics, and 0 from aquaponics. Transmission was studied using cohabitation of naïve and infected organisms or direct inoculation of the water. Pathogen control methods included ultraviolet (UV) irradiation, blue light-emitting diodes, media filtration, membrane filtration, heat, sonication, and three methods that fell outside these categories. Water quality and flow rate were identified as important factors influencing disinfection efficacy, along with parameters specific to each disinfection method. The lack of studies on pathogen transmission and control in aquaponics systems, paired with the risks associated with a disease outbreak, make this an important, yet neglected, area of research.
1.2. Introduction

Aquaponics systems combine plant production in a water-based medium (hydroponics) with raising fish in tanks (aquaculture) (Rakocy, Masser, and Losordo, 2006). Fish produce waste that is broken down by nitrifying bacteria into nitrates that the plants uptake, simultaneously cleaning the water and recycling nutrients (Rakocy, Masser, and Losordo, 2006). While the concept of aquaponics originated in China over a thousand years ago, its potential as a modern means of soilless food production has only recently begun to be explored (Jones, 2002). The simplest aquaponics system is a fish tank connected to a hydroponics unit, which is connected back to the fish tank to create a recirculating system (Rakocy, Masser, and Losordo, 2006). Additional components, such as separate biofilters and water clarifiers, are often incorporated depending on the size and needs of the facility.

Water quality is an important factor in all soilless production systems, but it is especially tricky in aquaponics systems that must balance the needs of fish, plants, and bacteria. For example, the ideal pH ranges for the three groups are different, so a compromise of a system-wide pH of 7 is maintained (Rakocy, Masser, and Losordo, 2006). Water temperature, dissolved oxygen, ammonia, and nitrite are other important water quality parameters that should be monitored and balanced between the fish, plants, and bacteria. Aquaponics requires more biological and technical knowledge than hydroponics or aquaculture, which is one of the reasons it is currently a niche agricultural method. However, its potential as a commercial means of food production has been explored in recent years. A survey by Love et al. (2015) of commercial aquaponics farmers found that though only 31% reported profitability in the past 12 months, almost 75% predicted profits in the next 3 years. Since aquaponics does not have the same land requirements as traditional agriculture, it offers a means of growing fresh, organic food in cities or other restrictive environments (Love, Uhl, and Genello, 2015). The possibility of aquaponics as a healthier and more environmentally friendly option for food production has motivated recent research into various aspects of this soilless production method.

Aquaponics has been investigated for its sustainability (Love, Uhl, and Genello, 2015; Forchino et al., 2017; Junge et al., 2017), financial feasibility and options for economic optimization (Petrea et al., 2016; Bosma et al., 2017; Quagrainie et al., 2018; Walraven et al., 2018). Several studies have been conducted on the general setup of aquaponics systems (Rakocy,
Masser, and Losordo, 2006; Kyaw and Ng, 2017; Rakocy et al., 2010; Selek et al., 2017), while others focused on more specific aspects of aquaponics operation, such as stocking density (Andriani et al., 2017), cultivation media (Roosta and Afsharipoor, 2012), ratios of plants to fish (Lam et al., 2015), and water recirculation rates (Ngo Thuy Diem, Konnerup, and Brix, 2017). Food safety in aquaponics is another topic of focus (Chalmers, 2004; Pantanella et al., 2015; Moriarty et al., 2018), as well as the presence and impact of non-pathogenic microorganisms on system functions (Gravel et al., 2015; Sirakov et al., 2016; Schmautz et al., 2017; Wielgosz, Anderson, and Timmons, 2017).

There was only one study that mentioned pathogens in aquaponics systems. Chitmanat, Pimpimol, and Chaibu (2015) examined the bacteria in tanks of channel catfish and quantified the prevalence of *Aeromonas hydrophila*, a bacterial fish pathogen, but did not investigate pathogen transmission or control. This bacterium could have entered the aquaponics system from incoming fish, the water supply, or from workers or equipment that touched the water. Aquaponics’ reliance on a recirculating water supply makes preventing the introduction of pathogens important, since recycling water provides the perfect environment for pathogen amplification (Yanong, 2003). Examples of outbreaks in aquaculture and hydroponics show the severe consequences of such an event. Barramundi kept in tanks inoculated with the bacterial fish pathogen *Streptococcus iniae* experienced 40% mortality within 48 hours of exposure (Bromage, Thomas, and Owens, 1999), and 100% of cucumbers became infected with the oomycete *Pythium aphanidermatum* within 3 days of exposure to contaminated water (Goldberg, Stanghellini, and Rasmussen, 1992). A disease outbreak can ruin an entire crop, or multiple crops if the problem is not addressed, and with the high startup cost of aquaponics, this can spell the end of an operation (Love et al., 2015).

A way to prevent or minimize the impact of disease is through disinfecting the water to reduce or eliminate pathogens. Disinfection can take place at various points in the system, depending on the method. Chemical methods like chlorination (Ewart and Chrimes, 1980), electrooxidation (Kraft, 2008), and ozonation (Summerfelt and Hochheimer, 1997) have been tested in aquaculture and hydroponics systems to improve water quality and kill pathogens. However, these disinfection methods, or their byproducts, can have negative health impacts on the fish or plants, so they must be administered carefully. When these methods are used, the water is typically pumped into a separate tank, or set of tanks, where it is disinfected, treated to
remove toxic compounds, and pumped back into the system. The tank(s) could be located before the production tank to process incoming water or after the production tank to process effluent. Antibiotics once commonly used in aquaculture (Cabello, 2006) are being supplanted by probiotics (Paulitz, 1997) and bacteriophages (Richards, 2014), which are applied directly to the tank water. Vaccination for fish also remains an option for a limited number of pathogens (Sommerset et al., 2005). These chemical and biological methods of pathogen control have been well reviewed (see previous citations), but such a review is lacking for physical disinfection methods. Physical disinfection or control is defined as any disinfection method that does not rely on the use of a chemical or biological agent. This category contains a broad range of options, including membrane filtration and heat, that have been studied over the past several decades. The purpose of this review was to gather and examine information regarding the transmission and physical disinfection of pathogens in aquaponics systems, while considering information from studies on aquaculture and hydroponics for its applicability to aquaponics and potential for future implementation.

1.3. Materials and Methods

1.3.1. Literature Search Process

The literature search for this review began in October of 2017 by searching pairs of keywords in Google Scholar, with the first term as the system type - “aquaponics”, “aquaculture”, and “hydroponics” - and the second term as the issue being investigated, which was “pathogen”, “disease”, or “control”. Every possible combination of these two groups of terms was searched. Sources that passed the initial round of screening were read in full, and if confirmed to be relevant, their references were searched for further literature. This method of tracing references was used until references became redundant or irrelevant. Two online databases, Web of Science and ScienceDirect, were searched on May 5, 2018 using the same combinations of keywords used for Google Scholar. The only restriction placed on the output from these searches was that ScienceDirect was limited to “research articles”. All available issues of four scientific journals, chosen for their applicability to the review, were also searched online. Books 1-1198 of Acta Horticulturae, volumes 1-82 of Aquacultural Engineering, volumes 1-494 of Aquaculture, and volumes 1-128 of Disease of Aquatic Organisms were examined for
relevant articles. Once the final collection of sources was read and assessed, the literature search was judged complete.

1.3.2. Inclusion Criteria

To be included in this review, sources had to be scientific journal articles, government reports, or theses containing primary research available online in English. The title or abstract had to mention aquaponics, aquaculture, hydroponics systems, or a synonym for these terms (ex. soilless system). These systems had to be in controlled environments, largely inside, which excluded types of setups like cage aquaculture, which is open to the environment. The title or abstract had to mention waterborne fish or plant pathogens and their horizontal transmission through the system, or physical disinfection or filtration methods. Physical disinfection was defined as any method of inactivating a pathogen that does not use chemical or biological agents. Any sources that failed to meet these criteria were not included in the results of this review.

1.3.3. Literature Bias Assessment

Sources that passed all screenings were subjected to a bias assessment based on the Mixed Methods Appraisal Tool (Version 2011) published by McGill University (Pluye et al., 2011). The criteria were tailored to this review, and included the following questions:

1. Is the objective clear?
2. Do the collected data address the objective?
3. Are the groups being compared actually comparable, and if not, do the researchers address this?
4. Is there an adequate sample size?
5. Are efforts made to prevent contamination of the system and samples?
6. Is the experiment replicated, or is there redundancy in the experimental setup?
7. Are the plants or fish acclimatized to their respective systems before the start of the experiment?
8. Is there an explanation of what results are deemed significant?
9. Are the experimental conditions explained well enough to replicate?
10. Is there an untreated/unexposed group (control)?
Sources received one point for every unmet or unreported criterion and could end up with a score from 0-10, with 0 meaning no apparent bias and 10 meaning extreme bias. If a study did not need to meet a criterion, an “NA” was used instead. An example of this would be a system lacking biosecurity protocols because they were looking at a system that was already infected. Sources with scores $\geq 5$ were automatically excluded.

1.3.4. Data Analysis

Information about the types of fish, plants, and disinfection methods found in the studies were displayed graphically and quantitative findings were either organized into tables or discussed individually in the relevant sections. The only alterations made to the data were unit conversions necessary for useful comparison.

1.3.5. Review Limitations

The review process was designed to reduce bias as much as possible. The keywords used for searching databases were kept broad to increase the number of results and multiple methods of finding sources were used to avoid the limitations of each approach. Even with precautions in place, this review has biases inherent to the inclusion criteria and search process. The limitation of sources to those published in English excludes any non-English sources and the keywords chosen for the database searches could have introduced limitations in the search results. There is also a natural human bias associated with one author doing the literature search. However, a concerted effort was made to reduce bias in this review and the results are believed to accurately represent the available literature.

1.4. Results and Discussion

1.4.1 General Findings

The total number of sources screened far surpassed 1,000, with 642 articles passing the title and abstract screening and 140 being included in the results of the review. The main reasons for excluding sources that passed the initial screening were that they did not address transmission or did not have systems in controlled environments. No sources were excluded for having bias scores greater than 5. Eighty-five of the sources included in the results dealt with aquaculture, 55 with hydroponics, and none investigated aquaponics systems. This reveals that waterborne
transmission and physical pathogen control methods are a complete unknown in the field of aquaponics, and while knowledge from similar systems can be applied, it remains a major research gap. The following sections will discuss waterborne transmission in soilless production systems, as well as different pathogen control methods.

1.4.2. Transmission in Soilless Production Systems

Understanding the details of transmission in soilless production systems is vital for managing the spread of pathogens. Seventy-two articles on pathogen transmission passed the inclusion screening, 57 about aquaculture and 15 about hydroponics. Studies examined pathogen transmission through water, risk factors for infection, variables that impacted spread and disease severity, shedding rate of infectious organisms by diseased fish or plants, and doses required to cause infection. A wide variety of fish and plant pathogens were found to spread through aquaculture and hydroponics systems, thereby also posing a threat to aquaponics systems.

Figures 1.1 and 1.2 show the sources that addressed transmission in aquaculture (Figure 1.1) and hydroponics (Figure 1.2), as broken down by the type of fish or plant examined. It should be noted that some studies are represented more than once for investigating multiple fish or plants. Figure 1.1 highlights that salmon are by far the most commonly studied fish, with 18 sources, followed by catfish at 8 sources, and trout at 7. Though salmon are a commonly farmed fish, findings from studies on subspecies that live in saltwater may not always be applicable to aquaponics, since plants cannot tolerate the salt. However, information about basic infection dynamics from saltwater aquaculture may be helpful for aquaponics, as long as systemic differences are accounted for. Tilapia, which is raised by around 69% of commercial aquaponics farmers in the U.S. (Love et al., 2015), is only represented 4 times, and most of the fish species only have a single publication (Figure 1.1). It is also apparent that viruses are the most commonly studied fish pathogen type, followed by bacteria (Figure 1.1).
Figure 1.1. Transmission of waterborne fish pathogens in aquaculture systems

The number of sources that investigate a pathogen type (x-axis) are provided for each type of fish (y-axis). Studies that investigate more than one pathogen and/or fish type are included multiple times. The ‘ornamental’ fish type consists of angelfish, goldfish, gourami, and mandarinfish. References for each fish type are as follows: Arctic charr (89), ayu (116), barramundi (32, 33, 96, 147), bass (96, 191, 232, 233), bream (57, 84, 96, 112, 232), carp (64, 88, 96, 229), cod (125), flounder (123, 171), grouper (96, 147), Murray cod (81), ornamental (64, 81, 96, 112), perch (96), rabbitfish (96), rohu (229), salmon (4, 9, 29, 85, 125, 143, 151, 152, 172, 176, 177, 180, 206, 213, 224, 245, 250, 269), snakehead (96), snapper (96), striped jack (7), tilapia (25, 96, 157, 193, 226), trout (5, 19, 62, 95, 145, 181, 197, 245), turbot (72, 125, 170), wolfish (234), yellowtail (108).

Figure 1.2 shows that only a handful of plant types have been studied in hydroponics. Viruses and oomycetes were the most commonly investigated plant pathogen types. Tomatoes were the most popular plant, with 18 studies, compared to 4 for cucumbers and even less for the other plants (Figure 1.2). A serious knowledge gap exists for herbs and salad greens, which are cultivated by over 75% of U.S. commercial aquaponics growers (Love et al., 2015). Lettuce, kale, bok choi, and chard are also popular crops that were not represented in the literature on pathogen transmission in hydroponics (Love et al., 2015). More experiments need to be conducted with fish and plants other than salmon and tomatoes and with a wider variety of
pathogens in order to improve recommendations for managing waterborne transmission of pathogens in soilless production systems.

*Figure 1.2. Transmission of waterborne plant pathogens in hydroponics systems*

The number of sources that investigate each pathogen type (x-axis) are provided for each plant type (y-axis). Studies that investigate more than one pathogen and/or plant type are included multiple times. References for each plant type are as follows: carnations (65), cucumber (38, 65, 156, 217), eggplant (54), lettuce (38), Pelargonium (18), peppers (188), potato (155), spinach (15), tobacco (38, 188), tomato (38, 39, 44, 54, 65, 155, 158, 187, 188, 239, 240, 266, 270).

Waterborne transmission occurs when the infectious organism is released into the aqueous environment by the diseased fish or plant, then is taken up by a previously healthy organism to cause infection. Fish typically shed pathogens through bodily fluids, like mucus, which enter the new host via a variety of portals, such as the skin (Bricknell, 2017). Plants shed pathogens from their root systems and the infectious agents adsorb to the roots of healthy plants (Büttner, Marquardt, and Führling, 1995; Mehle et al., 2014). The rate at which shedding takes place varies between pathogens. The release of *Flavobacterium psychrophilum* by infected fish peaked after 7 days (Madetoja, Nyman, and Wiklund, 2000), while the shedding of
*Renibacterium salmoninarum* reached its maximum 22 days after challenge (McKibben and Pascho, 1999). The rate of pathogen shedding, the organism’s ability to survive and multiply in the nutrient solution, and its ability to infect new hosts are what determine the amount of pathogen in the system at a given time. Tobacco mosaic virus has been shown to survive up to 5 days in a hydroponic environment (Park et al., 1999) and potato virus Y, pepino mosaic virus, and potato spindle tuber viroid stayed infectious at 20°C for up to 1, 3, and 7 weeks, respectively (Mehle et al., 2014). More research needs to be conducted to determine how long these organisms remain infectious to inform treatment options for growers experiencing outbreaks.

Natural waterborne transmission is simulated under experimental conditions by cohabitating infected and healthy individuals or exposing healthy fish or plants to water inoculated with the pathogen. It should be noted that the etiological agents of some fish and plant diseases remain unknown, like the cause of cold-water strawberry disease, even though transmission studies through cohabitation are still successful (Pond et al., 2008). Transmission studies examine either a monoculture or coculture of species, and cross-species transmission can occur in the case of co-culture, as is common in aquaponics (Love et al., 2015). An example of this is a study by Go and Whittington (2006) that demonstrated dwarf gourami (*Colisa lalia*) infected with megalocytivirus could transmit it to Murray cod (*Maccullochella peeli peeli*).

Some studies conducted in saltwater systems showed similar results, with Sitjà-Bobadilla and Alvarez-Pellitero (2003) finding that *Cryptosporidium molnari* could be spread between gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*). Korsnes et al. (2012) found that nervous necrosis virus was easily transmitted between turbot (*Scophthalmus maximus*) and Atlantic cod (*Gadus morhua*), but was not detected in Atlantic salmon (*Salmo salar*) cohabitants. Though the mechanism of transmission via pathogen shedding into the water is the same for both freshwater and saltwater, cross-species transmission should be investigated further in freshwater systems to allow direct application of the results to aquaponics. Interspecies transmission was also noted in a hydroponics system in which the fungal pathogen *Colletotrichum coccodes* was successfully spread from infected eggplants to tomatoes (Daughtrey and Schippers, 1980). There have also been reports of infections in aquaculture shrimp from fungi commonly classified as plant pathogens. Khoa, Hatai, and Aoki (2004) described an outbreak of *Fusarium incarnatum* - an herb fungus - in black tiger shrimp that caused severe gill damage and mortality. Karthikeyan and Gopalakrishnan (2014) reported
morbidity and mortality in black tiger shrimp from *Gilbertella persicaria*, a fungal pathogen of fruiting plants. These results show that outbreaks of certain pathogens can devastate multiple populations in a soilless production system and that co-culture of different fish or plant species does not guarantee a back-up crop in case of disease.

The issue of carriers was introduced in a study by El-Matbouli and Soliman (2011) that examined the role of goldfish (*Carassius auratus auratus*) as carriers of Cyprinid herpesvirus-3 and their ability to transmit the virus to common carp (*Cyprinus carpio*). The results indicated that asymptomatic goldfish can successfully infect common carp, emphasizing the importance of obtaining fish stock from certified disease-free sources. Biofilms may be another way that bacterial pathogens can persist in soilless systems, as was demonstrated in a study by Welker et al. (2005) that showed that *Flavobacterium columnare* formed a biofilm on the tank walls within 4 days of introduction into the system. Cai, De La Fuente, and Arias (2013) confirmed that biofilms of *F. columnare* grown in flow conditions remain virulent by exposing channel catfish fingerlings to the biofilm and observing 100% mortality within 48 hours of inoculation, indicating that accumulation of bacteria on system components could create reservoirs for pathogens to amplify and cause additional infections. The conditions and locations of biofilm formation, the rate at which it would occur, and possible control methods are unknown in the context of these systems, and without this knowledge, treatment and prevention methods may prove to be insufficient.

Pathogens may also be released into the nutrient solution from the tissues of dead organisms, which is why quick removal is recommended. Vike et al. (2014) showed that even though no particles of infectious salmon anemia virus were found in the water around deceased salmon, infectious particles were detected in body tissues for longer than 5 days after death. This suggests that if the tissues degraded further, these particles may be released and cause more infections. Removing diseased organisms is also important prior to death. A study by Shaw, Kent, and Adamson (1998) demonstrated that more naïve salmon (*Oncorhynchus tshawytscha and kisutch*) became infected *Loma salmonae* the longer the group was cohabitated with individuals afflicted with the parasite, with 3/10 naïve fish testing positive after 24 hours of exposure and 10/10 testing positive after 7 days. Becker, Speare, and Dohoo (2005) found that naïve rainbow trout (*Oncorhynchus mykiss*) exposed for 12 hours to individuals infected with *L. salmonae* experienced significantly higher infection rates than those exposed to infected
individuals for 1, 24, and 96 hours. This indicates that longer exposure times lead to increased infections, though there may be a time at which maximum transmission occurs.

Duration of exposure of naïve fish to contaminated water is another important factor, with Zhang, Xu, and Shoemaker (2016) showing that cumulative mortality of channel catfish from infection with *Aeromonas hydrophila* increased significantly when fish were exposed to inoculated water for 60 minutes (100% mortality) versus 30 minutes (85%). This relationship between exposure time and disease outcome makes sense, since longer exposure periods give the pathogen more opportunity to invade the host. A closely related factor, dose-response, is the relationship between the amount of pathogen received by the fish or plant and the disease outcome. Table 1.1 offers a summary of dose-response studies in both aquaculture and hydroponics systems, and reports whether there was a positive correlation between the amount of pathogen and the severity of response, often seen as a higher mortality rate. Most of the pathogens shown in Table 1.1 were found to have dose-dependent disease outcomes, though one pathogen, *A. salmonicida*, had conflicting results. This bacterium was examined in two separate studies with Atlantic salmon, and while one found a dose-response relationship (Rose, Ellis, and Monroe, 1989), the other did not (Nordmo, Ramstad, and Riseth, 1998). However, due to large differences between the two study setups and a lack of other studies, a definite assessment of the results cannot be made.

**Table 1.1. Dose-Response Relationships of Fish and Plant Pathogens**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Test Species</th>
<th>Positive Correlation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. hydrophila</td>
<td>I. punctatus</td>
<td>yes</td>
<td>Zhang, Xu &amp; Shoemaker, 2016</td>
</tr>
<tr>
<td>A. salmonicida</td>
<td>S. salar</td>
<td>yes; no</td>
<td>Rose, Ellis &amp; Monroe, 1989; Nordmo, Ramstad &amp; Riseth 1998</td>
</tr>
<tr>
<td>F. psychrophilum</td>
<td>O. mykiss</td>
<td>yes</td>
<td>Madetoja, Nyman &amp; Wiklund, 2000</td>
</tr>
<tr>
<td>Infectious salmon anemia virus</td>
<td>S. salar</td>
<td>yes</td>
<td>Raynard, Snow &amp; Bruno, 2001</td>
</tr>
<tr>
<td>L. salmonae</td>
<td>O. mykiss</td>
<td>no</td>
<td>Becker, Speare &amp; Dohoo, 2005</td>
</tr>
<tr>
<td>P. aphanidermatum</td>
<td>C. sativus</td>
<td>yes</td>
<td>Menzies, Ehret &amp; Stan, 1996</td>
</tr>
<tr>
<td>S. iniae</td>
<td>L. calcarifer</td>
<td>yes</td>
<td>Bromage and Owens, 2002</td>
</tr>
<tr>
<td>V. anguillarum</td>
<td>P. altivelis</td>
<td>yes</td>
<td>Kanno, Nakai &amp; Muroga, 1989</td>
</tr>
<tr>
<td>V. salmonicida</td>
<td>S. salar</td>
<td>yes</td>
<td>Nordmo and Ramstad, 1999</td>
</tr>
</tbody>
</table>
A study by Shoemaker, Evans, and Klesius (2000) demonstrated that dose-response relationships might be reliant on other factors, like stocking density. Fish exposed to *Streptococcus iniae* at doses of $2.9 \times 10^7$ and $1.2 \times 10^8$ cfu/mL had significant differences in mortality rates only when tilapia (*Oreochromis niloticus*) were kept at a high stocking density (22.4 g fish/L). Ogut and Reno (2004) found a highly significant positive association between the stocking density of rainbow trout and the prevalence of infectious hematopoietic necrosis virus. Hauge et al. (2017) determined that pairing oxygen stress with crowded conditions led to a higher prevalence of infection with Piscine orthoreovirus in Atlantic salmon. Davis, Griffin, and Gray (2002) supported the relationship between stress and disease when stressing channel catfish with low water levels for six hours led to a denser distribution of *Ichthyophthirius multifiliis* parasites in fish tissue. In contrast, no significant difference was found between stocking density of Atlantic salmon and time to 50% mortality from *A. salmonicida* (Nordmo, Ramstad, and Riseth, 1998) or cumulative mortality for infectious pancreatic necrosis virus (Bowden et al., 2003). These findings suggest that the pathogen and fish species determine the role of stocking density in transmission.

