# **Microbial Quality of Aquaculture Water Used for Produce Irrigation**

by

Evelyn Sophia Willmon

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Approved by

Sondra Jean Weese, Chair, Professor of Poultry Science
Tung-Shi Huang, Professor of Poultry Science
Terry Hanson, Professor of School of Fisheries, Aquaculture and Aquatic Sciences

## Abstract

The use of recycled water from an aquaculture fish tank to irrigate produce, or aquaponics, has grown rapidly in the past decades, with a large diversity of system designs. Since the water is reused from the fish tank, there are concerns about the produce grown becoming contaminated by foodborne pathogens such as *Escherichia coli, Salmonella*, *Listeria monocytogenes,* etc. that might be present in the fish waste. This study evaluated the microbial quality of the water from a tilapia production tank for irrigation and the soil used for tomato and cucumber growth. The possibility of *Listeria monocytogenes* and *Salmonella* contamination in the tomatoes and cucumbers was also examined. Populations of generic *E. coli* and coliforms, commonly used as indicators of fecal microbial contamination and water quality, were monitored every two weeks. Water effluents from the tilapia fish tank and the plant soils were collected, with 5 replicates for water samples and 15 replicates for soil samples. *E. coli* and coliform populations were detected using 3M Petrifilm *E. coli*/coliform plates, and the data was analyzed using ANOVA. *L. monocytogenes* and *Salmonella* were monitored on tomatoes and cucumbers by plating methods, and confirmed by PCR. The *E. coli* population in the tank effluent had a geometric mean (GM) of 49 CFU/100mL and a statistical threshold value (STV) of 62 CFU/100 mL in November 2016, decreased to an undetectable level during winter, and rose to a GM of 30 CFU/100 mL and a STV of 127 CFU/100 mL in May 2017. Coliform populations followed a similar trend, with the highest and lowest populations having a GM of 1,820 and 3 CFU/100 mL, respectively. Populations of *E. coli* and coliforms in soil were typically higher than in water, with

the highest at 2940 CFU/g for coliforms and 293 CFU/g for *E. coli*. *L. monocytogenes* was detected in five cucumber and one tomato samples; *Salmonella* wasn’t found in any produce samples. The generic *E. coli* population in irrigation water is lower than the regulation limits of 126 CFU/100mL (GM) and 410 CFU/mL (STV) set by the U.S. FDA's Produce Safety Rule.

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## List of Abbreviations

BS-Bismuth sulfate agar

CFU-colony forming unit

GM-geometric mean

HE-Henkoten enteric agar

MOX-modified Oxford agar

mTEC-modified thermotolerant *E. coli* agar

PCR-polymerase chain reaction

RV-Rappaport-Vassiliadis enrichment broth

STV-statistical threshold value

TT-tetrathionate broth

VRBA with MUG-violet red blue agar with 4-methylumbelliferyl-ß-D-glucuronide

XLD-xylose lysine deoxycholate agar

# Chapter 1 Introduction

Aquaponics is a novel and relatively new method of farming that has grown in popularity over the past decades. While aquaponics systems vary greatly in design, they all work off the basic principle and design where the aquaculture, or farming of aquatic animals such as fish, is integrated with the farming of produce, usually hydroponically. Wastewater generated by the aquatic animals contained in tanks is high in nitrates, an excellent source of fertilizer for plants, as *Nitrobacter* and *Nitrosomonous* spp. contained in the biofilter can convert ammonia excreted by the fish into nitrate usable by plants (Hu, 2015; Kowalchuk, 2001). This water is then removed from the system and delivered to the plants, and depending on the design of the system, the water may be returned to the fish tanks. A decoupled, linear system where water for irrigation is not returned to the fish component and the water removed from the fish tank is replenished by an outside source, can offer several advantages over a coupled system (Goddek, 2016). These advantages include the ability to manage both the fish and plant components separately, and conditions can be maximized for growth of each system (Goddek, 2016; Pickens, 2015). Conditions for plants, such as certain trace elements and nutrients lacking in aquaponics systems needed for plant growth, can be added without disrupting the water chemistry or threatening the health of the fish (Pickens, 2015; Rakocy, 2006). Certain species of plants, such as lettuce, tomatoes, and cucumbers, are more popular as they do well when grown in these types of systems (Love, 2014; Pickens, 2015).

Information regarding the safety of produce grown in aquaponic systems is minimal. It has been proven that plants grown in hydroponic systems can internalize bacteria including potentially pathogenic species such as *E. coli* and *Salmonella* into produce, presenting a health risk to humans (Hanning, 2009: Lopez-Galvez, 2014). Various species of bacteria, including Firmicutes (includes *Listeria monocytogenes)* and Proteobacteria (includes *E. coli* and *Salmonella*) have been found to inhabit various parts of the aquaponic setup, although *Cetobacterium*, a common fish gut inhabitant, makes up the majority of microbial system flora (Schmautz, 2017). Fish also can carry pathogens that could impact human health (Novoslavskij, 2016). *E. coli* is a major concern, since it may be introduced through various routes, including deposited from fecal matter from the fish or from water that is added to the system from outside sources to replace water lost to evaporation (Fox, 2012). A study examining bacterial populations of leafy greens grown in aquaponic systems identified the presence of non-pathogenic *E. coli*, *Enterobacteriacae* (which includes *E. coli* and *Salmonella* spp.), and members of the genus *Listeria* on the greens (Barnhart, 2015).