The temperature of the nutrient solution is important for maintaining the physical functions of the fish and plants but has also been shown to influence transmission dynamics and disease outcomes. Fouz et al. (2000) determined that the bacterial pathogen *Photobacterium damselae subsp. damselae* only caused fatalities in turbot when the water was warm (22°C). The percent mortality of Atlantic salmon from infection with *A. salmonicida* was found to increase when the water temperature was raised from 8 to 12°C (Nordmo and Ramstad, 1999). This phenomenon was seen in hydroponic systems as well, with *Pythium aphanidermatum* causing 100% mortality in spinach crops grown in water kept at 30°C, but 0% mortality in crops grown in water kept at 20°C (Bates and Stanghellini, 1984). Additionally, *Pythium dissotocum* caused 100% of plants to wilt when water was 30°C compared to the 69% that wilted when water was 20°C (Bates and Stanghellini, 1984). These differences in disease prevalence and severity are likely related to the optimal growth temperatures of these pathogens, with increased infection rates and mortalities resulting from water temperatures being more favorable to pathogen amplification. The section dealing with alternative disinfection methods (3.2.7) discusses the potential application of temperature control for pathogen management.
Injury as a factor in pathogen transmission has been examined in some aquaculture studies. Singhal, Jeet, and Davies (1987) determined that damaged skin is the main route of natural infection for the fish pathogenic oomycetes Achlya racemosa and the Saprolegnia parasitica-Saprolegnia diclina complex. Zhang, Xu, and Shoemaker (2016) found that channel catfish with clipped fins experienced 90% mortality due to infections with A. salmonicida, compared to 20% mortality in fish with light skin abrasions and 5% to uninjured fish. The idea that more severe injuries increase fish susceptibility to pathogens was supported by Madetoja, Nyman, and Wiklund (2000) when exposure to F. psychrophilum resulted in 100% mortality in rainbow trout with injured skin, but only 25% in fish whose mucus layer had been removed and 0% in uninjured fish. Injuries provide an easy entrance for pathogens into the deeper tissues and cause stress in the fish, making disease outcomes worse for wounded animals. Damaged plants have also been shown to be more susceptible to infections and have worse disease outcomes, but not in the context of soilless production systems (Banniza and Vandenberg, 2003). Another factor in disease transmission is the impact of coinfections on vulnerable populations. Xu et al. (2013) determined that channel catfish coinfectected with Edwardsiella ictaluri and I. multifiliis could transmit both pathogens to naïve catfish. This could be an issue in systems with multiple pathogens, but the dynamics of these coinfections are not well understood and require further research.

Though many pathogens transmit easily through the water of soilless production systems, not all pose a significant threat. Two studies conducted on tomatoes in hydroponics systems found that the pathogens Pyrenochaeta lycopersici, Phytophthora nicotianae, Corynebacterium michiganense (Staunton and Cormican, 1978), Colletotrichum coccodes, and Spongospora subterranea (Staunton and Cormican, 1980) did not cause disease at any time point in either experiment. The results for P. nicotianae conflict with findings from at least two other studies, one by Vanachter, Van Wambeke, and Van Assche (1983) and another by Van Voorst, Van Os, and Zadoks (1987), which both found that the oomycete spread easily and induced disease in tomatoes to dose-dependent degrees of severity. The study by Staunton and Cormican (1978) was carried out over four months, so there was ample time for infection and all three studies examined tomatoes in nutrient film technique systems. However, there may have been other systemic differences that led to the conflicting results, or issues with the culture of P. nicotianae used in the study under question. No other studies were conducted with the other pathogens, so
there is no definite conclusion for whether these agents are a threat to soilless production systems.

A study by Perera, Johnson, and Lewis (1997) presented conflicting results for transmission in an aquaculture system. When the spread of *S. iniae* was examined through cohabitating infected and naïve tilapia, there was no observed disease development in the naïve population. The tilapia in this experiment were a hybrid of *Oreochromis niloticus* and *O. aureus*, and the bacteria were shown to cause disease when administered via bath immersion, so the fish are known to be susceptible (Perera, Johnson, and Lewis, 1997). One explanation could be that the infected fish in the cohabitation challenge did not shed enough bacteria to expose the naïve individuals to the minimum dose required to cause disease. This study should be replicated to confirm or challenge the original results.

There are a wide variety of fish or plant pathogenic bacteria, viruses, parasites, oomycetes, nematodes, and fungi that can be spread through the water of soilless production systems. Several factors influence this spread, including fish and plant species, stocking density, pathogen dose, water volume and conditions, injury, duration of exposure, and duration of pathogen viability in solution. Currently, there are not enough studies exploring any of these concepts, and the field of aquaponics is entirely bereft of research regarding pathogen transmission. Additional research is needed to understand transmission dynamics in soilless production systems to create a basis for control measures and management policies.

1.4.3. Physical Disinfection and Filtration Methods

The six physical disinfection and filtration methods found in the literature were ultraviolet irradiation (UV), blue light-emitting diodes (LED), media filtration, membrane filtration, heat, and sonication. A category called “alternative” was also created for the three articles that fell outside those classifications. Figure 1.3 shows the distribution of disinfection methods by soilless production system type. It should be noted that some studies are represented multiple times if they examined more than one disinfection method. The following sections will report the findings of the studies for each disinfection method, as well as offer comparisons between studies and suggestions for future research.
Figure 1.3. Physical disinfection and filtration methods for waterborne pathogen removal in aquaculture and hydroponics

The number of studies (x-axis) that address each disinfection method (y-axis) are shown, broken down by system type. References for each system type are as follows: alternative (3, 153, 225), blue LED (210, 211), heat (3, 8, 109, 119, 144, 179, 207, 215, 248), membrane (20, 83, 138, 164, 182, 220, 249), media filtration (11, 12, 21, 22, 30, 43, 76-78, 115, 130, 148, 149, 159, 160, 178, 263-265, 274-276), sonication (3, 18, 248, 248, 277), and ultraviolet irradiation (1, 3, 8, 18, 35, 39, 66, 77, 86, 97, 98, 103, 102, 119, 133-137, 144, 146, 159, 160, 184, 207, 214, 220, 237, 238, 244, 248, 265, 276, 284, 286).

1.4.3.1 Ultraviolet Irradiation

Ultraviolet (UV) disinfection in soilless production systems involves exposing tank water to light in the germicidal range of roughly 225 to 312 nm (Hijnen, Beerendonk, and Medema, 2006; Sholtes et al., 2016). These UV lights typically disinfect effluent water from the fish tank but can be used to disinfect influent water as well (Liltved and Cripps, 1999; Mamane et al., 2010). Lamps used in these systems are often categorized as either low or medium-pressure, with low-pressure lamps producing a single wavelength of light at 254 nm, while medium-pressure lamps emit multiple wavelengths in the germicidal range (Loge et al., 1999). Low-pressure lamps were encountered in the literature more frequently than medium-pressure lamps.
The main measure of UV disinfection capacity is dose, which is the energy received per unit area over a certain time period (Mamane et al., 2000). Dose is influenced by factors like light reflection, refraction, intensity, and length of exposure time (Hijnen, Beerendonk, and Medema, 2006), and determines whether microorganisms within the water lose viability and become inactivated (Hijnen, Beerendonk, and Medema, 2006). The mechanisms of pathogen inactivation are damage to the DNA that disrupts replication and damage to mRNA (Xu et al., 2018), with bacteria generally being the most susceptible to this damage, followed by protozoa, then viruses and bacterial spores (Hijnen, Beerendonk, and Medema, 2006). However, this order of susceptibility is a trend rather than a rule, and UV sensitivity is highly variable between different species and even different strains of the same species (Hijnen, Beerendonk, and Medema, 2006). Ultraviolet irradiation is the most studied disinfection method for soilless production systems, with 37 sources meeting the inclusion criteria. Twenty-one dealt with aquaculture systems, while 16 involved hydroponics. Table 1.2 summarizes the UV doses, in mJ/cm², required for at least 99% inactivation of some fish and plant pathogens. Doses originally reported with alternative units were converted for easier comparison.

Table 1.2. UV Irradiation Dose Required for ≥99% Plant and Fish Pathogen Inactivation

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Dose (mJ/cm²)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. salmonicida subsp. salmonicida</td>
<td>2.34; 3.2</td>
<td>Liltved and Landfald, 1995; Liltved and Landfald, 1996</td>
</tr>
<tr>
<td>V. anguillarum</td>
<td>2.7; 2.8</td>
<td>Liltved, Hektoen, and Efraimsen, 1995; Liltved and Landfald, 1996</td>
</tr>
<tr>
<td>V. salmonicida</td>
<td>2.7</td>
<td>Liltved, Hektoen, and Efraimsen, 1995</td>
</tr>
<tr>
<td>Y. ruckeri</td>
<td>2.7; 1.2</td>
<td>Liltved, Hektoen, and Efraimsen, 1995; Liltved and Landfald, 1996</td>
</tr>
<tr>
<td>Koi herpesvirus</td>
<td>4</td>
<td>Kasia and Yoshimizu, 2005</td>
</tr>
<tr>
<td>Infectious salmon anemia virus</td>
<td>7.5</td>
<td>Liltved et al., 2006</td>
</tr>
<tr>
<td>P. aphanidermatum</td>
<td>15</td>
<td>Sutton et al., 2000</td>
</tr>
<tr>
<td>F. oxysporum f.sp. cyclaminis</td>
<td>40</td>
<td>Sutton et al., 2000</td>
</tr>
<tr>
<td>F. oxysporum f.sp. melongenae</td>
<td>70</td>
<td>Runia, 1994</td>
</tr>
<tr>
<td>Tomato mosaic virus</td>
<td>100</td>
<td>Runia, 1994</td>
</tr>
</tbody>
</table>
Table 1.2. (cont.) UV Irradiation Dose Required for $\geq 99\%$ Plant and Fish Pathogen Inactivation$^a$

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Dose (mJ/cm$^2$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlantic halibut nodavirus</td>
<td>104</td>
<td>Liltved et al., 2006</td>
</tr>
<tr>
<td>Infectious pancreatic necrosis</td>
<td>122; 246; 720-792</td>
<td>Liltved, Hektoen, and Efraimsen, 1995; Liltved et al., 2006; MacKelvie and Desautels, 1975</td>
</tr>
<tr>
<td>F. psychrophilum</td>
<td>126</td>
<td>Hedrick et al., 2000</td>
</tr>
<tr>
<td>P. cryptogea</td>
<td>237</td>
<td>Wohanka and Seidel, 2004</td>
</tr>
<tr>
<td>M. cerebralis</td>
<td>1,300</td>
<td>Hedrick et al., 2000</td>
</tr>
</tbody>
</table>

$^a$ Irradiation was administered using a collimated beam of UV with wavelength of 253.7-254 nm.

An important observation to make is that the range of doses in Table 1.2 is very wide. While some pathogens are highly susceptible to damage from UV rays, such as $A.\ salmonicida$, the parasite $M.\ cerebralis$ needs a large dose at 1,300 mJ/cm$^2$ (Table 1.2). Infectious pancreatic necrosis virus was 99% inactivated by a dose of 122 mJ/cm$^2$ in one study but required 246 mJ/cm$^2$ in another (Table 1.2). Both studies were conducted in the laboratory, but inconsistencies in the source of the pathogen, ambient laboratory conditions, UV device used, and other factors could have influenced the results. This emphasizes the importance of replicating UV inactivation experiments in soilless production systems and establishing a standardized method of testing and reporting the results.

Additional sources that support the efficacy of UV disinfection in soilless production systems include Spanier (1978), who determined that the bacterial fish pathogen Serratia marcescens was significantly reduced by UV treatment of recirculating water with lamps emitting light between 250 and 270 nm. Buyanovsky and Gale (1981) found that applying a UV radiation dose of 171.6 mJ/cm$^2$ to a recirculating hydroponics system for three hours a day reduced bacterial counts from $400 \times 10^3$ cells/mL to $50 \times 10^3$ cells/mL. Mamane et al. (2010) demonstrated that bacterial levels were cut in half from 1500 bacteria/mL to 750 bacteria/mL when water from a recirculating aquaculture system was passed through a low-pressure UV disinfection unit applying doses of 2-4 mJ/cm$^2$. However, Mamane et al. (2010) pointed out that only highly UV sensitive organisms would be affected by such low doses and recommended increasing these doses to control pathogenic bacteria. Zhang and Tu (2000) found that even though applying UV doses of up to 87.9 mJ/cm$^2$ significantly decreased Pythium
aphanidermatum counts in a hydroponic solution, it was not enough to reduce root rot caused by the oomycete. This emphasizes the importance of not only looking at dose and the level of pathogen reduction, but also the impact on disease outcomes, since this is the end goal of UV disinfection.

The question of whether periodic or continuous UV treatment is more effective was examined by Ewart and Chrimes (1980), and continuous UV treatment was shown to cause a more significant decrease in potentially pathogenic bacteria. Continuous UV treatment was also demonstrated to eliminate 96.4-99.7% of bacteria at a dose of 250 mJ/cm² and suppress growth of P. aphanidermatum in a rockwool hydroponics system (Van Os et al., 2004). Similarly, Rey et al. (2001) found that 300 mJ/cm² of UV light killed >95% of Pythium spp. in a rockwool hydroponic crop. Aquaculture systems also benefited from continuous UV sterilization, with Yoshimizu et al. (2005) demonstrating that many fish viruses can be 99% inactivated by a dose of 1 mJ/cm², though more resistant ones required doses around 100 mJ/cm². However, Vanachter and Leuven (1995) determined that partial sterilization, in which only 20% of the nutrient solution was irradiated, sufficiently prevented disease development in plants from Pythium spp. or Olpidium brassicae. This suggests that total elimination of pathogens from the recirculating water may not be necessary to control disease, and that the effectiveness of UV sterilization against both fish and plant pathogens make it potentially useful for disinfection in aquaponics.

Water quality has a strong influence on UV treatment in soilless production systems, with colored water and suspended solids limiting disinfection efficacy. When water is colored, the material responsible absorbs the UV light and blocks its transmission through the rest of the water (Basu, Page, and Wei, 2007). Suspended solids create an even greater barrier to UV transmission, with particles absorbing and deflecting the light, while also shielding particle-associated pathogens from the damaging rays (Qualls, Flynn, and Johnson, 1983; Sharrer et al., 2005). Yoshimizu et al. (2005) demonstrated that viruses can adsorb to solid particles, and the same process has been proposed for bacteria (Sharrer et al., 2005). Loge et al. (1999) demonstrated that UV light was not detected 10 μm inside the solid, which suggests that any pathogens contained deeper in the suspended solid were unaffected by the UV irradiation. This explains why particle-associated bacteria have been found to be more resistant to UV disinfection (Hijnen, Beerendonk, and Medema, 2006), and why filtration has been shown to increase the effectiveness of disinfection (Qualls, Flynn, and Johnson, 1983).
The issue of suspended solids is important in aquaculture and aquaponics systems because of the production of solid waste by the fish and the breakdown of uneaten fish food. Bazyar Lakeh et al. (2013) demonstrated that UV irradiation was significantly more effective in clear water than in turbid water, and problems with UV efficacy in hydroponics systems tested by Van Os et al. (2004) were due to dirty lamps and high turbidity. When Afonso et al. (2012) compared UV irradiation of two fish viruses – infectious hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV) - in either culture media or effluent blood water (EBW), a higher UV dose was required for viral inactivation in the EBW than the culture media. Doses of 3.82 and 3.84 mJ/cm² were needed for the IHNV and VHSV, respectively, in the culture media to obtain a 3-log reduction, while the viruses in the EBW given a dose of 4.0 mJ/cm² resulted in only a 2.26-log reduction (Afonso et al., 2012). Oye and Rimstad (2001) achieved a 3-log reduction of VHSV in freshwater at a dose of 3.3 mJ/cm², and a 3-log reduction in wastewater at a dose of 3.1 mJ/cm². Infectious salmon anemia virus (ISAV) was shown to be similarly sensitive, experiencing a 3-log reduction at a dose of 0.79 mJ/cm² in freshwater and 7.2 mJ/cm² in wastewater (Oye and Rimstad, 2001). Infectious pancreatic necrosis virus (IPNV) was much more resistant, requiring a dose of 118.8 mJ/cm² for 3-log reduction in freshwater and 336.7 mJ/cm² in wastewater (Oye and Rimstad, 2001).

These findings highlight the variability of UV sensitivity and how the presence of particulate matter in water impacts UV disinfection efficacy. For the highly UV-sensitive viruses – VHSV, IHNV, and ISAV – the water quality did not have as great of an effect, since exposure to low amounts of UV rays is enough to render them inactive. This is in direct contrast to the IPNV tested in Oye and Rimstad (2001), which required a dose almost 3 times greater when the virus was suspended in wastewater than the dose needed for 3-log inactivation in freshwater. This indicates that placing a UV resistant pathogen in a medium that blocks UV transmittance greatly increases the virus’s ability to survive UV exposure, raising concerns about the persistence of such dangerous pathogens in systems with higher levels of suspended solids. A study by Bullock and Stuckey (1977) offered contradictory results when UV irradiation was tested on a group of pathogenic fish bacteria in clear spring water and water with organic matter. A 99% inactivation efficacy was achieved with a dose of between 4 and 4.75 mJ/cm² for test strains in water with organic matter and 4.5 mJ/cm² for bacteria suspended in clear freshwater (Bullock and Stuckey, 1977). Since the dose for freshwater falls into the range for turbid water, it
does not appear that turbidity had an impact on UV efficacy, however, prefiltration could have removed many of the suspended solids prior to entry into the disinfection unit.

Prefiltration is the practice of removing larger suspended solids from the water before it enters the UV unit. In the literature found for aquaculture and hydroponics, prefilters were mesh screens with various pore sizes, though other alternatives exist. Bullock and Stuckey (1977) showed that employing a 25 nm prefilter decreased the range of UV doses needed to eliminate more than 99% of pathogenic bacteria from 3.3 to 5.3 mJ/cm² in water with organic matter to between 4 and 4.75 mJ/cm² in filtered water. A study by Liltved and Cripps (1999) showed that adding a 50 μm prefilter to a UV system led to reduction in bacterial counts 3 logs greater than unfiltered water. Increasing the UV dose for the unfiltered water did not result in a change in bacterial counts, supporting the idea that solids provide protection for the bacteria and introduce a threshold for effectiveness of UV treatment (Liltved and Cripps, 1999). A study of *M. cerebralis* conducted by Hoffman (1975) demonstrated that tanks receiving water treated with a 25 μm prefilter and a UV dose of 27.65 mJ/cm² had a much lower percentage of fish test positive for the parasite than tanks receiving water treated only with a UV dose of 18 mJ/cm², though it should be noted that the tanks receiving UV treatment alone had a lower dose than the tanks receiving both prefiltration and UV treatment.

This discussion of suspended solids and prefiltration is most relevant to aquaculture and aquaponics systems, which produce large amounts of suspended solids from fish food and waste. Water quality must be considered to determine if effective application of UV disinfection is feasible and what water quality management practices can be adopted to reduce suspended solids. Some management practices discussed in the review by Cripps and Bergheim (2000) include improving the quality of the fish feed to limit breakdown and improve the quality of fish feces and employing various solids separation techniques, such as sedimentation and microscreens. One consideration in aquaponics systems is the impact of prefiltration on the nitrification process critical to plant growth. Studies should be conducted to determine if adequate breakdown of fish waste and food occurs prior to filtration, or if different options need to be explored for aquaponics systems. Additional work needs to be done on UV disinfection in aquaponics to determine if there are any benefits or limitations unique to those systems. The energy usage and cost of maintaining UV lamps should be investigated to get an idea of their
environmental and economic impact. Overall, ultraviolet irradiation should be considered a well-researched and effective disinfection method for soilless production systems.

1.4.3.2 Blue light-emitting diode photoinactivation

Blue light-emitting diode (LED) photoinactivation involves placing these lights above fish tanks to expose the water. Two studies examined the bactericidal and antiprotozoal effects of light at the wavelengths 405 and 465 nm. The first compared infection rates and bacterial counts of *Edwardsiella piscicida* in tanks of carp when exposed to ambient light (control), 405 nm LED, or 465 nm LED (Roh and Kang et al., 2018). The average light intensities were 198 and 369 μmol/m²/s for the 405 nm and 465 nm LEDs, respectively (Roh and Kang et al., 2018). It was demonstrated that tanks exposed to the 405 nm LEDs had the lowest transmission rates when naive and infected carp were cohabitated, with 3/10 and 2/10 cohabitants becoming infected, in contrast with the 6/10 and 5/10 for the ambient light group and the 7/10 and 4/10 for the 465 nm group (Roh and Kang et al., 2018).

The second study examined the effectiveness of the same LEDs against *Miamiensis avidus*, a protozoan pathogen of olive flounder (*Paralichthys olivaceus*) (Roh and Kim et al., 2018). Tanks of flounder challenged by bath immersion with *M. avidus* were exposed to either ambient light (control) or the two wavelengths of LEDs and observed for mortality (Roh and Kim et al., 2018). Cumulative mortality was lowest for the group exposed to the 405 nm LEDs at 30% and 25%, compared to 100% for ambient light and 70% and 67% for 465 nm LEDs (Roh and Kim et al., 2018). Both studies also examined the potential for negative health effects on the fish and found none (Roh and Kang et al., 2018; Roh and Kim et al., 2018). The success of these two studies suggests that blue LEDs have the potential for application in soilless production systems, though effectiveness against plant pathogens and a wider range of fish pathogens remains unknown. Further research should also be done on the influence of water quality and system conditions on blue LED disinfection efficacy. Energy usage and cost-effectiveness of this technology must also be considered, but presently, blue LEDs are a promising area of investigation as another means of pathogen control in soilless systems.

1.4.3.3 Media Filtration

Media filtration in soilless production systems entails pumping nutrient solution through a granulated or fibrous material to capture and remove pathogens, with sand and rockwool as the
most common filter bed materials. Such filters are known to separate a variety of particulates from water, including microorganisms (Boller and Kavanaugh, 1995). Media filters can either filter inflow water before reaching the production tanks (Berkelmann, Wohanka, and Krczal, 1995) or filter the effluent from the production tanks to prevent recirculation of pathogens (Van Os et al., 2001). Filter efficacy is determined by multiple parameters, like media composition, organic load, water temperature, and buildup of debris (Arndt and Wagner, 2003). The costs incurred while running a media filter are from electricity usage by water pumps, waste removal, and replacement media (Boller and Kavanaugh, 1995). There are two main approaches to media filtration: slow or rapid media filtration.

Slow filtration is both a mechanical and biological process that uses water flow rates of between 42 and 334 L/m²h (Arndt and Wagner, 2003). Mechanical filtration occurs when particles are prevented from moving through the filter media, while biological filtration takes place when the microorganisms in the water interact with those growing on and within the media bed (Arndt and Wagner, 2003). These microorganisms, along with accumulated debris, form a layer on top of the media bed that restricts water flow and must be scraped off or backflushed with water every 4-6 weeks (Arndt and Wagner, 2003). Backflushing is when the normal flow of water is reversed to dislodge buildup. The formation of this layer is one major limitation of slow filtration, since it forms faster as the amount of suspended solids in the water increases. Effluent from aquaculture or aquaponics systems contains higher loads of particulate matter, which could pose a problem to implementing slow filtration. It should be noted that the literature did not provide any information on the maintenance and lifespan of fibrous media filters, such as rockwool or glasswool, so there could be additional problems with these materials that do not apply to granulated materials like sand. A second limitation of slow filtration is that the low water flow rates are insufficient for large soilless production systems. For large systems, a better alternative may be rapid media filtration.

Rapid media filtration handles water flow rates between 4167 and 19,792 L/m²h (Arndt and Wagner, 2003). The consequence of such a high flow rate is that debris accumulates faster and requires backflushing more often (Arndt and Wagner, 2003). New filters can be backflushed on a weekly basis, with older filters usually requiring backflushing two or more times a week (Arndt and Wagner, 2003). The main limitation of rapid media filtration is increased maintenance time and cost, compared to slow filtration. The benefit is the ability to handle the
higher flow rates needed for bigger soilless production operations. However, rapid media filters were examined less often in the literature highlighting a possible research gap. Twenty-three articles detailing media filtration in soilless production systems passed the inclusion screening, 21 of which dealt with hydroponics systems and 2 with aquaculture. Table 1.3 offers a summary of removal efficiencies for a variety of filter media types and system setups.

**Table 1.3. Media Filtration Pathogen Removal Efficacy**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Filter Material</th>
<th>Grain Size or Density</th>
<th>Flow Rate (L/m²h)</th>
<th>Filter Depth (m)</th>
<th>Efficacy (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. oxysporum f.sp. cyclaminis</td>
<td>sand</td>
<td>&lt;2 mm</td>
<td>200</td>
<td>-</td>
<td>99.9</td>
<td>Wohanka, 1995</td>
</tr>
<tr>
<td></td>
<td>rockwool</td>
<td>190 kg/m³</td>
<td>300</td>
<td>0.4</td>
<td>99.9</td>
<td>Bergstrand et al., 2011</td>
</tr>
<tr>
<td></td>
<td>glasswool</td>
<td>53 kg/m³</td>
<td>100 (300)</td>
<td>0.075</td>
<td>≥95.95 (≥46.50)</td>
<td>Van Os et al., 2001</td>
</tr>
<tr>
<td></td>
<td>rockwool</td>
<td>210 kg/m³</td>
<td>100 (300)</td>
<td>-</td>
<td>≥91.94 (≥96.91)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rockwool</td>
<td>135 kg/m³</td>
<td>100 (300)</td>
<td>-</td>
<td>≥93.31 (≥93.30)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sand</td>
<td>0-2 mm</td>
<td>100 (300)</td>
<td>-</td>
<td>≥99.92 (≥97.94)</td>
<td></td>
</tr>
<tr>
<td>F. oxysporum f.sp. lycopersici</td>
<td>glasswool</td>
<td>53 kg/m³</td>
<td>100 (300)</td>
<td>0.075</td>
<td>≥97.8 (≥95.97)</td>
<td>Furtner et al., 2007</td>
</tr>
<tr>
<td></td>
<td>sand</td>
<td>0.15-0.3 mm</td>
<td>100 (300)</td>
<td>-</td>
<td>≥97.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mineral wool</td>
<td>-</td>
<td>300</td>
<td>1</td>
<td>&gt;97</td>
<td>Lee and Oki, 2013</td>
</tr>
<tr>
<td></td>
<td>sand</td>
<td>Fine-coarse</td>
<td>1.2 L/h</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pelargonium flower break virus</td>
<td>sand</td>
<td>Rhine-sand</td>
<td>200</td>
<td>0.8</td>
<td>88.9</td>
<td>Berkelmann, Wohanka, and Krczal 1995</td>
</tr>
</tbody>
</table>
Table 1.3. (cont.) Media Filtration Pathogen Removal Efficacy

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Filter Material</th>
<th>Grain Size or Density</th>
<th>Flow Rate (L/m²h)</th>
<th>Filter Depth (m)</th>
<th>Efficacy (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. capsici</td>
<td>sand Rhine-sand</td>
<td>200</td>
<td>1</td>
<td>100</td>
<td></td>
<td>Lee and Oki, 2013</td>
</tr>
<tr>
<td>P. cryptogea</td>
<td>sand 0.3 mm</td>
<td>0.15 m/h</td>
<td>1</td>
<td>100</td>
<td></td>
<td>Calvo-Bado et al., 2003</td>
</tr>
<tr>
<td></td>
<td>sand 0.12 mm</td>
<td>300</td>
<td>-</td>
<td>100</td>
<td></td>
<td>Wohanka and Seidel, 2004</td>
</tr>
<tr>
<td>P. aphanidermatum</td>
<td>mineral wool</td>
<td>-</td>
<td>300</td>
<td>1</td>
<td>100</td>
<td>Furtner et al., 2007</td>
</tr>
<tr>
<td></td>
<td>rockwool 190 kg/m³</td>
<td>300</td>
<td>0.4</td>
<td>100</td>
<td></td>
<td>Bergstrand et al., 2011</td>
</tr>
<tr>
<td>P. oligandrum</td>
<td>sand 0.2-2 mm</td>
<td>100</td>
<td>-</td>
<td>100</td>
<td></td>
<td>Belbahri et al., 2007</td>
</tr>
<tr>
<td></td>
<td>rockwool 136 kg/m³</td>
<td>100</td>
<td>-</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. dahliae</td>
<td>sand &lt;2 mm</td>
<td>200</td>
<td>0.8</td>
<td>12</td>
<td></td>
<td>Martinez et al., 2005</td>
</tr>
</tbody>
</table>
Table 1.3. (cont.) Media Filtration Pathogen Removal Efficacy

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Filter Material</th>
<th>Grain Size or Density</th>
<th>Flow Rate (L/m²h)</th>
<th>Filter Depth (m)</th>
<th>Efficacy (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>X. campestris pv. pelargonii</td>
<td>sand</td>
<td>0.2-2 mm</td>
<td>200</td>
<td>0.8</td>
<td>83.24</td>
<td>Wohanka et al., 1999</td>
</tr>
<tr>
<td></td>
<td>anthracite</td>
<td>0.8-1.6 mm</td>
<td>200</td>
<td>0.8</td>
<td>81.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rockwool</td>
<td>136 kg/m³</td>
<td>200</td>
<td>0.8</td>
<td>98.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pumice</td>
<td>0.4-4 mm</td>
<td>200</td>
<td>0.8</td>
<td>85.06</td>
<td></td>
</tr>
<tr>
<td>X. campestris pv. pelargonii</td>
<td>sand</td>
<td>0-2 mm</td>
<td>100 (300)</td>
<td>-</td>
<td>≥99.99 (≥99.99)</td>
<td>Van Os et al., 2001</td>
</tr>
<tr>
<td></td>
<td>sand</td>
<td>0.15-0.3 mm</td>
<td>100 (300)</td>
<td>-</td>
<td>≥99.99 (≥99.91)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>glasswool</td>
<td>53 kg/m³</td>
<td>100 (300)</td>
<td>-</td>
<td>≥92.95 (≥97.73)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rockwool</td>
<td>135 kg/m³</td>
<td>100 (300)</td>
<td>-</td>
<td>≥97.85 (≥96.67)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rockwool</td>
<td>210 kg/m³</td>
<td>100 (300)</td>
<td>-</td>
<td>≥96.56 (≥93.61)</td>
<td></td>
</tr>
</tbody>
</table>

Removal efficacies greater than 90% were observed in all but four of the studies, with many over 95% (Table 1.3). With all the flow rates placing the systems in the category of slow filters, it appears that slow filtration works best against the oomycete plant pathogens of the *Pythium* and *Phytophthora* species at 100% removal efficiency (Table 1.3). Slow filters also appear to work well against the bacteria *Xanthomonas campestris*, but results appear a bit more mixed for fungal plant pathogens (Table 1.3). While all the studies examining *Fusarium oxysporum f.sp. cyclaminis* reported removal efficacies of > 90%, one of the three studies investigating *F. oxysporum f.sp. lycopersici* found that the slow sand filter did not eliminate any of the fungi (Lee and Oki, 2013). A different study on *Verticillium dahliae* found the slow sand filter only removed 12% of the fungi from the recirculating water (Martinez et al., 2005). This could be due to characteristics unique to these two fungi, but it is more likely because of differences in study setups. The grain size of the sand was not specified in Lee and Oki (2013),
so it might have been too coarse to properly filter out the fungi. Though a general grain size of  
<2 mm was reported for Martinez et al. (2005), no size distribution was given, so most of the  
sand could have been too large. These discrepancies highlight the importance of specificity when  
reporting study setups, especially for the key parameters of the disinfection system.  

Reduction of disease prevalence in a hydroponics system by media filtration was another  
area of investigation, and these findings are summarized in Table 1.4. Slow filtration was shown  
to significantly reduce disease prevalence in hydroponics systems in all but one study, though  
that single study presented conflicting results (Table 1.4). In one trial with slow sand filtration,  
Minuto et al. (2008) found no significant difference between tanks receiving filtered or unfiltered  
water, while another trial did reveal a significant difference. The other studies in Table 1.4  
determined that slow sand filtration reduces disease prevalence by at least 50%, with Garibaldi,  
Minuto, and Salvi (2004) and Minuto et al. (2005) finding that employing this filtration method  
led to a disease prevalence around one-fourth that of untreated hydroponic tanks (Table 1.4).
Table 1.4. Disease Prevalence with Media Filtration

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Filter Material</th>
<th>Flow Rate (L/m²h)</th>
<th>Grain Size or Density</th>
<th>Filter Depth (m)</th>
<th>Prevalence a (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. oxysporum f.sp. chrysanthemi</td>
<td>sand</td>
<td>200</td>
<td>&lt;2 mm</td>
<td>-</td>
<td>5.8 (20.3) b</td>
<td>Minuto et al., 2008</td>
</tr>
<tr>
<td></td>
<td>sand</td>
<td>200</td>
<td>&lt;2 mm</td>
<td>-</td>
<td>5.9 (55.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rockwool</td>
<td>200</td>
<td></td>
<td>-</td>
<td>4.3 (20.3) b</td>
<td></td>
</tr>
<tr>
<td>Pelargonium flower break virus</td>
<td>sand</td>
<td>200</td>
<td>Rhine-sand</td>
<td>0.8</td>
<td>25 (100)</td>
<td>Berkelmann, Wohanka, and Krczal, 1995</td>
</tr>
<tr>
<td>P. cactorum</td>
<td>sand</td>
<td>100-300</td>
<td>0.15-0.35 mm</td>
<td>1</td>
<td>0 (17 and 34)</td>
<td>Martinez et al., 2010</td>
</tr>
<tr>
<td>P. cryptogea</td>
<td>sand</td>
<td>200</td>
<td>&lt;2 mm</td>
<td>-</td>
<td>11.1 (22.8)</td>
<td>Garibaldi et al., 2003</td>
</tr>
<tr>
<td></td>
<td>sand</td>
<td>300</td>
<td>1.2-1.8 mm</td>
<td>-</td>
<td>5.4 (33.4)</td>
<td>Garibaldi, Minuto, and Salvi, 2004</td>
</tr>
<tr>
<td></td>
<td>sand</td>
<td>200</td>
<td>&lt;2 mm</td>
<td>-</td>
<td>5.4 (33.4)</td>
<td>Minuto et al., 2005</td>
</tr>
</tbody>
</table>

a Disease prevalence in experimental group (prevalence in the positive control group)

b Not significantly different, according to Tukey’s multiple range test (p = 0.05)

While the results presented in Tables 1.3 and 1.4 are promising, there are reporting and research gaps that need to be filled. The omission of information regarding filter bed depth, flow rate, and grain size or material density is an important issue with reporting, since this is important information for replicating experimental setups. When these details are lacking, it becomes harder to accurately compare systems and limits applicability of the results. There is also the lack of studies about rapid media filtration, which is an issue for larger aquaponics and aquaculture systems that can deal with flow rates >22,000 L/h (Rakocy, Masser, and Losordo, 2006; Rakocy et al., 2010). This is likely why all the studies in Tables 1.3 and 1.4 are from hydroponics systems, which can operate well at lower flow rates. Additional research needs to be
conducted on adapting media filtration methods to aquaculture and aquaponics and determining its effectiveness at removing pathogens from these systems.

Filter substrate and its role in pathogen removal is another factor that needs consideration. Removal efficacy of the bacterial plant pathogen *Xanthomonas campestris* was shown to be dependent on the filter material, with Wohanka et al. (1999) finding that rockwool removes 15% more of the bacteria than sand, anthracite, or pumice. In contrast, Van Os et al. (2001) found that there was no significant difference between sand, rockwool, or glasswool for elimination of *X. campestris*. A thesis written by Elizabeth Nyberg at Clemson University compared the removal efficiency of *P. nicotianae* by sand, calcined clay, crushed brick, polyethylene beads, and Kaldnes® medium at varying medium depths and a flow rate of 200-300 L/m²h. Polyethylene beads and Kaldnes® medium never exceeded a 45% removal efficiency, while the brick and clay exceeded 96% at a depth of 60 cm (Nyberg, 2011). Sand was found to be the best material, eliminating 100% of oomycetes at 40 cm and more than 98% at 20 cm (Nyberg, 2011). In another study, a filter medium bed depth of at least 60 cm was found to be optimal for slow filters containing rockwool (Wohanka et al., 1999). The subject of filter depth did not appear often in the literature, highlighting a lack of understanding of one of the key elements in the design of a media filter.

The depths required for adequate filtration depend on grain size, since this determines the pore size, with larger grains creating larger pores. A study of *M. cerebralis* showed that only 2 cm of 355-424 μm diameter sand was required to filter out the same amount of parasite as 4 cm of 425-499 μm diameter sand at a flow rate of 1920 L/h (Arndt and Wagner, 2003). In this same study, none of the rainbow trout exposed to inoculated water filtered with sand between 180 and 300 μm in diameter became infected, while 49% became diseased when exposed to water filtered with sand > 300 μm in diameter, thereby suggesting that the organism is too large to fit through the pores created by sand smaller than 300 μm (Arndt and Wagner, 2003). This relationship between grain size and disease outcomes can also be seen in a study of *Phytophthora cinnamomi* that found filtration of inoculated water through a 0.8 m filter bed of sand ≤ 0.8 mm resulted in no infections, regardless of whether the flow rate was 0.1 or 0.3 m/h (Van Os et al., 1999). The nematode *Radopholus similis* was not able to infect plants when water was filtered through sand 0.15-0.3 mm in diameter, while infections occurred with 0.2-0.8 mm sand (Van Os et al., 1999). Knowing the appropriate grain size distribution is necessary for constructing an effective media
filter, but the issue of fouling needs to be considered as well. Smaller grains create smaller pores, which clog easier and could require more maintenance. The relationship between the type of soilless production system, grain size, and fouling is largely unknown, so more research should be conducted to allow for more specific design guidelines.

Filter age, or biological activation status, has been shown to be another important factor in media filter efficacy. Kametani and Umezawa (1966) demonstrated that a filter operated for more than 10 weeks had a removal efficiency of 99.75% compared to a new filter with a maximum efficiency of 95.3%. Nyberg (2011) found that the sand filter removed a higher percentage of *P. nicotianae* zoospores on Day 21 of the experiment than on Day 0. Van Os et al. (2004) observed that hydroponics systems with old filters ended up with fewer plants infected with *P. aphanidermatum* (60%) than new filters (70%). One study did find that the effects of biological activation could be pathogen dependent. Filters exposed to a biologically activated nutrient solution eliminated significantly more *X. campestris* bacteria (98.48%) than filters exposed to sterile solution (68.79%), but no significant difference was noted for *F. oxysporum f.sp. cyclaminis* (Brand and Wohanka, 2001). These results suggest that exposing new filters to nutrient solution before use in a system could be beneficial, but how important this factor is should be further examined.

Flow rate was found to have a significant negative correlation with filter efficacy by Wohanka et al. (1999), meaning that as the flow rate increased from 100 to 300 L/m²h, the removal efficacy of *X. campestris pv. pelargonii* dropped. However, this finding was challenged by Van Os et.al (2001), who found that the filtration rates of 100 and 300 L/m²h did not have significantly different removal efficacies for the same organism. The two media filtration systems are described in Table 1.3, and it is notable that Van Os et al. (2001) did not provide the filter bed depth, which could explain the discrepancy. More studies need to be conducted in order to determine the role of flow rate on media filter efficacy so that future design of these disinfection systems can be better informed.

The success of the studies with controlling *M. cerebralis* with media filtration and the lack of others in the fields of aquaculture and aquaponics highlight that this control method has unexplored potential. Media filters are cheap and easy to construct, relatively simple to maintain, and do not introduce chemicals into the system. One difficulty in employing media filtration technology, especially in aquaculture and aquaponics, is fouling from solid waste. Periodic
backflushing of the medium can remove waste and clarifying technologies can be employed prior to the filter unit, but the extent of media filtration’s practical application is unknown outside of hydroponics. Due to the economic risks associated with commercial soilless production systems, the focus of future research should be to determine if, and under what conditions, media filtration can be used to control disease in aquaculture and aquaponics.

1.4.3.4 Membrane Filtration

Membrane filtration involves forcing water through a woven or spun material that retains any matter larger than its pore size, with microfiltration membranes rejecting particles >0.1 µm, ultrafiltration rejecting particles >0.01 µm, nanofiltration retaining particles >1 nm, and membrane-reverse osmosis blocking materials >0.1 nm (Lekang, 2013). The membrane can be as simple as a sheet stretched over an opening, or a series of cartridges or bags, and is typically located before the production tanks (Ohtani et al., 2000; Schuerger and Hammer, 2009). Water is pumped into the filtration unit and all materials smaller than the pores move through the membrane and flow into the rest of the system, while larger materials are rejected and removed (Lekang, 2013). Filters can be made from many different materials, such as spiral wound cellulose (Liu, Lau, and Lo, 2008) or polyester felt bags (Moen's and Hendrickx, 1992). Prefilters are often used to remove much larger solid materials before reaching the membranes with smaller pores, which can foul easily. The two studies in which prefiltration was mentioned used filters of 10 µm (Ohtani et al., 2000) and 100 µm (Schuerger and Hammer, 2009). Seven sources passed the inclusion screening, all for hydroponics systems. Membrane filtration was shown to be effective against a range of pathogens, with the pore size or molecular weight cutoff being the key factor for filter efficacy. Table 1.5 shows the results of studies conducted with various filter sizes.
Table 1.5. Membrane Filter Pore Size for \( \geq 99\% \) Efficacy

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Size a</th>
<th>Filter Type</th>
<th>Flow Rate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. incognita</td>
<td>150-25-0.5 µm b</td>
<td>cartridge</td>
<td>1000 L/h</td>
<td>Moens and Hendrickx, 1992</td>
</tr>
<tr>
<td>P. solanacearum</td>
<td>0.3 µm (10 µm)</td>
<td>membrane</td>
<td>45.83 L/h</td>
<td>Ohtani et al., 2000</td>
</tr>
<tr>
<td>P. aphanidermatum</td>
<td>5 µm (20 µm)</td>
<td>cartridge</td>
<td>-</td>
<td>Tu and Harwood, 2005</td>
</tr>
<tr>
<td>P. oligandrum</td>
<td>150,000 Da</td>
<td>membrane</td>
<td>-</td>
<td>Belbahri et al., 2007</td>
</tr>
<tr>
<td>R. similis</td>
<td>150-25-1 µm b</td>
<td>cartridge</td>
<td>1000 L/h</td>
<td>Moens and Hendrickx, 1992</td>
</tr>
<tr>
<td>Tomato mosaic virus</td>
<td>30,000 Da</td>
<td>cartridge</td>
<td>400 L/m²h</td>
<td>Liu, Lau, and Lo, 2008</td>
</tr>
<tr>
<td></td>
<td>5,000 Da</td>
<td>cartridge</td>
<td>30 L/m²h</td>
<td></td>
</tr>
</tbody>
</table>

a prefILTER size in parentheses  
b Investigated a series of cartridges, with the first having a pore size of 150 µm, and so on

Table 1.5 shows that membrane filtration is effective at removing a variety of pathogens. While an efficacy of \( \geq 99\% \) was achieved in each study, the membrane pore sizes ranged from 150 µm to 5 kDa due to the size differences between the pathogens (Table 1.5). Four of the seven systems employed at least one prefILTER for the purpose of reducing fouling, which can be a major problem with membrane filters, especially as pore size decreases (Table 1.5). A study by Schuerger and Hammer (2009) found that using single filters with pore sizes as small as 1 µm still resulted in at least half of the plants having disease symptoms but pairing these 1 µm membranes with another membrane of 4.5 µm or less led to zoospore removal of \( >99.5\% \). Goldberg, Stanghellini, and Rasmussen (1992) showed that filtering water through a 20 µm filter, then a 7 µm filter, greatly slowed the onset of infection by \( P. \) aphanidermatum (11 and 14 days) in comparison to filtration through a single 20 µm filter (7 and 9 days). When the filters were tested, no zoospores were found in the inner core of the 7 µm filter, suggesting that all zoospores were removed from the nutrient solution and infection in the dual filter system occurred due to transmission via shore flies (Goldberg, Stanghellini, and Rasmussen, 1992). These results indicate that the setup of membrane filtration units, with filters alone or in series,
should be further investigated to determine what arrangement is best in terms of economics and system health.

Backflushing, a concept introduced in the section on media filtration, is also relevant to membrane filtration. When the membrane filter traps particulate matter, it begins to form a layer of debris that builds up over time (Lekang, 2013). This layer blocks water flow and raises the pressure difference across the membrane, which can cause damage (Lekang, 2013). Backflushing is a physical removal method that is accomplished by forcing water in the opposite direction of normal flow, but this does not help with biofilm formation or the accumulation of mineral scaling (Lekang, 2013). Chemicals like hydrogen peroxide can be applied to the membranes as antifouling treatments to reduce or slow the buildup of the filter cake, but this introduces potentially toxic compounds to sensitive systems (Lekang, 2013). Chemicals can also be used during backflushing to improve its effectiveness, but raises the same problem (Lekang, 2013). The containment and removal of these chemicals must therefore be considered if utilized in aquaculture, hydroponics, or aquaponics systems. The frequency at which backflushing should occur is another matter of debate. In the study by Ohtani et al. (2000), backflushing was performed every four hours, although the effects of this practice on membrane performance were not examined. Future research should investigate different approaches to membrane maintenance in order to make the disinfection method more economical, safer for fish and plants, and less laborious.

The impact of membrane composition on filter efficacy at different pore sizes is another area that requires more research in soilless production systems. Possible filtration materials range from plastic polymers to metals and ceramics, with each material having a certain cost and effectiveness. The optimal membrane materials for application in aquaponics are unknown, since the pairing of fish with plants introduces additional variables and system conditions. Further studies need to be conducted to determine what kinds of filters can handle *in vivo* conditions, what their ideal operating conditions are, filter lifetime, and limitations of this disinfection method.
1.4.3.5 Heat

Heat denatures the proteins of pathogens, rendering them harmless. Effluent water from the production tanks is pumped into a separate tank, heated to the desired temperature for a specified amount of time, and cooled for return to the production tanks (Runia and Amsing, 2001). Nine articles about heat treatment qualified for inclusion, with five for hydroponics systems and four for aquaculture. It should be noted that seven of the nine articles conducted experiments exclusively in laboratory settings, with only Runia and Amsing (2001) and Rey et al. (2001) investigating the in vivo application of heating for disinfection in hydroponics systems. Heat is effective for inactivating both fish and plant pathogens in water, but the temperatures and exposure times required varies widely between organisms, as can be seen in Table 1.6. If a study investigated more than one temperature, only results for the lowest temperatures with high inactivation rates were included, since lower temperatures are more desirable due to energy costs.
Table 1.6. Heat Treatment for ≥ 99.9% Pathogen Inactivation

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Temperature (C)</th>
<th>Time (min)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. anguillarum</td>
<td>44</td>
<td>3</td>
<td>Jacobsen and Liltved, 1988</td>
</tr>
<tr>
<td>Pythium spp.</td>
<td>95</td>
<td>0.5</td>
<td>Rey et al., 2001</td>
</tr>
<tr>
<td>P. aphanidermatum</td>
<td>45</td>
<td>8</td>
<td>Tu and Zhang, 2000</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>F. oxysporum</td>
<td>80</td>
<td>0.75</td>
<td>Runia and Amsing, 2001</td>
</tr>
<tr>
<td>R. similis</td>
<td>48</td>
<td>5</td>
<td>Runia and Amsing, 2001</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>52.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Tomato mosaic virus</td>
<td>81</td>
<td>2.75</td>
<td>Runia and Amsing, 2001</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>87</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Infectious pancreatic necrosis virus</td>
<td>60</td>
<td>300</td>
<td>MacKelvie and Desautels, 1975</td>
</tr>
<tr>
<td>Infectious pancreatic necrosis virus</td>
<td>80</td>
<td>5</td>
<td>Nygaard, Modahl, and Myrmel, 2012</td>
</tr>
<tr>
<td>Koi herpesvirus</td>
<td>≥ 50</td>
<td>1</td>
<td>Kasai, Muto, and Yoshimizu, 2005</td>
</tr>
</tbody>
</table>

A notable trend in Table 1.6 is that the higher the temperature, the shorter the time it takes to completely inactivate Pythium oomycetes, tomato mosaic virus, and R. similis. 95°C is a common disinfection temperature for this reason, but several studies showed that lower temperatures are sufficient without significant increases in treatment duration (Table 1.6). This opens up possibilities for making heat treatment more energy efficient and less demanding on the equipment. There was not enough data to make more specific conclusions, which means more research needs to be conducted to better determine the efficacy of heat treatments and inactivation rates for a wider range of fish and plant pathogens.

Instead of directly looking at pathogen inactivation, two studies looked at health outcomes for the plants and fish. Arimoto et al. (1996) found that heating the water in which striped jack larvae were kept to 60°C significantly improved survival rates when the larvae were exposed to striped jack necrosis virus. Albright et al. (2007) examined the impact of heat
treatment on *P. aphanidermatum* in a hydroponics system and found that heating the water to 60°C for 1 minute reduced root damage due to infection by > 95%. It can be extrapolated that had the researchers measured pathogen levels in the nutrient solutions, inactivation of these pathogens would have been observed.

*In vivo* application of heat treatment in soilless production systems is the main topic lacking information. The two studies in hydroponics systems showed that this technology could be beneficial to the field (Runia and Amsing, 2001; Rey et al., 2001). Whether heat would be an effective disinfection method in systems containing fish is currently unknown. The ability to kill both fish and plant pathogens without introducing harmful elements into the water would be useful in aquaponics and could also serve as a means of controlling the water temperature. These promising results indicate that heat treatment should be researched further as a disinfection method in soilless production systems.

1.4.3.6 Sonication

Sonication, in the context of soilless production systems, involves the application of high frequency waves to the influent nutrient solution as a means of inactivating pathogens before entry into the production tanks (Bazyar Lakeh et al., 2013). A machine is used to generate these waves, usually 20-40 kHz, which are transmitted to the water through a probe (Albright et al., 2007). These waves induce the formation of low-pressure pockets inside cells and cause their collapse in a process called cavitation (Albright et al., 2007). The use of sonication as a disinfection method is relatively new and only five studies passed the inclusion criteria. Two articles dealt with aquaculture systems and three with hydroponics.

In laboratory studies, it was found that waves with a frequency of 20 kHz and amplitude of 120 μm were optimal for destroying *P. aphanidermatum* in solution (Misonix; Albright et al., 2007; Tu and Zhang, 2000). Sonication at these settings reduced damage to plant roots from this oomycete by 100% when paired with a flow rate of 200 mL/min (Albright et al., 2007) and inactivated 100% of *P. aphanidermatum* zoospores in 150 mL of solution after 1.5 min (Tu and Zhang, 2000). Though sonication effectively inactivates this major plant pathogen *in vitro*, the small volumes raise the question of applicability to soilless production systems that use thousands of liters.
In vivo studies conducted with aquaculture systems had mixed results regarding the effectiveness of sonication against parasitic worms. Sonication at 25 kHz was shown completely ineffective against Anguillicoloides crassus (Bayzar Lakeh et al., 2013), while sonication at 20 kHz (Hielscher) reduced prevalence of Bucephalus polymorphus infection from 76.3% in the control to 26.4% in fish exposed to treated water (Wolber and Pietrock, 2004). The drastic difference in the effectiveness of sonication against these two species could be due to the different frequencies used and biological variability. This variability, between species and pathogen types, could determine whether sonication is a practical disinfection option.

Combination with another disinfection method is a potential solution to the limited effectiveness of sonication. Tesoriero (2008) found that a combined sonication-UV unit was 100% effective against P. aphanidermatum and F. oxysporum, and eliminated 97% of Thielaviopsis basicola at a water flow rate of 300 mL/s. However, since no information regarding the UV dose or sonication frequency was provided, the benefits of combining the technologies cannot be properly assessed. Results from the study by Bazyar Lakeh et al. (2013) showed that sonication at 25 kHz, when combined with UV doses of 7.3 and 40 mJ/cm², was not significantly more effective against Anguillicola crassus than UV treatment alone. This calls into question whether combining UV and sonication is worthwhile, but further studies should be conducted to provide a more definite conclusion. More research needs to be done on the overall efficacy of sonication alone, and what frequencies and exposure times would be necessary to make it effective. Improvements need to be made for scaling up the technology, so it can be better applied in vivo, and the economic benefits of disinfection need to be evaluated so growers can make informed decisions regarding the use of sonication as a water disinfection technique.

1.4.3.7 Alternative Disinfection Methods

While most of the studies that passed the inclusion screening fit into the defined categories of disinfection, there were three articles that did not. Two dealt with alternative means of disinfection in aquaculture and one in hydroponics. Shinn et al. (2009) proposed a method of mechanically removing the parasite Ichthyophthirius multifiliis from the bottom of fish raceways using a suction device. A field trial at a farm raising rainbow trout found that vacuuming the bottom of these canals increased the survival of the fish from 70.6% in the control to 84.5% in the experimental group over 3 months (Shinn et al., 2009). Though this makes intuitive sense,
since the parasite is being physically removed from the system, issues such as stress in the fish, cost, and potential problems with the device itself should be examined.

McVicar and White (1982) researched a possible control method for a fish disease called black patch necrosis. At the time of publication, the etiological agent of the disease was unknown, but it was later discovered to belong to the *Flavobacterium* genus, most likely *F. maritimus* (Bernardet et al., 1990). McVicar and White (1982) proposed the use of sand in the fish rearing tanks to prevent and cure infections, and 20 mm of fine-grained sand was placed in the bottom of experimental tanks at either the beginning of the experiment, or after several days. When all tanks were challenged with an infected Dover sole (*Solea solea*), tanks that contained sand during the whole experimental period experienced very low mortalities (<2.6%) compared to control tanks (>92%) (McVicar and White, 1982). Tanks that received sand later experienced a cease in mortalities six days after the sand was added, with cumulative mortality plateauing at 17.3% (McVicar and White, 1982). This offers evidence that the presence of sand prevents the transmission of the bacterium, though how is unknown. Further research should be conducted to evaluate this simple solution as a potential alternative to medication for treating black patch necrosis.

In the article focusing on hydroponics, Albright et al. (2007) investigated temperature reduction as a means of curing *P. aphanidermatum* outbreaks. A severe *P. aphanidermatum* infection was established in a spinach hydroponics facility before the water temperature was decreased from 27°C to 20°C (Albright et al., 2007). The spinach crop was shown to almost completely recover from the outbreak and ended up producing at least as much as the negative control systems (Albright et al., 2007). Production in the inoculated system at 27°C remained around zero for the duration of the experiment (Albright et al., 2007). When this method was attempted with lettuce, the results were not as favorable, with production returning to pre-infection levels, then dropping to around 50% (Albright et al., 2007). However, this could have been due to the addition of another, unidentified, stressor. These findings are in line with what is known about growth rates of *P. aphanidermatum*. Gold and Stanghellini (1985) demonstrated that the oomycete grows faster as the temperature increases, so the temperature reduction from 27°C to 20°C in Albright et al. (2007) may have slowed down the growth rate and allowed the relatively fast-growing spinach crops to move through the system without major contamination. Albright et al. (2007) also found that reducing the time crops spent in the system eliminated root
damage, supporting the idea that decreasing exposure time reduces the impact of disease. More research needs to be put into temperature reduction and crop rotation duration to confirm and possibly extend these findings to other systems.

These articles show the benefit of creative thinking and simple solutions when dealing with pathogen control in soilless production systems. Using sand instead of medications offers a cheap, safe means of dealing with an outbreak of black patch necrosis and mechanical removal of parasites from the bottom of raceways opens possibilities for fish tank designs that would streamline this process. Temperature reduction and shortening crop rotations offer basic methods of handling a common and persistent plant pathogen without decreasing production. The findings of these studies should be replicated, and more effort needs to be put into devising innovative means of pathogen control in soilless production systems.

1.5. Conclusion

This review examined the literature available on waterborne transmission of fish or plant pathogens in soilless production systems - aquaculture, aquaponics, and hydroponics - and physical disinfection or filtration methods employed to control these pathogens. In the one-hundred and forty sources containing primary research on these subjects, none addressed aquaponics. Ultraviolet disinfection and media filtration were the most commonly studied, with blue light-emitting diodes and sonication being the least studied. Heat, membrane filtration, and alternative methods were also examined. The major benefit of using physical disinfection methods was that none of those examined produced harmful byproducts, a disadvantage of some common chemical treatments.

Ultraviolet irradiation was shown to be an effective water disinfection method. The presence of suspended solids in the water impeded performance but practicing prefiltration mitigated this problem. The cost of installing, operating, and maintaining a UV system is the main disadvantage, though these costs may be offset by preventing production losses due to disease outbreaks. Blue LEDs had similar costs and benefits as UV irradiation, with the additional benefit of allowing placement of the LEDs directly over the tank. The limitation of blue LEDs is lack of research, since it is such a new technology. Media filtration was also demonstrated to eliminate fish and plant pathogens from the nutrient solution with a simple and cost-effective setup. However, most of the studies were for slow media filtration, which cannot
handle the water flow rates typically used in aquaponics or aquaculture. Though rapid media filtration is an alternative that can function with high flow rates, there is little research on its application in soilless production systems.

Membrane filtration, though affected by flow rate, relies more on the composition of the water, which impacts filter design and the speed of filter cake formation. The cost of these filtration systems varies depending on the number and type of filters, but the specificity of the membrane pore size is the greatest limitation of this method. The smaller the pathogens that need to be removed, the smaller the pore size, and clogging increases as the pore size shrinks. Backflushing can reduce this problem, though, and the simplicity of operation and allowance for customization offers many benefits (Lekang, 2013). Heat was able to inactivate some fish and plant pathogens from water, but most of the studies were conducted in vitro, so the practicality of this water treatment method in soilless production systems is still questionable. The cost of the equipment and its operation may be prohibitive depending on the volume of water contained in the system, and the time required for disinfection might introduce a limitation for flow rate that is below the acceptable level.

Sonication also had questionable applicability to soilless production systems. Though certain frequencies were able to inactivate some of the pathogens examined in the literature, the findings from in vivo studies were mixed in their support for sonication. Whether the technology can be scaled up and made more effective has yet to be concluded. The alternative disinfection methods showed the benefits of unique approaches to the problem of pathogens in soilless production systems. By thinking outside the box, disinfection methods can be invented that are effective, easy, and inexpensive. Overall, the in vivo application of these pathogen control methods needs to be further studied, with a focus on use in aquaponics.
Chapter 2

Predictive modeling of pH in an aquaponics system using Bayesian and non-Bayesian linear regression to inform system maintenance

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2.1. Abstract

Aquaponics - the farming of fish and plants in a soilless system - is a growing niche industry ideal for urban environments and water stressed regions. These systems can be a good source of fresh food but must maintain a delicate balance between the water quality requirements of the fish, plants, and nitrifying bacteria, or risk decreased production, disease, and death. One of the most important water quality parameters is pH, which should be maintained between 6.4 and 7.4 to meet the needs of all three organisms. However, pH is often unstable and must be continually monitored and adjusted. The general method for pH maintenance is a triage approach of measurement, diagnosis of a range violation, and application of either an acidic or basic compound to lower or raise the pH. A consequence of this approach can be overcompensation when adding corrective chemicals or system shock from too sudden of a shift in pH. This could have negative effects on the health of all the organisms in the system, so a better alternative would be to predict pH values in advance and slowly add the necessary balancing compounds over a longer period of time. In order to predict pH in an aquaponics system, we conducted both traditional linear regression and Bayesian linear regression using aquaponics water quality data to develop a predictive model. It was found that the pH values measured one and two days prior to the target date could predict the pH in an aquaponics system with an adjusted $R^2$ of 0.74 and a
root mean square error of 0.15. The sensitivity and specificity of the predictions for range violations were 0.68 and 0.99, respectively. A web application was developed to host this model, as well as provide options for basic data analysis and visualization.

2.2. Introduction

Aquaponics is the coculture of plants and fish in a system that recirculates water. This is a cyclical system in which the fish produce waste that is broken down by nitrifying bacteria, which is then used as food by the plants. These aquaponics systems can be constructed in almost any environment, making them prime candidates for farming in urban areas and places experiencing water stress (Kyaw and Ng, 2017; Vermeulen and Kamstra, 2013). These systems have a high startup cost (Love et al., 2015), but they, in theory, use less water, electricity, and fertilizers than traditional agricultural systems. Though the body of research on this subject is growing, our understanding of water quality parameter dynamics remains limited.

In aquaponics, the driving force behind fish, plant, and bacteria health is the water quality. Important parameters include pH, carbonate hardness (kH), water temperature, dissolved oxygen (DO), and concentrations of ammonia species (Sallenave, 2016). These are measured with a variety of methods, ranging from test strips to automatic continuous sensors located within the system (personal communication), at frequencies ranging from continuous to once a week. The choice of method and measurement schedule is based on equipment and labor costs, as well as ease of use, resulting in high between-system variability. In general, all aquaponics systems monitor their water quality regularly for proper system health. Water chemistry shifts, primarily in the ammonia species levels and pH, are caused by the bacterial nitrification of the fish waste, which is an acidifying process. Over time, the pH of the system drops, and interventions are needed via the application of a base, typically calcium carbonate or potassium bicarbonate (Sallenave, 2016). This buffers the system by raising the kH, which in turn stabilizes the pH within the appropriate range (Sterling, 2020). Most aquaponics farmers adjust water chemistry using a daily triage method, adding base when necessary. This approach runs the risk of shocking the system with too dramatic of a pH shift and resulting in toxic levels of ammonia that are lethal to fish (Sallenave, 2016). Therefore, it is vital to monitor pH daily to avoid such large shifts (Sallenave, 2016).
There has been research in aquaponics on the subject of water chemistry monitoring approaches, (Kyaw and Ng, 2017; Guerrero and Edwards, 2013) as well as nutrient dynamics (Cerozi and Fitzsimmons, 2017) and the impact of pH on different system processes (Cerozi and Fitzsimmons, 2016). It has been shown that an increase in pH leads to a decrease in phosphorus availability to the plants, which makes it important to maintain pH in the recommended range to facilitate healthy plant growth (Kaewwiset and Yooyativong, 2017). Fluctuations in pH also directly affect the ratio of toxic to non-toxic ammonia, as well as the rate of nitrification in the system (Sallnave, 2016). A linear regression with data collected from automatic sensors in a hydroponics system allowed prediction of pH with an accuracy of 95% based on the parameters of pH lagged by one time step and the amount of nitric acid added to adjust pH (Kaewwiset and Yooyativong, 2017). Highly accurate predictions of pH were also accomplished with a neural network that had one layer of nine hidden states and used a quasi-Newton backpropagation algorithm (Ferentinos, Albright & Scott, 2000). However, there are currently no such models for aquaponics systems.

This project proposes to develop a predictive model for pH in aquaponics systems to forecast pH shifts and allow for a slower pH adjustment. The model will incorporate data from two different aquaponics systems to enhance its external validity and widen the applicability of the findings. Constructing this model, and the associated examination of the relationships between water quality parameters, will further our knowledge about water quality dynamics in aquaponics and provide a stepping stone for future modeling of these systems.

2.3. Materials and Methods

Water quality data were acquired from two educational aquaponics systems at Loyola University in Chicago, Illinois between January 1, 2016 and December 31, 2018. One system used deep water culture and nutrient film technique to grow lettuce and basil, while also raising tilapia. The other system grew a variety of herbs in a vertical arrangement of flood and drain media beds, with a fish tank containing koi located at ground level. The tilapia system was on the second floor of the greenhouse, and the koi system was in the lobby. The water from both systems was recirculated and heated by the ambient air. The measured variables were pH, temperature, dissolved oxygen (DO), ammonium (NH₄⁺), nitrate (NO₃⁻), nitrite (NO₂⁻), and kH. Arduino sensors were used for pH, temperature, and DO, while ammonium, ammonia, and nitrite
were measured with a manual Vernier probe (Vernier Software & Technology, Beaverton, OR). kH was measured with API brand color test strips (Mars Incorporated, McLean, VA). Data were collected daily on temperature, pH, and dissolved oxygen, while kH, ammonium, nitrate, and nitrite were measured weekly. In addition, the daily amount of feed provided to the fish was recorded. This was a volumetric measurement using a standard measuring cup. Two separate systems were examined, with the first being a deep-water culture setup with tilapia, basil, and lettuce. This was used as the training system, while the media bed system with koi and a variety of herbs was used as the testing system. Both systems were on different floors of the same building.

Once the data were obtained from Loyola University, they were cleaned and analyzed using the R platform (v.4.0.2). Impossible or improbable values were removed - for example, a pH outside the range of 0 to 14 - and summary statistics were calculated for each variable. Table 2.1, shown below, provides the healthy ranges for these water quality parameters in a tilapia aquaponics system. A correlation analysis was conducted to examine relationships among the water quality parameters.

### Table 2.1. Aquaponics Water Quality Parameter Ranges

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Temp (F)</th>
<th>DO (ppm)</th>
<th>NO₂⁻ (ppm)</th>
<th>NO₃⁻ (ppm)</th>
<th>NH₄⁺ (ppm)</th>
<th>kH (dKH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>6.4</td>
<td>65</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>2.8</td>
</tr>
<tr>
<td>Maximum</td>
<td>7.4</td>
<td>85</td>
<td>10</td>
<td>1</td>
<td>150</td>
<td>1</td>
<td>5.6</td>
</tr>
</tbody>
</table>

¹Sallenave (2016)

Following the preliminary data analysis, the data values for pH, temperature, and DO were lagged one to seven days in order to account for time-delayed effects. The other variables were not lagged, since they were already measured at one week intervals, and it was not felt that two weeks was biologically relevant to predicting future pH values. After lagging the data, a model of all the original and lagged variables was constructed, and a procedure using the bootStepAIC function in R (v.1.2.0) was utilized for model selection. The two models with the highest adjusted R² and lowest Akaike’s information criterion (AIC) scores were selected for further analysis.
A second modeling approach using Bayesian linear regression was also employed to examine the stability of the models to fitting assumptions. The same lagged data and full model were used as the initial input, and the bas_lm (v.1.5.5) function was used to perform a Bayesian fitting for potential regression models using Markov chain Monte Carlo (MCMC) and Bayesian adaptive sampling. The MCMC was run for 10,000 iterations and the prior for the models was set at “uniform” to give all possible models an equal weight. The prior distribution was selected with the “AIC” procedure. As with the original linear regression models, the two models with the highest adjusted $R^2$ and lowest AIC scores were chosen for additional analysis.

The goal of this analysis was to select the model that best predicts pH values, preferably one with an adjusted $R^2$ of $\geq 0.9$. The ability of each of the four models to accomplish this was assessed using the predict.lm (v.2.3.1) function. The models trained on the data from the tilapia aquaponics system were used to predict values in the koi system. To further evaluate the model, the koi data was used to train the model, with the tilapia data used for testing to see if there would be a different outcome in model selection. The choice to train with data from one system and test on data from a separate system was made to maximize the amount of information used in the model development process. This decision assumed that the relationships between the variables of interest are independent of the system structure and types of crops grown. The dynamics between pH, temperature, kH, dissolved oxygen, nitrite, nitrate, and ammonium are driven by nitrification, which is a process that is carried out the same way, with the same results, regardless of the system configuration. It was therefore felt that data could be used from systems that varied in their design, but not in their function, especially given that the two aquaponics setups in question were in the same building. An additional benefit of this training and testing procedure was to prevent the model from being too site-specific, which would limit the applicability of our findings beyond the systems studied.

Once trained and tested, the model with the highest accuracy and lowest root-mean squared error (RMSE) was then selected as the overall best model to predict pH in an aquaponics system with the available variables. The sensitivity (Equation 2.1) and specificity (Equation 2.2) were also calculated for the chosen models to evaluate their ability to correctly categorize healthy range violations.
Sensitivity = true positives / (true positives + false negatives) \hspace{1cm} (2.1)
Specificity = true negatives / (true negatives + false positives) \hspace{1cm} (2.2)

The selected best model was then incorporated into a web application, developed in RShiny (v.1.5.0), that allows users to predict the pH of their systems, as well as perform basic data analysis and visualization functions. The link to this web application is provided in the Supplementary Materials.

2.4. Results

Table 2.2 provides the summary statistics for each of the water quality variables measured in the aquaponics systems. The percentage of time spent in the ideal ranges is also included.

<table>
<thead>
<tr>
<th>Measure</th>
<th>pH</th>
<th>Feed</th>
<th>Temp</th>
<th>DO</th>
<th>NH$_4^+$</th>
<th>NO$_2^-$</th>
<th>NO$_3^-$</th>
<th>kH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min</td>
<td>5</td>
<td>0</td>
<td>50</td>
<td>0.7</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Q1</td>
<td>6.4</td>
<td>1.9</td>
<td>68</td>
<td>5.3</td>
<td>0.25</td>
<td>0</td>
<td>55.75</td>
<td>2</td>
</tr>
<tr>
<td>Median</td>
<td>6.7</td>
<td>2.25</td>
<td>71</td>
<td>6.2</td>
<td>0.25</td>
<td>0</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>Mean</td>
<td>6.66</td>
<td>2.53</td>
<td>71.3</td>
<td>6.2</td>
<td>0.39</td>
<td>0.09</td>
<td>102</td>
<td>2.3</td>
</tr>
<tr>
<td>Q3</td>
<td>6.9</td>
<td>3</td>
<td>75</td>
<td>6.9</td>
<td>0.5</td>
<td>0.25</td>
<td>150</td>
<td>3</td>
</tr>
<tr>
<td>Max</td>
<td>7.7</td>
<td>5.4</td>
<td>100</td>
<td>13</td>
<td>2.8</td>
<td>2</td>
<td>180</td>
<td>6</td>
</tr>
<tr>
<td>Time Spent in Ideal Range (%)</td>
<td>74.6</td>
<td>NA</td>
<td>91.5</td>
<td>80.4</td>
<td>95.5</td>
<td>99.5</td>
<td>76.2</td>
<td>30.2</td>
</tr>
</tbody>
</table>

Most of the parameters have minimums and maximum values outside the healthy range for a tilapia aquaponics system, as defined by Table 2.1. For kH, only 30.2% of measured values fell within the optimal range. The results for pH indicate that there are sizable fluctuations in this variable that enter into unsafe territory. The temperature has a wide range as well, likely due to the fact that these systems were not heated in the winter and relied on ambient building
temperature. Dissolved oxygen dipped well into the hypoxic zone at its lowest point but remained above 5 ppm from the first quartile onward. The statistical summary reports a maximum DO level of 13, which is possible, but unlikely given these water temperatures and biological demands. Ammonium (NH$_4^+$) and nitrite (NO$_2^-$) typically remained below their thresholds of 1 ppm but can exceed that by a notable amount. Nitrates (NO$_3^-$) can both drop too low for proper plant health and raise too high for proper fish health. Overall, though, this system usually falls within the range of a normal, healthy aquaponics system, with all but the kH spending more than 74% of the time in the ideal range. The low value for kH suggests there may be an issue with carbonate hardness in the system.

As can be seen in Table 2.3, the strongest correlation is a negative relationship between dissolved oxygen and fish feed. This makes sense, given that increased fish feed raises the biological oxygen demand of the system and depletes the DO through absorption by the nitrifying bacteria and fish. There is a negative correlation between NO$_2^-$ and DO, since higher levels of NO$_2^-$ indicate increased bacterial activity and oxygen depletion. The relationship

<table>
<thead>
<tr>
<th></th>
<th>feed</th>
<th>pH</th>
<th>Temp</th>
<th>DO</th>
<th>NH$_4^+$</th>
<th>NO$_2^-$</th>
<th>NO$_3^-$</th>
<th>kH</th>
</tr>
</thead>
<tbody>
<tr>
<td>feed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>-0.021</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp</td>
<td>-0.291</td>
<td>0.160</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO</td>
<td>-0.610</td>
<td>0.103</td>
<td>0.434</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>-0.124</td>
<td>-0.027</td>
<td>-0.188</td>
<td>0.125</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO$_2^-$</td>
<td>0.485</td>
<td>-0.081</td>
<td>-0.261</td>
<td>-0.460</td>
<td>-0.215</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>0.136</td>
<td>-0.183</td>
<td>-0.137</td>
<td>0.046</td>
<td>0.586</td>
<td>-0.106</td>
<td></td>
<td></td>
</tr>
<tr>
<td>kH</td>
<td>0.112</td>
<td>-0.281</td>
<td>-0.455</td>
<td>-0.159</td>
<td>-0.154</td>
<td>-0.051</td>
<td>0.124</td>
<td></td>
</tr>
</tbody>
</table>
between temperature and kH is also moderately negative, though the reason for this is not readily apparent. NO\textsubscript{2}\textsuperscript{−} and temperature, feed and temperature, pH and kH, and NO\textsubscript{2}\textsuperscript{−} and NH\textsubscript{4}\textsuperscript{+} all have weakly negative correlations as well. Weak positive correlations were observed between temperature and DO, which seems counterintuitive given that DO decreases with increased water temperature. There may be some underlying dynamic related to the biological activity of the system that drives this positive correlation. Fish feed and NO\textsubscript{2}\textsuperscript{−} also have a positive correlation, as do NO\textsubscript{3}\textsuperscript{−} and NH\textsubscript{4}\textsuperscript{+}, due to fish feed being an indirect addition of ammonia to the system, and each ammonia species being related via water chemistry and ionization. The main takeaway from this analysis is that pH has no strong correlators, so this analysis does not provide any additional information for our modeling building process.

After conducting the bootstrapped linear regression model selection procedure, models 1 and 4 were produced (Table 2.4). The Bayesian linear regression modeling yielded models 2 and 3. The RMSE and Nash Sutcliffe Efficiency (NSE) are provided (Table 2.4). The NSE is a measure that compares simulated to observed values to determine the goodness of fit of a model to the data. Possible NSE values range from negative infinity to 1, with 1 equaling a perfect model fit. The numbers next to the variable names indicate how many days the values were lagged.

<table>
<thead>
<tr>
<th>Model</th>
<th>RMSE</th>
<th>NSE</th>
<th>Fitting Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH\textasciitilde pH1+PH2</td>
<td>0.15</td>
<td>0.74</td>
<td>Linear Regression</td>
</tr>
<tr>
<td>pH\textasciitilde feed1+PH1+PH2</td>
<td>0.187</td>
<td>0.682</td>
<td>Bayesian Regression</td>
</tr>
<tr>
<td>pH\textasciitilde feed1+PH1+PH2+DO2</td>
<td>0.196</td>
<td>0.65</td>
<td>Bayesian Regression</td>
</tr>
<tr>
<td>pH\textasciitilde feed1+feed3+feed7+Temp1+PH1+PH2+DO2</td>
<td>0.22</td>
<td>0.563</td>
<td>Linear Regression</td>
</tr>
</tbody>
</table>

The chosen model, shown in bold, was selected based on having the smallest RMSE and highest NSE. This means that the average deviation of the predictions from the actual values was
within 0.15, which is a reasonable level of error for pH in an aquaponics system, given that deviations this small do not have serious impacts on system health. The regression coefficients and standard errors are shown in Table 2.5.

**Table 2.5. Linear Regression Coefficients**

<table>
<thead>
<tr>
<th>Measure</th>
<th>pH1</th>
<th>pH2</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>0.6626</td>
<td>0.23797</td>
<td>0.67193</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.0526</td>
<td>0.05259</td>
<td>0.18252</td>
</tr>
</tbody>
</table>

*Figure 2.1. Actual vs. Predicted Values*

Actual pH measurements from the koi systems (x axis) plotted against the predicted values (y axis). The points will cluster around the solid line if the predicted values are close to the actual values.

The slope of Figure 2.1 was 1.03 and the intercept was -0.24, indicating that the predicted values were a close match to the actual measurements. In order to see if the time series nature of
the data were introducing error into the regression results, the residuals of the chosen model were plotted over time, and a loess regression line was fit to the output, shown as a solid line (Figure 2.2). The relatively horizontal nature of the solid fit line shows that there is minimal effect of seasonality on the regression outcome. It can therefore be concluded that time is not a significantly influential factor in this model and time series regression is not necessary.

*Figure 2.2. Residuals Plot Over Time*

Residual values (y-axis) for the “pH–pH1+pH2” model plotted over time (x-axis) to see if time of year has any impact on model outputs. The gray shading around the solid line is the confidence interval of the model fit.

The sensitivity of the predictions for range violations was 0.68 and the specificity was 0.99. This means that the model is very good at classifying values within the safe range but is not as good at identifying range violations. Particularly, the model is more likely to falsely classify a range violation as being in the safe range than it is to classify a safe value as a violation. However, since the RMSE is low, the severity of these misclassifications is minimal, with the
model catching all the larger pH deviations. The analysis in which the training and testing data sets were reversed yielded the results shown in Table 2.6.

### Table 2.6. Reversed Test and Train Data Regression Outcomes

<table>
<thead>
<tr>
<th>Model</th>
<th>RMSE</th>
<th>NSE</th>
<th>Fitting Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH~pH1+pH2</td>
<td>0.157</td>
<td>0.762</td>
<td>Linear Regression</td>
</tr>
<tr>
<td>pH~feed1+pH1+pH2+DO2</td>
<td>0.164</td>
<td>0.74</td>
<td>Bayesian Regression</td>
</tr>
<tr>
<td>pH~feed1+pH1+pH2</td>
<td>0.19</td>
<td>0.651</td>
<td>Bayesian Regression</td>
</tr>
<tr>
<td>pH~feed1+feed3+feed7+Temp1+pH1+pH2+DO2</td>
<td>0.4</td>
<td>-0.563</td>
<td>Linear Regression</td>
</tr>
</tbody>
</table>

The major observation from this analysis is that it switched the orders of the second and third models. Also, the NSE score for the last model went from positive to negative, indicating that the model fit was worse when it was trained on the koi data set and tested on the tilapia system. However, the same model was determined to be the best, with the NSE score increasing slightly along with the RMSE. This analysis supports the selection of the “pH~pH1+pH2” linear regression model.

### 2.5. Discussion and Conclusion

The model structure for pH indicates that the pH values for the two days prior can be used to predict the next day's pH reading, with an accuracy of 0.74 and a RMSE of 0.15. This gives aquaponics farmers a day to address the issue, most likely through gradually adding the basic compound. There are a few potential reasons for such a simple model structure, one being that some of the variables were only measured weekly, and as such were not incorporated into the final model. Were they measured daily, some may have contributed to the predictions in a meaningful way. It is also possible that there may be variables that could predict pH fluctuations which were not monitored in this system, such as salinity or total dissolved solids. The simplicity of the model is helpful, however, since an aquaponics producer would only need to keep up a regular pH monitoring schedule to make predictions and anticipate problems.

The model’s accuracy is below the desired 0.9, but the small RMSE indicates that the extent of the inaccuracy is minimal. The sensitivity of the model to identifying pH boundary
violations is 0.68, while the specificity is 0.99, showing that the model is vulnerable to false negatives, but robust against false positives. There were 9 instances in which a false negative occurred, and 3 in which there was a false positive. However, given the small RMSE, the difference between the actual and predicted values remained negligible, so even if the boundary was technically violated, it was not by much. For the boundary violations that were caught (true positives), the measured pH value was on average 0.15 ± 0.0816 away from the safe threshold. For the boundary violations that were not caught (false negatives), the measured pH value fell outside the safe threshold by 0.1333 ± 0.0485 on average. This means that the model was better at catching the larger deviations than the smaller ones, which is preferred. Overall, there were only 26 boundary violations for both the actual and predicted, some of which overlapped and some that did not, and all serious boundary violations were successfully caught by the model. One important observation is that this model was trained and tested on data from separate aquaponics systems. Though located in the same building, each system operates independently and contains different types of fish and plants. The koi system uses a flood and drain media bed for its hydroponics setup, in contrast with the deep-water culture and nutrient film technique used in the tilapia system. This is why the additional analysis of swapping the testing and training data sets was conducted, which gave similar results to the original analysis. This lends the model some external validity, since the accuracy of the model when applied to another system (74%) was not too far of a departure from the original training model accuracy (81%), and this result remained consistent regardless of which data set was used for training and which for testing.

A possible limitation to this model is that it hinges on a daily pH monitoring schedule, which is not universal in aquaponics production. The accuracy of <80% and sensitivity to range violations of <0.7 are also two limitations to the model and indicate room for improvement. Future research should focus on increasing data collection on the variables examined through more frequent measurements, more advanced measurement equipment, and data from additional aquaponics systems. Outfitting the aquaponics system with automatic sensors to continually measure water quality parameters would provide a large amount of accurate data with which to build a better model. Additional data at more frequent time points would also allow more exploration of the impact of time lagged regression inputs and possibly allow forecasting that gives farmers more advanced notice. It should also be emphasized that water chemistry
measurements should take place at the same location in the system every time to avoid inconsistencies. Another future area of study should be the relationships between some of the parameters observed in the correlation analysis. Looking at the interactions between feed and dissolved oxygen, dissolved oxygen and nitrite, and temperature and kH could yield insights into the dynamics of this system. Overall, this study provides a predictive model for pH with a moderate accuracy and a low error. The web application makes data analysis, visualization, and pH prediction broadly available to aquaponics producers.

2.6. Supplemental Materials

The web application and the code used for the analysis can be accessed at:
https://jamesonjmori.shinyapps.io/AquaponicsData/
Chapter 3

Quantification and comparison of risks associated with wastewater use in spray irrigation

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Published

3.1. Abstract

In the U.S., spray irrigation is the most common method used in agriculture and supplementing with animal wastewater has the potential to reduce water demands. However, this could expose individuals to respiratory pathogens such as *Legionella pneumophila* and non-tuberculosis *Mycobacteria* (NTM). Disinfection with methods like anaerobic digestion is an option but can increase concentrations of cytotoxic ammonia (personal communication). Our study aimed to model the annual risks of infection from these bacterial pathogens and the air concentrations of ammonia and determine if anaerobically digesting this wastewater is a safe option. Air dispersion modeling, conducted in AERMOD, generated air concentrations of water during the irrigation season (May-September) for the years 2013-2018. These values fed into the quantitative microbial risk assessments for the bacteria and allowed calculation of ammonia air concentrations. The outputs of these models were compared to the safety thresholds of $10^{-4}$ infections/year and 0.5 mg/m$^3$, respectively, to determine their potential for negative health outcomes. It was determined that infection from NTM was not a concern for individuals near active spray irrigators, but that infection with *L. pneumophila* could be a concern, with a
maximum predicted annual risk of infection of $3.5 \times 10^{-3}$ infections/year and 25.2% of parameter combinations exceeding the established threshold. Ammonia posed a minor risk, with 1.5% of parameter combinations surpassing the risk threshold of 0.5 mg/m$^3$. These findings suggest that animal wastewater should be anaerobically digested prior to use in irrigation to remove harmful pathogens.

3.2. Introduction

In the United States, agriculture accounts for 80% of all the water consumed in the country (USDA, 2019), with over 58 million acres of farmland requiring irrigation (USDA, 2017). Such a high demand for freshwater can be stressful on the environment, but replacing a percentage of that freshwater with animal wastewater could reduce this stress. There are over 64,000 swine farms in the U.S. and these operations produce large quantities of wastewater (USDA, 2017). This wastewater is a combination of fecal material, urine, and municipal water used to fill the swine lagoon and flush the waste collection area of the facility (Owusu-Twum & Sharara, 2020). Utilizing this effluent could help prevent water scarcity as climate change worsens, however, assessments of the human health risks need to be conducted for some areas of concern. This is particularly important for the question of treated versus untreated wastewater, since there are risks and benefits to both. Untreated wastewater contains higher levels of nutrients like nitrogen but can also contain human pathogens, both enteric and respiratory (Hamilton et al., 2007). Treating wastewater reduces those pathogens, but also the nutrients, while sometimes creating toxic compounds as a result (Massalha, Dong, Plewa & Nguyen, 2018). Therefore, a comparison between the two types of health risks – pathogens and toxins - is an important component of decision-making for implementing wastewater irrigation in the United States.

Quantitative microbial risk assessment (QMRA) is a formal process of calculating human health risks from pathogen exposure, where hazard identification is followed by exposure assessment, dose-response modeling, and risk characterization (WHO, 2016). For spray irrigation, the most common irrigation method in the United States (USDA, 2019), hazard identification starts with determining the pathogen levels in the source water. This is followed by air dispersion modeling to predict the air concentrations and spatial distribution of the pathogens around the source. Studies of human health risks from spray irrigation have used Gaussian plume
model equations (Katzenelson & Teltch, 1976; Dungan, 2014; Courault et al., 2017; Hamilton et al., 2018), and advanced Gaussian dispersion models, such as the US EPA recommended AERMOD (Perry et al., 2005) and the UK-CERC-ADMS models (Carruthers et al., 1994), as well as the hybrid Lagrangian HYSPLIT model (Stein et al., 2015) to yield these ambient concentration estimates. Gaussian and Lagrangian dispersion models offer the ability to generate predictions on large spatial and temporal scales, along with having established computer programs that can be used to conduct these analyses. Gaussian models operate within a static spatial grid, while Lagrangian models track a specific emitted element as it moves through time and space. These methods are powerful and can be tailored to the area of interest, but they can be computationally intensive. As with any model, there are also limitations to what these models can calculate, such as the current inability of standard Gaussian plume models to include evaporation as a factor. However, the thorough documentation and validation of these models make Gaussian and Lagrangian models popular options.

Empirical methods have also been employed to directly measure air concentrations of sprayed wastewater using air samplers and droplet catch methods (Shuval, Guttman-Bass, Applebaum & Fattal, 1989; Brooks et al., 2002; Donnison et al., 2004). Empirical predictions of dispersion have also been made using a modified version of Pasquill’s diffusion equation (Sorber, Bausum, Schaub & Small, 1976) and distance modelling (Burch et al., 2014). The empirical approach has the benefit of site-specificity and direct model validation but can lack external validity and relies on data that may miss some of the variability of the model parameters, especially variation in weather conditions. Thus, the costs and benefits of each modeling approach must be weighed for each individual research scenario since there is no single best model for all situations. Regardless of the method chosen, the output of an air dispersion model is predicted air concentrations of the hazard of interest. These air concentrations are used to determine the dose inhaled by an individual and the individual’s response to that exposure (Van Leuken et al., 2016). The severity and likelihood of this response are then used to make decisions about risk management.

QMRA is a powerful tool, but there are gaps in the research on the risks of wastewater irrigation. There have been numerous studies regarding the dispersion (Katzenelson & Teltsch, 1976; Teltsch, Kedmi, Bonnet, & Katzenelson, 1980; Shuval, Guttman-Bass, Applebaum, & Fattal, 1989; Donnison, Ross, Noonan, Fisher, & Waller, 2004; Molle et al., 2016) and human
health risks (Dungan, 2014; Moazeni et al., 2017) of enteric pathogens in agricultural settings, but few studies examining respiratory pathogens (Hamilton & Haas, 2016; Hamilton et al., 2018; Hamilton et al., 2020). There is concern regarding *Legionella pneumophila*, the bacterial causative agent of Legionnaire’s disease, which is known to be easily aerosolized and able to survive transport through the air (Hamilton et al., 2018). *L. pneumophila* naturally occurs in soil and a variety of water sources and has been found in these environments in swine farms (Hong, Yannarell, Dail, Ekizoglu & Mackie, 2013). This opportunistic bacterium is present in both human (Loenenbach et al., 2018; Caicedo et al. 2019) and swine wastewaters (He et al., 2019) and inhalation of the organism can lead to serious infections, especially with close exposure (Fields, Benson & Besser, 2002). Environmental contamination from soils and natural water sources in which the swine lagoons come in contact with is also a possible route of introduction for *Legionella spp.* into the wastewater. Another group of bacterial respiratory pathogens of concern is non-tuberculosis *Mycobacteria* (NTM), which is a subcategory of *Mycobacteria spp.* that includes microorganisms like the *Mycobacterium avium* complex and *Mycobacterium parafortuitum* (Primm, Lucero, & Falkingham III, 2004). Like *L. pneumophila*, NTM are often found in wastewater (Radomski et al., 2011; Ajibode et al., 2013), though studies of NTM are few. The hardiness and ubiquity of NTM and *L. pneumophila* in water environments make their neglect in modeling for an agricultural context a notable gap in our understanding of this system (Primm, Lucero, & Falkingham III, 2004; WHO, 2007).

Disinfection of wastewater is used to control these bacterial populations, with one effective method being thermophilic anaerobic digestion, which uses heat to inactivate microorganisms (Cervero-Aragó, Rodríguez-Martín, Puertas-Bennasar & Araujo, 2015; Schulze-Robbecke & Buchholtz, 1992). Anaerobic digestion is a method of animal wastewater processing that utilizes bacteria to break down organic material under high temperatures and anaerobic conditions to produce renewable biogas and processed wastewater effluent (Massalha et al., 2020). Operating the anaerobic digester at a thermophilic temperature (55 °C) would effectively kill *L. pneumophila* (Cervero-Aragó et al., 2015) and NTM (Schulze-Röbbecke & Buchholtz, 1992). However, anaerobic digestion increases the concentration of ammonia in the wastewater, which is a cytotoxic compound (Massalha et al., 2020). Inhalation of water containing ammonia by individuals close to the source, such as farm workers, could cause irritation and other harmful effects on the respiratory tract (US EPA, 2013).
The purpose of our study is to evaluate the options of disinfection with anaerobic digestion and irrigation with untreated wastewater. The first option would eliminate the bacterial pathogens of concern but increase the ammonia levels, while the second option would involve irrigation with potentially bacteria-laden wastewater that has less ammonia. To determine the safer choice, we compared the risk of respiratory infection with *L. pneumophila* and NTM to the risk of ammonia toxicity.

### 3.3. Materials and Methods

#### 3.3.1. Laboratory Wastewater Quantification of Target Pollutants

#### 3.3.1.1. Ammonia Concentrations

Ammonia data were obtained from two identical 2 L anaerobic reactors consisting of glass Erlenmeyer flasks sealed with rubber stoppers that were connected to PVC tubes for discharge of gas and kept in temperature-controlled water baths (Massalha et al., 2020). One reactor was operated under mesophilic conditions (35 °C) and another under thermophilic conditions (55 °C). These reactors had a target residence time of 31 days after the start-up of 45 days. During the start-up time, the reactors were seeded with 1500 mL of inoculum from a full-scale anaerobic digester of a domestic wastewater treatment plant and 500 mL of swine slurry. This swine slurry was sampled from two separate swine farms in Illinois, USA (Massalha et al., 2020). One of the sources was a swine wastewater collection pond and the other was a drainage collection channel downstream of swine pens (Massalha et al., 2020). The wastewater from each source was kept separate through the entire process (Massalha et al., 2020).

Every week during the start-up time, 500 mL supernatant was replaced by 500 mL of fresh swine wastewater. Subsequently, 1300 mL of the liquid from each reactor was replaced with a fresh swine slurry sample every 20 days. Biogas was discharged via a poly(vinyl chloride) tube submerged in water to prevent air from entering the reactors. Biogas composition was not measured. The anaerobic digesters were operated for 21 days following system startup, as described in Massalha et al. (2020). Ammonia concentrations were measured using the HACH Ammonia-Nitrogen Method 8155 (Loveland, CO) in units of mg/L (Massalha et al., 2020).
3.3.1.2. Floodwater Sampling in North Carolina

Bacterial concentrations of *Legionella pneumophila* and *Mycobacteria* spp. were obtained from samples of runoff flood water taken downstream of swine farms after Hurricane Florence from both flooded and unflooded coastal zones in North Carolina on October 7, 2018. Water samples were collected in triplicate at each sampling area using 15 x 7.5-inch sterile Whirl-Pak Stand-Up Bags (Nasco, Fort Atkinson, WI), each containing approximately 2 L. The individuals collecting the samples were wearing disposable gloves disinfected with 70% ethanol. The water bags were sealed by tightening the metallic strip at the bag mouth and covering it with transparent tape. A small volume of air was kept above the water in the bags to avoid leaking. After collection and labeling, the water was stored in coolers filled with ice packs frozen at -20 °C. After returning to the laboratory, the water samples were stored at 4 °C.

To collect the microorganisms, the water samples were flocculated and filtered. MgCl₂ (25 mmol MgCl₂ / L water sample) was added, with 30 minutes of shaking for flocculation. The flocculated water samples were then filtered with 1.6 μm glass fiber prefilters (MilliporeSigma, Burlington, MA) and 0.22 μm polyethersulfone (PES) stericup vacuum filters (MilliporeSigma, Burlington, MA), successively. Multiple 1.6 μm prefilters were used for each bag, depending on different flow conditions. Only one 0.22 μm PES filter was used for filtering all replicates at one location by cutting a 1.6 μm prefilter into quarters for four different analyses. The 0.22 μm PES filters were cut into halves for two different analyses. Each DNA sample was extracted from either ¼ - ½ of the 1.6 μm prefilter or ½ of the 0.22 μm PES filter. DNA was extracted with Fast DNA SPIN Kit for Soil (MP Biomedicals) following the instructions provided in the kit, with the exceptions of shortening lysis to 15 seconds and lengthening the centrifugation time to 15 minutes. Lastly, each DNA extract was dissolved in the 100 μL DES (DNase/Pyrogen-Free Water) provided in the kit.

The samples were tested for *Enterococcus spp.*, *E. coli*, *Mycobacteria* spp., *Salmonella typhimurium*, *Pseudomonas* spp., and *Legionella pneumophila*. The presence of the fecal indicator organisms alongside the *Mycobacteria* spp. and *L. pneumophila* was interpreted as meaning the samples contained wastewater from the swine lagoons. It was also assumed that the concentrations observed in flood water collected near flooded swine wastewater lagoons was representative of the concentrations typically found in farm operations that dilute wastewater with freshwater prior to irrigation, as is the common practice (Dungan, 2014).
3.3.1.3. Bacterial Quantification with mfqPCR

Assay designs for the genes targeted in the microfluidic quantitative PCR (mfqPCR) were adapted from previous studies to detect *Legionella pneumophila* and *Mycobacterium* spp. (Ishii et al., 2014; Ishii, Segawa, & Okabe, 2013; Radomski et al., 2013). Forward and reverse primers for all assays were purchased from Custom DNA Oligos (Integrated DNA Technologies, Coralville, IA). Probes were obtained from the Universal Probe Library (UPL) (Roche, Basel, Switzerland) and were labeled with 6-Carboxyfluorescein (6-FAM) at the 5’ end and a dark quencher dye at the 3’ end. Standards were bought from gBlock Gene Fragments (Integrated DNA Technologies, Coralville, IA). Standard curves were generated by performing qPCR using serial dilutions (2 x 10^0 to 2 x 10^6 copies/μL) of a pool containing 23 DNA standards for assay validation prior to their use in mfqPCR, following the procedure used in Ishii et al. (2014).

*Pseudogulbenkiania* NH8B was used as an internal amplification control in the environmental samples to check for PCR inhibition. All DNA samples and standard pool dilutions underwent 14 cycles of standard target amplification PCR to increase template DNA yields before quantification. The standard pool dilutions (2 x 10^0 to 2 x 10^6 copies/μL) were used to generate standard curves for mfqPCR. The reaction (5 μL) contained 2.5 μL 2X TaqMan PreAmp Master Mix (Thermo Fisher), 0.5 μL 0.2X TaqMan primer probe mix and 1.25 μL of template DNA. The PCR plate was processed with the following thermal cycle on a thermocycler: 95°C for 10 min, 14 cycles of 95°C for 15 sec, and 60°C for 4 min.

The standard target amplification products were diluted 25-fold with TE buffer and 5 μL aliquots of each sample and duplicates of each assay were loaded onto a 48.48 chip (Fluidigm, South San Francisco, CA). mfqPCR was performed in a Biomark HD Real-Time PCR machine (Fluidigm, South San Francisco, CA) under the following thermal conditions: 70°C for 30 min, 25°C for 10 min, 95°C for 1 min, 35 cycles of 96°C for 5 sec, and 60°C for 20 sec.

Quantification cycle (C_q) values and standard pool dilutions (log copies/μL) were used to generate standard curves for each mfqPCR assay. Linear regression analysis was performed to fit the standard curves. The model’s goodness-of-fit was assessed by calculating R^2. The efficiency of each assay was calculated based on the slope of its respective standard curve to validate adequate target amplification (Bustin et al., 2009). Standard curves were accepted as quantifiable if the efficiency achieved was ≥ 90% and if the lower limit of detection was ≤ 2 copies/μL, based
on the work of Ishii et al. (2014). The final output was the number of bacterial gene copies per liter of water (gc/L), with the assumption that every gene copy was equivalent to a single, viable, and infectious microorganism (Figure 3.1).

Figure 3.1. Bacterial Source Concentrations

Source concentrations of *L. pneumophila* and *Mycobacteria spp.*, obtained from swine farm floodwaters using mfqPCR. Units are gene copies/L and 69 samples are represented.

3.3.2. Air Dispersion Modeling

Separate models were created for the pathogen and ammonia assessments due to differences in the biologically relevant droplet sizes. Both bacteria require alveolar deposition to cause infection, while ammonia can be cytotoxic at any point of contact in the respiratory tract, so droplets up to 11 μm in aerodynamic diameter were considered for the bacteria and droplets up to 110 μm for the ammonia. These size cutoffs are typically 10 μm (Heyder, 2004) and 100 μm (Van Leuken et al., 2016), respectively, but based on the droplet size distributions generated by the software utilized in the following sections, these values were rounded up rather than down to account for more droplets as a conservative estimate. A flowchart of the air dispersion modeling process, as well as the subsequent risk assessments, is provided below in Figure 3.2.
3.3.2.1. Weather Data Processing (AERMET)

Surface and upper air weather data from Greensboro, North Carolina were processed with the program AERMET (v18081) for use in AERMOD (v19191). North Carolina was chosen because it was the state from which the floodwater samples were obtained, and Greensboro was the location of the closest NOAA weather station that provided both surface and upper air data. The years 2013 through 2018 were considered to account for a wide range of weather conditions and input parameters were obtained from the AERMET manual (US EPA, 2018).

3.3.2.2. Droplet Size Distribution Generation (AGDISP)

The USDA Forest Service software AGDISP (v8.26) was used to calculate water droplet shrinkage from an ASABE medium droplet size distribution to the final droplet size distribution 50 meters downwind (Teske & Curbishley, 2011). This calculation was based on an evaporation rate of 84.76 $\mu$m$^2$/°C/sec and the median wind speed, temperature, and relative humidity for the
irrigation season (May-September) in Greensboro, North Carolina. Emission was modeled from a ground-level sprayer. The atmospheric stability class was set to thinly overcast, which was the most common cloud cover condition for the area and years modeled, and the downwind droplet size distributions and volume fractions were obtained from the trajectory files. Figure 3.3 compares the original (dashed) and downwind (solid) droplet size distributions to show the importance of considering evaporation when dealing with water droplets.

Figure 3.3. Droplet Size Distributions

The original (dashed) and 50 meters downwind (Downwind) droplet size distributions from AGDISP are shown. Droplet diameter (x-axis) and the relative volume fraction of each droplet diameter (y-axis) are plotted. The downwind droplet size distribution (solid) was used in AERMOD.

3.3.2.3. Air Dispersion Modeling (AERMOD)

These droplet size distributions served as inputs for the air dispersion modeling conducted in AERMOD (v19191). AERMOD was chosen for its computational ability and for
being the US EPA recommended air dispersion model (Perry et al., 2005), as well as its previous use in modeling infection risks from spray irrigation (Dungan, 2014). Water droplets were approximated as PM$_{10}$ with a density of 1 g/m$^3$. The averaging time for the air concentrations was set to 24 hours. The spray irrigator was modeled as 53 adjacent volume sources for a total length of 800 meters, a length based on the North Carolina Irrigation Guide’s (2010) description of maximum irrigator dimensions. Separate North-to-South and East-to-West source orientations were modeled, and their predictions combined to account for the impact and uncertainty of source location. The total water emission rate was 850 gallons/minute, selected from Dungan (2014) and supported by practices in North Carolina (North Carolina Irrigation Guide, 2010). The water emission rate was multiplied by the total volume fraction of droplets with sizes relevant for the pathogens (0.0027) or ammonia (0.1755) – calculated from the downwind droplet size distributions produced by AgDisp - then divided among the individual volume sources. Deposition and low wind conditions were considered, and the terrain was assumed flat. A Cartesian grid of receptors surrounding the source allowed predictions of air concentrations up to 500 meters away in all directions, and a polar ring of receptors was used to make predictions between 1.6 and 2 kilometers from the center of the source. Only the months of May through September were considered, as this was when irrigation could occur. The output of this air dispersion model was predicted air concentrations of sprayed irrigation water for the irrigation seasons of the years 2013 to 2018 in units of µg/m$^3$.

3.3.3. Ammonia Air Concentration Assessment

The assessment of ammonia air concentrations was conducted in the R platform (R Core Team, 2018). These air concentrations were used to determine risk of ammonia toxicity through comparison to the US EPA’s reference concentration for inhalation of ammonia, which is 0.5 mg/m$^3$ (US EPA, 2013). This reference concentration is meant to prevent respiratory symptoms and reduced lung function from acute exposure to aerosolized ammonia (US EPA, 2013). Concentrations were calculated using Equation 3.1, where “$A_a$” is the air concentration of dispersed wastewater droplets in the size range relevant to ammonia (µg/m$^3$), calculated by AERMOD, and “$S_a$” is the concentration of ammonia in the irrigation wastewater (mg/L). The constant in the denominator allows for unit conversions between the parameters. The values of
both parameters are given in Table 3.1. Ammonia concentrations exceeding 0.5 mg/m$^3$ were considered harmful to human health.

$$NH3 = \frac{(Aa \cdot Sa)}{10^9} \quad (3.1)$$

To capture the range of possible scenarios, the parameters were Latin Hypercube sampled for 1000 iterations. We assumed that no chemical reactions occurred following aerosolization and that the ammonia was evenly dispersed within and among the water droplets.

**Table 3.1. Ammonia Air Concentration Assessment Input Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol (Units)</th>
<th>Values</th>
<th>Distribution</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air concentration of wastewater</td>
<td>$Aa$ (μg/m$^3$)</td>
<td>99.9th percentile = 2.5 x 10$^5$</td>
<td>Empirical</td>
<td>AERMOD output</td>
</tr>
<tr>
<td>containing ammonia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source concentration of ammonia</td>
<td>$Sa$ (mg/L)</td>
<td>Min = 500, Max = 1800</td>
<td>Uniform</td>
<td>Lab results</td>
</tr>
</tbody>
</table>

3.3.4. Bacterial Viability Factor

To account for the impact of environmental exposure on the aerosolized bacteria, we calculated a bacterial viability factor ($V$) based on the equation from Dungan (2014) (Equation 3.2). We defined the bacterial viability factor as the fraction of bacteria that are still viable when inhaled. To calculate this factor, non-solar aerosol decay rates ($a$) were chosen for *L. pneumophila*, since there are presently no solar inactivation rates for this bacterium. The distance parameter ($d$) assumed individuals were between 50 and 500 meters downwind of the irrigator and the wind speed ($ws$) was obtained from the North Carolina weather data. The values for these parameters are given in Table 3.2.

$$V = e^{-a \cdot \frac{d}{ws}} \quad (3.2)$$
Table 3.2. Bacterial Decay Correction Factor Parameter Values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol (Units)</th>
<th>Values</th>
<th>Distribution</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wind speed</td>
<td>ws (m/s)</td>
<td>95th percentile = 6.5</td>
<td>Empirical</td>
<td>AERMET output</td>
</tr>
<tr>
<td>Downwind distance</td>
<td>d (m)</td>
<td>Min = 50, Max = 500</td>
<td>Uniform</td>
<td>Assumption</td>
</tr>
<tr>
<td>M. parafortuitum decay rate</td>
<td>a (s⁻¹)</td>
<td>Min = 0.0002, Max = 0.002</td>
<td>Uniform</td>
<td>Paez-Rubio and Peccia (2005)</td>
</tr>
<tr>
<td>L. pneumophila decay rate</td>
<td>a (s⁻¹)</td>
<td>Shape1 = 7.9, Shape2 = 36341.2</td>
<td>Beta</td>
<td>Hamilton et al. (2018)</td>
</tr>
</tbody>
</table>

3.3.5. Quantitative Microbial Risk Assessment

To determine the annual probabilities of infection with *L. pneumophila* and NTM, a QMRA was performed in the R platform (R Core Team, 2018). It was assumed that the bacteria were evenly dispersed within and among the droplets, there was no bacterial growth after emission, and exposed individuals were adults with healthy immune systems.

First, the viability factor and other parameter distributions were Latin Hypercube sampled 1000 times (Table 3.3). In lieu of direct information regarding farm worker exposure time to irrigation water, a distribution of values was generated from a survey of U.S. farmers published in 2013 by the Farm Journal, in which 1607 respondents reported the number of hours they spent on “farm-related work” every day (Schafer, 2013). This daily amount of “farm-related work” was assumed to correspond exactly with each irrigation event so that the individual considered in the model was exposed to the aerosols for the entire duration of the irrigation event at the exposure distance of interest. It was recognized that this is an overestimation of exposure time, since “farm-related work” is a broad term encompassing many activities far from the active irrigator, but it was felt that this information offered a worst-case scenario that would better quantify the upper limits of the health risks examined.

The number of irrigation events was based on the irrigation frequencies during peak use periods for North Carolina’s three largest crops: corn, peanuts, and tobacco (Natural Resources Conservation Service, 2010). The length of the irrigation season (153 days) was divided by the lowest (3 days) and highest (11 days) of these frequencies to obtain the minimum and maximum values for the uniform distribution of the number of irrigation events (Table 3.3). All other parameters were obtained from the literature (Table 3.3).
The first step in the exposure assessment was calculating the distribution of single exposure doses (Equation 3.3). This equation was based on the method for calculating downwind microbial air concentrations proposed by the US EPA (US EPA, 1982) and supported by subsequent publications (Dungan, 2014; Hamilton & Haas, 2016). The “Sb” parameter corresponds to the source concentration of the bacteria (Figure 3.1). The other parameters are the air concentration of irrigation wastewater in the size category relevant to bacteria (Ab), the inhalation rate (IR), the duration of exposure to the aerosol (E), the qPCR correction factor for \( L. \) *pneumophila* (C), the bacterial viability factor (V), and the aerosol retention rate in the lungs (RR). This qPCR correction factor is necessary because several studies have shown large discrepancies between qPCR and culture of *Legionella* (Lee et al., 2011; Ditommaso, Ricciardi, Giacomuzzi, Rivera & Zotti, 2015). The length of exposure to the aerosol (E) is assumed to perfectly correspond to the duration of the irrigation event in terms of timing and duration. The constant in the denominator is for unit conversions between the parameters.

\[
Dose = \frac{(Ab \cdot IR \cdot Sb \cdot E \cdot C \cdot V \cdot RR)}{9.97 \times 10^9} \quad (3.3)
\]
The output of Equation 3.3 was a distribution of doses received by an individual after spending the entire exposure period (E) either within 500 m or between 1.6 and 2 km of the active irrigator. This dose distribution was applied to an exponential dose-response equation to calculate the probabilities of infection for each exposure event (Equation 3.4), since the exponential equation is often used for both pathogens (Hamilton & Haas, 2016; Hamilton, Weir, & Haas, 2017). The dose-response parameters (r) were obtained from the literature for *L. pneumophila* and the *Mycobacterium avium* complex (Table 3.3).

\[ P_s = 1 - e^{(-r \cdot \text{Dose})} \]  \hspace{1cm} (3.4)

The final step of the QMRA was to determine the distributions of the annual probabilities of infection (Equation 3.5) using Monte Carlo sampling of the single exposure infection probabilities and the number of irrigation events (N) (Table 3.3) (Karavarsamis & Hamilton, 2010). Sampling was conducted 1000 times, with the number of single exposure infection probabilities sampled at each of those times set equal to the selected number of irrigation events. The exposed individual was assumed to be present for the entire duration of all these irrigation events.

\[ P_a = 1 - \prod_{k=1}^{N} (1 - P_{s_k}) \]  \hspace{1cm} (3.5)

The medians of the distributions produced by Equation 3.5 were compared to the threshold of $10^{-4}$ infections/year to assess the human health risks associated with the modeled scenario (WHO, 2016).

3.3.6. Sensitivity Analysis

A Sobol sensitivity analysis was performed for both the ammonia and pathogen models to examine the impact of input parameters on the risk output. The Sobol method divides the variance of the model output into fractions that can be attributed to individual parameters and their interactions with other parameters (Zhang, Trame, Lesko, & Schmidt, 2015). These fractions can have values between 0 and 1, with the sum of all these fractions equal to 1 (Zhang et al., 2015). This allows quantification and ranking – called “indices” - of parameters from
having the least to most influence on the model results, with an index of 0 indicating no
collection and an index of 1 indicating sole contribution to output variance (Zhang et al.,
2015). The first order and total sensitivity indices for the model parameters were calculated in
this study. First order indices quantify that amount of variance exclusively caused by each
parameter, while total order indices also include the impact of each parameter’s pairwise and
higher order interactions with the other parameters (Zhang et al., 2015). Parameters with total
indices greater than 0.05 were considered sensitive, with larger values indicating stronger
influence (Zhang et al., 2015). All parameters were represented as uniform distributions to give
them equal weight and to capture the full impact of the variability and uncertainty of their values
on the model output. The minimums and maximums for these uniform distributions are provided
in Table 3.4. Parameter units are the same ones used in the risk assessments.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>air concentration of wastewater containing bacteria (Ab)*</td>
<td>0</td>
<td>1.00E+05</td>
</tr>
<tr>
<td>exposure duration (E)</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>L. pneumophila source concentration (Sb)</td>
<td>0</td>
<td>1.00E+06</td>
</tr>
<tr>
<td>NTM source concentration (Sb)</td>
<td>0</td>
<td>1.00E+06</td>
</tr>
<tr>
<td>retention rate (RR)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>viability factor (V)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>qPCR correction factor (C)</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>exponential dose-response parameter (r)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>inhalation rate (I)</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>ammonia source concentration (NH3)</td>
<td>0</td>
<td>5000</td>
</tr>
<tr>
<td>air concentration of wastewater containing ammonia (Aa)*</td>
<td>0</td>
<td>9.00E+05</td>
</tr>
</tbody>
</table>

3.3.7. Scenario Analyses

3.3.7.1. Aerosol Deposition

A scenario analysis was performed to determine the length of time the wastewater aerosol
remained in the air after irrigation ceased. To do this, an irrigation schedule with start and stop
times was modeled in AERMOD for July 2018 and the time increment following the end of
irrigation at which the air concentration of water to returned to 0 μg/m³ was found.
3.3.7.2. *L. pneumophila* Regulatory Concentration Assessment

A scenario analysis was conducted to examine the applicability of the regulatory threshold of 1000 cfu/L for *L. pneumophila* to spray irrigation systems (WHO, 2007). The same QMRA model was used as in the original risk assessment, with the source concentration of bacteria set at 1000 cfu/L. The output distributions of the annual probabilities of infection were compared to the safety limit of $10^{-4}$ infections/year for exposure at 500 m and 1.6-2 km.

3.4. Results

3.4.1. Air Dispersion Modeling

The average air concentrations of aerosolized irrigation water to which a farm worker may be exposed are shown on the right in Figure 3.4, along with the wind speeds and directions for the years modeled (left). Predictions for both source orientations were combined to account for the uncertainty in the irrigator’s location.

*Figure 3.4. Wind Rose and Contour Map of Air Dispersion of Sprayed Wastewater*

The wind directions (left) and average air concentrations at each AERMOD receptor (right) for the combined North to South and East to West source orientations (white rectangles) for the pathogen emission model.

This dispersion model shows that the highest air concentrations occur closest to the irrigator and rapidly decrease with distance. These maximums also occur directly
downwind of the predominant wind directions (northeast and southwest).

### 3.4.2. Ammonia Air Concentration Assessment

The ammonia assessment yielded a maximum predicted air concentration of 1.7 mg/m$^3$ for receptors within 500 meters and 1.6 mg/m$^3$ for receptors between 1.6 and 2 km (Table 3.5). Both predictions exceed 0.5 mg/m$^3$, indicating that ammonia exposure could be hazardous for the conditions modeled under extreme conditions. However, the 95th percentiles for both distances are well below 0.5 mg/m$^3$, indicating that even a highly conservative risk estimate puts the risk caused by ammonia inhalation at less than half of the regulatory threshold (Table 3.5).

<table>
<thead>
<tr>
<th>Exposure Scenario</th>
<th>Median</th>
<th>95th Percentile</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia (500 m)</td>
<td>3.7e-03</td>
<td>8.1e-02</td>
<td>1.7e+00</td>
</tr>
<tr>
<td>Ammonia (1.6-2 km)</td>
<td>5.2e-03</td>
<td>2.1e-01</td>
<td>1.6e+00</td>
</tr>
</tbody>
</table>

### 3.4.3. Quantitative Microbial Risk Assessments

Risk estimates for *L. pneumophila* and NTM are provided in Table 3.6, in units of infections/year. The risk assessment for NTM yielded maximum risk estimates that were 3 to 4 orders of magnitude lower than $10^{-4}$ infections/year. More extreme scenarios were explored in which the source concentration of NTM was increased by orders of magnitude to determine a rough concentration at which the annual risk of infection exceeded $10^{-4}$ infections/year. It was found that the source concentration of NTMs would have to be around $10^7$ gc/L to surpass the limit for 500 m exposures, and around $10^8$ gc/L for exposures at 1.6-2 km. For reference, the maximum concentration of NTMs detected in the samples taken for this study was 1800 gc/L. This indicates a lack of concern for NTMs as a pathogen in this system.

<table>
<thead>
<tr>
<th>Exposure Scenario</th>
<th>Median</th>
<th>95th Percentile</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. pneumophila</em> (500 m)</td>
<td>5.4e-05</td>
<td>1.6e-03</td>
<td>3.5e-03</td>
</tr>
<tr>
<td><em>L. pneumophila</em> (1.6-2 km)</td>
<td>7.6e-06</td>
<td>1.2e-04</td>
<td>3.1e-04</td>
</tr>
<tr>
<td>NTM (500 m)</td>
<td>8.7e-07</td>
<td>3.7e-06</td>
<td>8.0e-06</td>
</tr>
<tr>
<td>NTM (1.6-2 km)</td>
<td>8.8e-08</td>
<td>4.2e-07</td>
<td>9.6e-07</td>
</tr>
</tbody>
</table>
The medians for both *L. pneumophila* exposure scenarios examined remained below the threshold of $10^{-4}$ infections/year, though the 95\textsuperscript{th} percentile and maximum risk estimates did go over the threshold (Table 3.6). Additionally, 25.2\% of parameter combinations resulted in more than $10^{-4}$ infections/year for workers within 500 meters of the source. 0.3\% of parameter combinations resulted in an outcome that exceeded the threshold for exposure at 1.6-2 km. The final risk distributions are shown in Figure 3.5, offering a stark contrast between the risks of infection with the two bacteria.

*Figure 3.5. Strip Chart of Annual Probabilities of Infection*

Distributions of the annual probabilities of infection for *L. pneumophila* (black) and NTM (grey) at both exposure distances (500 meters and 1.6-2 kilometers). The dashed line represents the safety threshold of $10^{-4}$ infections/year.
These results indicate that though respiratory infection with *L. pneumophila* likely will not occur 75% of the time, scenarios exist in which the risk is above the safety threshold. However, this is only for the 500 m exposure scenario, with >99% of scenarios at 1.6-2 km remaining below the risk threshold. High-risk scenarios are likely due to a combination of favorable weather conditions for bacterial survival, a long exposure duration, a low qPCR conversion factor, and a particularly deep breath with a high retention rate.

An additional analysis was run in which single exposure infection probabilities for *L. pneumophila* were examined to see if these large risk outputs were being driven by a small handful of exposure events. The threshold of $10^{-6}$ infections/year was used due to its establishment as a general limit for infections from drinking water (Pepper & Gerba, 2018). It was found that 6.5% of scenarios at 500 m exceeded this threshold, while 4.6% of scenarios at 1.6-2 km went above the limit. This, when paired with the finding that the third quartile risks for both distances were equal to 0, indicates that the annual probabilities of infection are driven by a small number of parameter combinations.

### 3.4.4. Sensitivity Analysis

The first order and total sensitivity indices from the Sobol sensitivity analyses are provided in Table 3.7, listed in order of most to least sensitive based on the first order index score.
Table 3.7. Sobol Sensitivity Analysis Indices

<table>
<thead>
<tr>
<th>Risk</th>
<th>Variable</th>
<th>1st Order Index</th>
<th>Total Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. pneumophila</td>
<td>exponential dose-response parameter (r)</td>
<td>0.12</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>qPCR correction factor (C)</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>viability factor (V)</td>
<td>0.11</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>retention rate (RR)</td>
<td>0.1</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>air concentration of wastewater (Ab)</td>
<td>0.075</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>L. pneumophila source concentration (Sb)</td>
<td>0.046</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>exposure duration (E)</td>
<td>0.034</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>inhalation rate (I)</td>
<td>7.60E-05</td>
<td>0.17</td>
</tr>
<tr>
<td>NTM</td>
<td>exponential dose-response parameter (r)</td>
<td>0.15</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>air concentration of wastewater (Ab)</td>
<td>0.14</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>retention rate (RR)</td>
<td>0.13</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>NTM source concentration (Sb)</td>
<td>0.11</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>exposure duration (E)</td>
<td>0.1</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>viability factor (V)</td>
<td>0.07</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>inhalation rate (I)</td>
<td>0.0</td>
<td>0.17</td>
</tr>
<tr>
<td>Ammonia</td>
<td>air concentration of wastewater (Aa)</td>
<td>0.46</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>ammonia source concentration (NH3)</td>
<td>0.43</td>
<td>0.54</td>
</tr>
</tbody>
</table>

These scores represent the influence each parameter has over the outcome of the model and scores above 0.05 were considered significant (Zhang et al., 2015). Differences between model parameters were relatively small, indicating that no single parameter drives the model output. It is interesting to note that the inhalation rate (I) had the lowest sensitivity for both bacteria and made very little, if any, contribution to the model output variance. This means that variation in inhalation rate does not have a large impact on the model results, which is largely irrelevant, since this value is well characterized and has little biological room for variability. In general, having a model that is less influenced by slight inaccuracies in variable distribution values is preferable to models that hinge on a single, poorly characterized parameter. However, care must be taken to avoid the compounding of inaccuracies in multiple distributions, given that they are all of relatively equal importance. An interesting outcome of this analysis was the difference between the first and total order indices for both the bacteria and the ammonia. Except for the retention rate in the NTM model, none of the first and total order indices lined up in their rankings. This is likely due to the greater importance of parameter interactions than individual contributions. Thus, even if single contributions to the output variance were higher for some parameters, the strong interactions of
other parameters, combined with their individual contributions, surpassed the less synergistic parameters in their total indices.

The importance of pairwise and higher order parameter contributions to output variance has important implications for future studies of these parameters. To improve the model and our understanding of this system, better quantification of these parameters is necessary. Based on this sensitivity analysis, though, the relationships between the parameters have a larger influence than individual parameters, suggesting that examining multiple parameters simultaneously in an experimental setting may be a better approach to understanding these types of agricultural exposures, though this is more difficult to carry out. Regardless of this challenge, these interactions should be kept in mind in future investigations.

3.4.5. Scenario Analyses

3.4.5.1. Aerosol Deposition

The examination of wastewater deposition indicated that its air concentration around the irrigator dropped to zero inside 500 meters of the source within an hour of irrigation ending, suggesting that delaying activities near the irrigator until 1 hour after irrigation ends may be a protective measure for farm workers.

3.4.5.2. L. pneumophila Regulatory Concentration

The scenario analysis of applying the regulatory maximum source water concentration of 1000 cfu/L to the model led to the conclusion that this threshold is far too high for a consistent source concentration of *L. pneumophila*. At this source concentration, 100% of scenarios at 500 m from the source resulted in annual probabilities of infection exceeding 1e-4, while 88% of scenarios at 1.6-2 km exceeded this safety limit. This suggests that a threshold for concentrations of *L. pneumophila* in water for agricultural use is necessary and that current standards based on drinking water are not adequate to address this health risk.

3.5. Discussion and Conclusion

This study offers the first comparison of the risk of respiratory infection to the risk of ammonia cytotoxicity in the respiratory tract from exposure to sprayed swine wastewater in an agricultural setting. Though this study compared a chemical risk to a biological one, it was felt
that the similar exposure scenario and shared hazard source allowed for a reasonable comparison of the risks. Our findings establish anaerobic digestion of swine wastewater as the preferred alternative to irrigation with untreated wastewater and highlight *L. pneumophila* as a bacterial pathogen of concern for this system. Another protective measure that can be used by farmers that is supported by the results of this analysis is waiting an hour after irrigation ends to begin work within 500 meters of the irrigator. It was also found that the regulatory concentration of 1000 cfu/L for *L. pneumophila* is much too high for a consistent source concentration in an irrigation system like this, under the conditions modeled.

This study also supports the hypothesis that proximity to the irrigator is an important risk factor, with farm workers within 500 meters of the active source having a higher risk of respiratory infection than those further away, reflecting the setback distance of around 625 meters suggested by Hamilton et al. (2018) for residential areas. This agrees with past research on farmer exposure to enteric (Moazeni et al., 2017; Dungan, 2014) and respiratory pathogens (Fattal, Bercovier, Derai-Cochin, & Shuval, 1985), and bolsters the findings of Pepper and Gerba (2018), who found a risk for respiratory infection with *L. pneumophila* from small-scale spray irrigator that exceeded $10^{-4}$ infections/year. The inverse relationship between distance and infection risk is likely due to aerosol dilution and increased bacterial decay over longer durations of aerosolization. Individuals therefore should remain upwind of the irrigator, or farther than 500 m downwind, and personal protective equipment should be worn if work is required on or near the irrigator during its operational hours.

One unexpected outcome of this study was the finding that the risk of respiratory infection from *L. pneumophila*, in the conditions modeled, was driven by a few “bad” parameter combinations. These combinations provided ideal conditions for bacterial survival and infection, and resulted in high individual probabilities of infection that, when included in a general annual probability of infection, almost guaranteed a yearly risk above $10^{-4}$ infections/year. Given the use of the Monte Carlo method for risk estimation, this means that if one or more of these “bad” combinations is selected during the sampling process, it will dominate the resulting annual probability of infection. Thus, in the case of *L. pneumophila* in irrigation, it might be better to focus more on single infection probabilities, rather than the annual risk of infection, especially since it is a pathogen that causes acute infection and is not an exposure that requires accumulation over time to have an effect.
The main limitation of our study was the absence of direct farm worker exposure data. Since this information was unavailable, our model used survey data of total hours worked on the farm, which likely overestimated exposure times. Better characterization of farm worker exposures would be beneficial for future risk assessments of agricultural hazards. Another limitation was our lack of direct sampling from the swine lagoons. Future efforts should be made to better characterize *L. pneumophila* and NTMs in these lagoons. Our atmospheric dispersion model also has not yet been validated in the field. Generally speaking, validation of the use of AERMOD for modeling a spray irrigator has not been conducted and should be pursued. Thus, our simulation serves as a preliminary exploration of the risks of spray irrigation with swine wastewater and its associated parameters, and is a starting point for further investigation and a source of advice, rather than definite calculations of risk. Other limitations lie in our characterizations of the parameters. Non-solar decay rates were used to calculate the viability factor for *L. pneumophila*, since this was preferred to the substitution of solar decay rates from other bacteria. However, these rates are lower than would be observed on a sunny day (Teltsch, Shuval, & Tadmor 1980; Paez-Rubio & Peccia, 2005). This might have led to an overestimation of the number of viable bacteria in the air, which may have overestimated the risk of infection. It should also be noted that different NTM species were used for the bacterial viability factor and dose-response calculations. This discrepancy likely introduced some error into our risk calculations for NTM.

Even recognizing these limitations, the results of our study are scientifically sound and based on the best available data. We determined that there is some risk of ammonia toxicity and negligible risk of respiratory infection with NTMs from airborne exposure to anaerobically digested and untreated wastewater. However, many scenarios do exist in which the risk of infection with *L. pneumophila* from untreated wastewater exceeds $10^{-4}$ infections/year for workers within 500 meters of the active irrigator for the duration of every exposure event in the irrigation season. Therefore, we recommend that wastewater should undergo thermophilic anaerobic digestion, or another effective disinfection method, prior to use in spray irrigation to eliminate *L. pneumophila* and non-tuberculosis *Mycobacteria*.
3.6. Supplemental Materials

Model code and supporting documents are provided in a repository at
https://github.com/brennac2/Quantification-and-comparison-of-risks-associated-with-
wastewater-use-in-spray-irrigation.git
Chapter 4

Risk of Legionellosis in residential areas around farms irrigating with municipal wastewater

Jameson Mori\(^1\) and Rebecca L. Smith\(^1\)

\(^1\)University of Illinois Urbana-Champaign, Department of VMS-Pathobiology

4.1. Abstract

The threat of a world with limited freshwater resources is not a problem for the future, but an issue already being faced today. One of the largest consumers of freshwater is agriculture, which drains local surface or groundwater sources to produce food. A balance must be struck between agriculture and its water use, and one of the potential options is to irrigate with diluted wastewater. Instead of releasing wastewater into lakes or rivers, as is commonly done, this wastewater could be used to supplement the freshwater applied to crops, thereby recycling an otherwise lost resource. However, this wastewater can contain pathogens that are harmful to human health, such as *Legionella pneumophila*. This bacterium can cause severe pneumonia when inhaled and is readily found in these types of waters. Aerosolized *L. pneumophila* can travel at least 6 km from its point of origin and cause illness, raising potential concerns for people residing near agricultural operations. Thus, an assessment was conducted to determine the single exposure event risk posed to individuals living downwind of farms using wastewater in their irrigation. The assessment found that using low pressure irrigation systems to irrigate with wastewater could be health risks for people living 1 km downwind of the operation, while high pressure systems posed a risk up to 2 km downwind.

4.2. Introduction

Irrigation has been used in agriculture to supplement rainfall for thousands of years. The most common method in the United States is spray irrigation, which applies water from nozzles located a few meters above the crops (USDA). There exists a large body of work dedicated to characterizing various aspects of this irrigation, focusing on subjects ranging from calculation of
droplet size distributions (Kincaid, Solomon & Oliphant, 1996; Montero, Tarjuelo & Carrion, 2003; Ferguson et al., 2020) to evaporation rates (Uddin, Smith, Hancock & Foley, 2010; Yan, Bai, He & Li, 2010) and spray transport (Molle, Tomas, Hendawi & Granier, 2012; Molle et al., 2016; Tomas, Molle, Chevarin & Serra-Whittling, 2019). Another area of research involves the potential health effects of exposure to this sprayed irrigation water, either for farm workers or individuals living downwind of these farms (Dungan, 2014). Quantifying these health effects requires knowledge of the air concentrations of the water over space and time, and under various weather conditions. Depending on the research question being asked and the options available to the researcher, there are two main approaches to addressing this issue: empirical data collection and modeling.

Several studies have employed the former approach, gathering information from field tests of spray irrigators (Katzenelson and Teltch, 1976; Shuval et al., 1989; Molle et al., 2016). Air samplers and catch cans are common methods for measuring air concentrations, but these empirical methods suffer from some drawbacks, including an inability to capture the full range of weather conditions experienced over the irrigation season, as well as the natural errors associated with aerosol collection. Modeling, on the other hand, can incorporate historical weather data and provide dispersion predictions for all the days in the selected time range. Modeling also allows for examination of large spatial and temporal scales, and is commonly used by regulatory agencies like the US Environmental Protection Agency to assess the risks posed by pollution sources (US EPA, 1982). However, these models are built on equations that make approximations and are thus inherently limited in their accuracy.

Irrigation can be modeled several ways, but one of the most common is with a Gaussian plume model (Lighthart and Mohr, 1987; Dungan, 2014). This model type calculates the air concentrations of water emitted from a source in a specified area over time. The majority of Gaussian plume models that have been used to predict infection risks have focused on enteric pathogens (Katzenelson and Teltch, 1976; Bausum et al., 1983; Lighthart and Mohr, 1987; Shuval et al., 1989; Dungan 2014). Respiratory pathogens have only been examined by a few studies, even though research is starting to suggest that respiratory pathogens like Legionella pneumophila in irrigation water can pose a risk to exposed individuals (Pepper and Gerba, 2018).

*L. pneumophila* is a human respiratory pathogen that is ubiquitous both in natural and man-made environments (Heijnsbergen et al., 2015; Caicedo et al., 2016). This bacterium can
cause severe pneumonia when inhaled and deposited in the alveoli of the lungs (Hamilton and Haas, 2016). Some studies have identified the presence in and quantified the emission of this bacteria from human wastewater (Medema et al., 2004; Blatny et al., 2008; Mirzaee et al., 2015), indicating that this organism may be of concern when spray irrigating with this water. However, there are no known studies that investigate the risk of Legionellosis for individuals living downwind of the irrigator (>1 km).

In this study, we sought to explore this question of the risk of respiratory infection with L. pneumophila for residences downwind of agricultural operations. A Gaussian plume model was used to simulate the air dispersion of diluted municipal wastewater after spray irrigation. Infection risks for single exposure events are examined at distances of 1 to 7 kilometers from the center of the irrigator and compared to the general safety threshold of $10^{-6}$ infections/exposure event to determine areas of concern for those potentially exposed (Pedrero et al., 2010). The results of this assessment are then utilized to make recommendations regarding best practices for farmers and nearby residents.

4.3. Materials and Methods

Air dispersion modeling was the initial step of the risk assessment to determine where the aerosolized irrigation water was located and at what concentrations. This was conducted using the US EPA’s software AERMOD (v19191) and its weather data-processing software AERMET (v18081). Weather data was obtained from NOAA at the Lincoln-Logan Airport in Lincoln, Illinois, USA. The years examined were between 2017 and 2019 and included only the months in which irrigation typically takes place: May through September. In AERMET, the land was classified as flat and a correction for low wind speeds was applied.

In addition to the weather data, a key input to AERMOD was the droplet size distributions of the water particles emitted from the modeled sources. This distribution was determined for low (10 psi) and high (30 psi) pressure irrigation systems with the USDA Forest Service software AGDISP (v8.26), which reports the final droplet sizes after evaporation in the trajectory files. The median wind speed (2.36 m/s), temperature (77.09 °F), stability class (sunny), and relative humidity (66%) for Lincoln, IL were used as inputs for AGDISP, as determined from the AERMET files. The original droplet size distribution was calculated in AGDISP with the “parametric” option using a $D_{v0.5}$ of 757 μm and a relative span (RS) of 1.22
(Ferguson et al., 2020). The terrain was specified as having no overhead canopy and all other settings were AGDISP defaults (Teske and Curbishley, 2011).

Once the weather data and droplet size distributions were obtained, AERMOD was used to calculate the air concentrations of water around the spray irrigator. The irrigator - modeled as a series of 53 volume sources with dimensions 15 x 15 x 3 m for a total length of 800 m- was oriented both North to South and East to West to account for the impact of source orientation on dispersion. The water was approximated as PM$_{10}$ with a density of 1 g/m$^3$, since there is no specification for water in AERMOD’s list of pollutants (US EPA, 2019). Rings of receptors were specified around the irrigator at distances of 1, 2, 3, 4, 5, 6, and 7 kilometers from the center of the source. These distances were chosen because viable *L. pneumophila* has been detected up to 6 km from its point of origin, with the possibility of further spread (Nguyen et al., 2006).

A bacterial decay factor for *L. pneumophila* was calculated to allow consideration of bacterial death from the time of emission to inhalation (Equation 4.1). The parameters included in this calculation were bacterial decay rate, downwind distance, and wind speed. The values of these input parameters are provided in Table 4.1.

\[
LD = e^{-\lambda d \cdot \left(\frac{\text{dist}}{w_s}\right)} \quad (4.1)
\]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>wind speed</td>
<td>ws (m/s)</td>
<td>Figure 4.1</td>
<td>AERMET</td>
</tr>
<tr>
<td>bacterial decay rate</td>
<td>ld (hr$^{-1}$)</td>
<td>Beta(shape1=7.9, shape2=36341.2)</td>
<td>Hamilton et al., (2018)</td>
</tr>
<tr>
<td>distance downwind</td>
<td>dist (m)</td>
<td>Uniform(min=1000, max=7000)</td>
<td>AERMOD receptor distances</td>
</tr>
</tbody>
</table>
The distributions of the air concentration of wastewater and the calculated bacterial decay factor were then incorporated into the equation for the inhaled dose of *L. pneumophila* received by an exposed individual downwind of the active irrigator (Equation 4.2). The other input parameters considered in the inhaled dose equation were inhalation rate, source concentration of bacteria, exposure duration, percent wastewater, and alveolar retention rate. The values associated with these parameters are shown in Table 4.2. The number in the denominator of Equation 4.2 is for unit conversions between all the parameters. It was assumed that the irrigator was active for the entire duration of the exposure.

\[
Dose = \frac{(air \cdot I \cdot LP \cdot E \cdot P \cdot LD \cdot RR)}{9.97 \times 10^7} \quad (4.2)
\]
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol (Units)</th>
<th>Value(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>exposure duration</td>
<td>E (hr/day)</td>
<td>Figure 4.2</td>
<td>Beyer, Szabo, Hoormann &amp; Stolley (2018)</td>
</tr>
<tr>
<td>wastewater air concentration (low pressure)</td>
<td>air (μg/m³)</td>
<td>Empirical(min=0, max=765.3)</td>
<td>AERMOD output</td>
</tr>
<tr>
<td>wastewater air concentration (high pressure)</td>
<td>air (μg/m³)</td>
<td>Empirical(min=0, max=1506.1)</td>
<td>AERMOD output</td>
</tr>
<tr>
<td>inhalation rate</td>
<td>I (m³/hr)</td>
<td>Uniform(min=0.6, max=1.5)</td>
<td>US EPA (2011)</td>
</tr>
<tr>
<td>percent wastewater</td>
<td>P (%)</td>
<td>Uniform(min=0.05, max=0.2)</td>
<td>Dungan (2014)</td>
</tr>
<tr>
<td>alveolar retention rate</td>
<td>RR (%)</td>
<td>Uniform(min=0, max=0.5)</td>
<td>Heyder (2004)</td>
</tr>
<tr>
<td><em>L. pneumophila</em> source concentration</td>
<td>LP (cfu/L)</td>
<td>Uniform(min=0, max=125000)</td>
<td>Johnson, Jjemba, Bukhari &amp; LeChevallier (2018)</td>
</tr>
<tr>
<td>exponential dose-response parameter</td>
<td>rlp (unitless)</td>
<td>0.06</td>
<td>Armstrong and Haas (2007)</td>
</tr>
</tbody>
</table>
Once the distribution of inhaled doses was obtained, it was used in Equation 4.3, which is an exponential dose-response model, commonly used in risk assessments involving L. pneumophila. This dose-response model requires inputs for two parameters: an amount of pathogen received by the individual (dose) and a dose-response parameter, with the latter acquired from literature involving laboratory tests with animals or back calculations from well characterized outbreaks. The output of the dose-response equation is a distribution of single exposure event infection probabilities based on the included parameter values (Table 4.2). These probabilities were compared to the recommended risk threshold of $10^{-6}$ infections/exposure (Pedrero et al., 2010).

$$P_s = 1 - e^{(-rlp \cdot Dose)}$$ (4.3)
A diagram is provided in Figure 4.3 outlining the air dispersion and risk assessment process.

Following the risk assessment, a Sobol sensitivity analysis was carried out in order to determine what fraction of the output variance was attributable to each input parameter, both individually and in conjunction with the other parameters (Zhang, Trame, Lesko & Schmidt, 2015). This information was used to highlight parameters of importance and areas requiring further investigation, as judged by comparison to the general lower bound significance threshold of 0.05 (Zhang, Trame, Lesko & Schmidt, 2015). Scores could have values between 0 and 1. The best fit distributions for the parameters in this analysis are provided in Table 4.3. These distributions are either from fitting to data (air), using vast overestimations of potential values (I and LP), and bounds that span the natural range of possible parameter values (remaining parameters).
Table 4.3. Sobol Sensitivity Analysis Parameter Values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol (Units)</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>wastewater air concentration</td>
<td>air (µg/m³)</td>
<td>Gamma(shape=0.151, scale=0.004)</td>
</tr>
<tr>
<td>exposure duration</td>
<td>E (hours/day)</td>
<td>Uniform(min=0, max=24)</td>
</tr>
<tr>
<td>bacterial viability fraction</td>
<td>LD (%)</td>
<td>Uniform(min=0, max=1)</td>
</tr>
<tr>
<td>inhalation rate</td>
<td>I (m³/hour)</td>
<td>Uniform(min=0, max=10)</td>
</tr>
<tr>
<td>percent wastewater</td>
<td>P (%)</td>
<td>Uniform(min=0, max=1)</td>
</tr>
<tr>
<td>alveolar retention rate</td>
<td>RR (%)</td>
<td>Uniform(min=0, max=1)</td>
</tr>
<tr>
<td><em>L. pneumophila</em> source concentration</td>
<td>LP (cfu/L)</td>
<td>Uniform(min=0, max=1,250,000)</td>
</tr>
<tr>
<td>exponential dose-response parameter</td>
<td>rlp (unitless)</td>
<td>Uniform(min=0, max=1)</td>
</tr>
</tbody>
</table>

4.4. Results

Figure 4.4 provides a visualization and quantification of the air concentration of wastewater around the active irrigator for the low pressure system, while Figure 4.5 shows this for the high pressure irrigator. These figures reveal strong similarities between the dispersion patterns for both irrigators, indicating that the wind dispersal affected the majority of the aerosols the same. What differed between the two systems was the magnitude of the air concentrations of wastewater. The low pressure systems saw a maximum air concentration of 765.3 µg/m³ (Figure 4.4), in contrast with the high pressure system, which had a maximum closer to 1506.1 µg/m³ (Figure 4.5). This means that as the pressure of the spray increases, the fraction of droplets in the size range of concern (≤ 10 µm) also increases. A high pressure system therefore poses a greater risk to human health than a low pressure system because it generates a larger volume of aerosols that are biologically relevant to infection with *L. pneumophila*.
Figure 4.4. Air Concentrations of Wastewater Around the Low Pressure Irrigator

Dispersion of airborne wastewater aerosols around the active low pressure (10 psi) irrigator. The x and y axes show distance in meters. The air concentration for each receptor (μg/m³) is the median value for the years examined (2017-2019).
Dispersion of airborne wastewater aerosols around the active high pressure (30 psi) irrigator. The x and y axes show distance in meters. The air concentration for each receptor (μm/m³) is the median value for the years examined (2017-2019).

The farthest distance from the irrigator at which the mean probability of infection exceeded the $10^{-6}$ infections/event threshold was 1 km for the low pressure system (Table 4.4) and 2 km for the high pressure system (Table 4.5). All the maximum values surpassed this threshold, yielding highly right skewed distributions that can be seen in Figure 4.6. This plot shows the single exposure event infection probabilities for both the low (L) and high (H) pressure irrigators at each receptor ring distance, with one ring every kilometer from 1 to 7 km.
Boxplots of single exposure event probabilities. On the y-axis, “L” stands for “low pressure” and “H” stands for “high pressure”. The numbers represent the distance, in kilometers, from the source. The mean risk value for each combination is marked as a black dot. Due to extreme right skew, only values up to the 90th percentile are shown. The safety threshold is $1 \times 10^{-6}$ infections/event.

Tables 4.4 and 4.5 provide the summary statistics for the risk estimates for both the low (Table 4.4) and high pressure systems (Table 4.5). For the low pressure system, the only notable risk is at 1 km from the source, where the mean risk of infection exceeds the $10^{-6}$ infections/exposure threshold. At every distance, even the third quartile remains below the safety limit, which emphasizes the skew shown in Figure 4.6 and suggests that risky exposure events are the product of particular combinations of parameter values that are favorable for infection. Such parameter values include long exposure times, low levels of bacterial decay, and high source concentrations. For the high pressure system, the mean risk of infection was over the threshold for both 1 and 2 km, with the medians of all distances one or two magnitudes lower than that threshold. At 1 km, the third quartile risk also exceeded $10^{-6}$ infections/exposure, reflecting the greater risk posed by high pressure irrigation. No other third quartile risk exceeded
or even approached the threshold, echoing the finding for the low pressure system that unacceptably high risk exposures are caused by infection-promoting parameter value combinations. These results lead to the conclusion that most exposure events will not cause respiratory infections with *L. pneumophila*, however, some conditions - such as high bacterial source concentrations and long exposure durations - exist in which that risk is of an undesirable magnitude.

**Table 4.4. Risk of Infection for Low Pressure Irrigation Systems**

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Probability of Infection per Exposure Event</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 km</td>
</tr>
<tr>
<td>Min</td>
<td>0</td>
</tr>
<tr>
<td>Q1</td>
<td>4.7e-8</td>
</tr>
<tr>
<td>Median</td>
<td>2.2e-7</td>
</tr>
<tr>
<td>Mean</td>
<td>2.6e-6</td>
</tr>
<tr>
<td>Q3</td>
<td>8.6e-7</td>
</tr>
<tr>
<td>Max</td>
<td>1.5e-4</td>
</tr>
</tbody>
</table>

**Table 4.5. Risk of Infection for High Pressure Systems**

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Probability of Infection per Exposure Event</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 km</td>
</tr>
<tr>
<td>Min</td>
<td>0</td>
</tr>
<tr>
<td>Q1</td>
<td>1.1e-7</td>
</tr>
<tr>
<td>Median</td>
<td>5.0e-7</td>
</tr>
<tr>
<td>Mean</td>
<td>4.4e-6</td>
</tr>
<tr>
<td>Q3</td>
<td>1.8e-6</td>
</tr>
<tr>
<td>Max</td>
<td>1.9e-4</td>
</tr>
</tbody>
</table>
The Sobol sensitivity analysis revealed that the air concentration of wastewater contributed the most to the output variance of the probabilities of infection, as indicated by its large total order index in relation to the other parameters (Table 4.6). All parameters had a total order index score greater than the cutoff of 0.05, but only the first order index for the air concentration of wastewater surpassed 0.05. This indicates that synergistic effects between the parameters are more influential on the risk estimate variance than single parameter effects.

Table 4.6. Sobol Sensitivity Analysis Results

<table>
<thead>
<tr>
<th>Input Parameter</th>
<th>First Order Index Score</th>
<th>Total Order Index Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>air concentration of wastewater</td>
<td>0.351</td>
<td>0.941</td>
</tr>
<tr>
<td>exposure duration</td>
<td>0</td>
<td>0.347</td>
</tr>
<tr>
<td>bacterial viability fraction</td>
<td>0.013</td>
<td>0.284</td>
</tr>
<tr>
<td>inhalation rate</td>
<td>0.002</td>
<td>0.447</td>
</tr>
<tr>
<td>percent wastewater</td>
<td>0</td>
<td>0.220</td>
</tr>
<tr>
<td>alveolar retention rate</td>
<td>0.0005</td>
<td>0.164</td>
</tr>
<tr>
<td>source concentration of bacteria</td>
<td>0</td>
<td>0.358</td>
</tr>
<tr>
<td>exponential dose-response parameter</td>
<td>0</td>
<td>0.141</td>
</tr>
</tbody>
</table>

4.5. Discussion and Conclusion

The outcome of this analysis reveals several insights into the public health implications of and best management practices for irrigation with municipal wastewater. Two findings of note are that the air concentrations of wastewater-laden aerosols decrease with distance, and that lower pressure irrigation systems pose less of a risk to those 1 km or greater from the active irrigator. This first revelation highlights the importance of planning appropriate setback distances for residential areas located near farms. Medical professionals practicing in agricultural areas where municipal wastewater is being used for spray irrigation should also be made aware of the potential risk of Legionnaire’s disease or Pontiac fever for their patients. Based on the results of this model, areas with similar weather and geographical conditions should ensure that residential
areas are more than 1 km away from low pressure irrigators and more than 2 km from high pressure irrigators. That conclusion about decreased risk with increased distance from the source can be applied to other public health questions regarding spray irrigators, such as concerns about lawn or golf course sprinkler irrigation.

The second key finding is that scenarios modeled with low pressure irrigators yield lower risk estimates than high pressure systems. The difference between the low and high pressure irrigators and the risks they each pose is due to the mechanics of aerosol generation. At higher pressures, water is split into aerosols with droplet diameters smaller than would be observed at lower pressures (Montero, Tarjuelo & Carrion, 2003; El-Berry et al., 2009). These smaller droplets can travel much farther than their larger counterparts, which tend to settle sooner due to the effects of gravity (Bautista-Capetillo et al., 2009). The small droplets are also more biologically relevant to respiratory infection with *L. pneumophila*. These factors result in aerosol plumes that can carry harmful bacteria kilometers from the point of origin. For these reasons, it is recommended that lower pressure irrigation systems be adopted when irrigating with municipal wastewater.

The Sobol sensitivity analysis revealed that the input variables for which accuracy is most important are the air concentration of wastewater, inhalation rate, source concentration of bacteria, and exposure duration. These four variables contribute the most to the overall variability of the risk output, so limiting their uncertainty is vital for a robust analysis. It should also be noted that the first order indices for all the variables, except the air concentration of wastewater, were relatively low. This indicates that variable interactions were more influential than individual contributions to the output variance, which should be taken into consideration when planning future studies. Having the air concentration of wastewater be the exception shows that it is the driving factor behind the risk prediction. This makes sense, given that the presence or absence of bacteria laden aerosol determines if there is any risk at all, and it is on this parameter that all the others are dependent. The external parameter on which this air concentration relies - weather - is also highly variable, which is likely why the air concentration first order index score is much larger than the rest. Future investigations should focus on improving the characterization and quantification of these four variables. Another future investigation could look at the application and associated risks of using this type of source water.
for lawn irrigation in suburban areas, since these locations would be closer than agricultural operations to the wastewater treatment plants.

Additional recommendations that can be made for managing the risks associated with municipal wastewater irrigation are to irrigate at times when individuals living nearby may be away from their home. Irrigating during weather conditions that are sunny, hot, and dry may also help kill bacteria contained in the aerosols (Katz and Hammel, 1987; Dennis and Lee, 1988; Chang et al., 2012). Lastly, avoiding irrigation during windy days would also help to limit the distance traveled by these aerosols.

In conclusion, *L. pneumophila* poses a health risk to those spending time outside up to 2 km from an irrigator actively spraying diluted municipal wastewater. However, given proper safety precautions, this wastewater can be used to supplement freshwater and reduce demands on local water supplies.

4.6. Supplementary Materials

The code used for this analysis can be found at:

https://github.com/brennac2/Legionella-Irrigation-Risk-Assessment
Conclusion

The world is running out of freshwater, and with agriculture as its main consumer, it is becoming increasingly important to investigate ways to reduce this negative impact. This dissertation explores both the direct and indirect public health implications of two major solutions: alternative agricultural methods that use less water and alternative water sources for traditional agriculture. Chapter 1 focused on pathogen transmission and control in hydroponics, aquaculture, and hydroponics systems, and found that research is entirely lacking for aquaponics in this subject area. It was also found that transmission happens easily in these systems, and each disinfection method has its own benefits and limitations, often specific to the type of system in which it’s employed. Chapter 2 dealt with issues of maintaining proper water chemistry in aquaponics systems and modeling shifts in pH using standard and Bayesian linear regression. A minimalist model was generated for the two aquaponics systems for which data was obtained, with a day’s pH being able to be predicted by the pH of the prior two days, though the main conclusion drawn in this study was that larger amounts of more frequently collected data are necessary to build stronger models. These two chapters demonstrated that research in the field of aquaponics is absent for questions regarding system health factors such as infectious diseases and water chemistry.

When examining alternative water sources to supplement freshwater for use in traditional agriculture, a common source is wastewater, either animal or human (municipal). Chapter 3 studied the potential risks to farmers and farmer workers of using swine wastewater in spray irrigation, specifically comparing the threat of respiratory infection with \textit{L. pneumophila} and non-tuberculosis \textit{Mycobacteria} to the danger of ammonia toxicity. It was found that Legionella posed the greatest risk, though the annual probabilities of infection were driven by a small subset of single exposure infection probabilities, making respiratory infection an uncommon occurrence for the scenarios modeled. Ammonia was rarely a hazard and non-tuberculosis \textit{Mycobacteria} were not a concern for the simulated conditions. Chapter 4 built on this analysis by examining the risk of infection with \textit{L. pneumophila} for individuals living within 7 kilometers of low and high pressure spray irrigators using municipal wastewater that was both diluted and treated prior to use. This analysis determined that low pressure irrigators could be harmful up to 1 km downwind of the irrigator, while the risk for high pressure irrigators extended to 2 km
downwind. The findings of Chapter 3 and 4 show that different kinds of wastewater can be safely used as a supplement to freshwater when spray irrigating in traditional agriculture if the water is disinfected prior to use and a setback distance of more than 2 km is established for nearby residential areas.

This dissertation answers some questions and raises others. The work presented here on aquaponics highlights how, currently, little is done to prevent or control pathogens in these systems. Research on this subject is scarce, though conclusions drawn from studies in hydroponics and aquaculture can be used in lieu of direct information. Since the publication of “Transmission of waterborne fish and plant pathogens in aquaponics and their control with physical disinfection and filtration: a systematized review”, there have only been three other publications regarding pathogens in aquaponics systems: two articles (Sirakov et al., 2019; Rivas-García et al., 2020) and one book (Goddek, Joyce, Kotzen, and Burnell, 2019). These works have added to our general knowledge of the potential use of biological control agents for plant pathogen control in aquaponics (Rivas-García et al., 2020), including seaweed extracts (Sirakov et al., 2019), as well as various aspects of aquaponics technology, system design and maintenance, and economic concerns (Goddek, Joyce, Kotzen, and Burnell, 2019), but there is still much research to be conducted. Specifically, additional investigation must be done into pathogen control options that are safe for both fish and plants, as well as practical and effective preventative biosecurity procedures. Interactions between fish and plant pathogens, or plants and fish pathogens, should also be studied in more depth.

In terms of water quality modeling in aquaponics, there exists no other model for pH in this type of agricultural system. The fact remains that pH is a key water quality parameter that drives the health of the entire system, and prediction of this parameter would aid aquaponics farmers in their decision-making for system maintenance. The current limitations of the presented model lie in the aquaponics water quality data itself. Some variables were measured daily and others weekly, which were frequencies determined inadequate for building a model of this kind. In future data collection efforts in aquaponics systems, it is recommended that continual digital monitoring be employed for as many water quality parameters as possible, given technological and budgetary constraints. This monitoring should be done at the same locations in the system with the same equipment. Other modeling methods should also be attempted to see if there are better ways to predict pH, such as machine learning. It is also critical
to examine data from a variety of system types and configurations, as well as crops, other than those studied here to broaden the applicability of the model results.

For traditional agriculture, it was determined that there are risks associated with spray irrigation with diluted wastewater, but that these risks can be managed through disinfection and maintaining a safe distance. Some recommendations that can be made based on the findings of these two chapters include remaining more than 2 km downwind of active irrigators when possible, and to wear personal protective equipment such as masks when work near the active irrigator is required. It would also be preferable to avoid spray irrigation on windy days since higher wind speeds promote further dispersion of contaminated aerosols. Lastly, scheduling irrigation on warm, sunny, and/or dry days to increase bacterial inactivation once it is emitted into the environment would be ideal.

Validation of the air dispersion model would be a beneficial area of future research. This could be done either empirically through field sampling or through modeling the same conditions using different air dispersion models. Better characterization of parameter distributions would also improve the model and reduce output uncertainty, with emphasis on parameters whose distributions were based on extrapolation from related data, like exposure duration. Collecting samples of wastewater directly from swine lagoons for bacterial quantification would also enhance the QMRA, given that the samples gathered for this study were an indirect approximation of the source concentrations. Exploration of related questions regarding the use of either animal or municipal wastewater in other applications, such as irrigation of lawns or golf courses, would provide additional insight into the public health implications of this water-saving option. Finally, increasing our understanding of aerosol droplet size evolution from the point of emission to inhalation or deposition would allow better source characterization for air dispersion modeling.

Overall, this dissertation has highlighted the gaps in research on water-based system health questions for both alternative and traditional agriculture, while also filling some of those identified gaps. The findings herein should be utilized as a guide and springboard for future work regarding the improvement of aquaponics systems and the advancement of more environmentally friendly practices in traditional spray irrigation.
References