Food safety regulations regarding aquaponics food safety are minimal, often different from each other depending on the regulatory agency. The World Health Organization has published guidelines regarding the use of waste-fed aquaculture, but they are more targeted towards the use of wastewater from human sources and storm runoff water to fertilize fish ponds and not aquaponics operations (WHO, 2006). The Food and Drug Administration (FDA) is currently the agency in the United States that monitors both seafood (excluding Siluriformes fishes, which fall under the USDA) and produce, but on the state level, regulations is much more patchwork (FDA, 2015 ; Stivers, 2016). State agencies may find themselves sharing oversight along with other agencies, and many have little or no experience or knowledge of regulating aquaponics

operations (Stivers, 2016). With the passage of the Food Safety Modernization Act (FSMA) and the Produce Safety Rule, the monitoring of the microbial quality of the water used in irrigation of produce is now required by law (FDA, 2015). The requirements for agricultural irrigation water are a geometric mean of less than 126 CFU/100 mL and a statistical threshold value of less than 410 CFU/100 mL for generic *E. coli* (FDA, 2015). Aquaponics water is currently classified by the FDA as close to agricultural water but within its own category, as it does not directly contact the edible portions of the plant (such as leafy greens or fruit) (FDA, 2015), but some state agencies range from classifying it as closer to raw manure to the FDA's view of it as agricultural water (Stivers, 2016).

Confusion about regulations, as well as the lack of knowledge on potential microbial risks in aquaponics systems, can also cause issues for commercial produce growers, as buyers and retailers are reluctant to buy product without assurances such as a third-party audit for food safety control measures (Aquaponics Association, 2015). Within the industry itself, the Aquaponics Association have issued a Good Agricultural Practices guide in Aquaponics (Aquaponics Association, 2015). They recommend practices that echo FDA recommendations, such as not allowing edible portions of the plant to contact the water and keeping good hygiene practices like the exclusion of vermin and hand washing. (Aquaponics Association, 2015; FDA, 2015)

Aquaponics was specifically addressed by the FDA in the Final Rule on Produce (FDA, 2015). Subpart E, concerning agricultural water standards, only applies if edible parts of the plants contact/are likely to come in contact with water from the fish. Subpart F, concerning biological soil amendments (such as manure) only applies if plants are not grown in a liquid only matrix. Subpart I, concerning the contamination from domestic or wild animals, will only apply if the

facility is not located indoors or in a greenhouse. Subpart E is considered by the FDA to be the best to regulate concerns that come from possible contamination by the fish (FDA, 2015; Stivers, 2016). They also recommend using potable water sources as the only water to be added to the system to prevent contamination by outside sources, a sentiment echoed by other sources (Aquaponics Association, 2015; Fox, 2012; Hollyer, 2009). The main sources of contamination in aquaponics operations are contamination from humans and from water sources used for irrigation; for example, farms that were using surface water were found to have higher instances of circulating coliforms (Fox, 2012; Hollyer, 2009).

Aquaponics offers many advantages and innovations to food production in both aquaculture and produce production fields, but due to the lack of research, the food safety risks remain a significant hurdle. This negatively affects both the agencies trying to establish a regulatory framework to protect consumers and the growers who try to sell their products on a commercial scale. The objectives of this study were to 1) evaluate the populations of general coliforms and *E. coli* that are commonly found in an aquaponics system over the course of a whole year: 2) determine the populations of general coliforms and *E. coli* present in the substrate for plant growth over the course of a whole year: and 3) determine if the produce grown in the aquaponics system carries foodborne pathogenic bacteria.

# Chapter 2 Literature Review

## 2.1 Functionality of Aquaponics Systems

Aquaponics systems are a marriage of recirculating aquaculture, or the farming of fish in a recirculating system, with hydroponics, where plants are grown in water, into a single arrangement. The fish are usually held in a recirculating system that consists of a tank attached to a filter that allows for both the settling and removal of solids and the bio-filtration (use of microorganisms to remove harmful contaminants) of the water. Tilapia is the most commonly used species, as it is a hardy species that is very tolerant of varying water conditions, high density stocking, and that grows quickly when fed with fish feed (Rakocy, 2006).

Traditional recirculating aquaculture systems contain a large number of fish grown in a relatively small volume of water. The fish excrete wastes such as ammonia from their gills and as solid wastes, which can be toxic to fish if not removed or converted into a less-toxic form. Recirculating systems usually have some form of a biofilter, which provides an aerobic site for *Nitrosomonous* and *Nitrobacter* bacteria to convert ammonia into nitrite, and then into nitrate (Kowalchuk, 2001). Ammonia and nitrite are very toxic to fish, and can cause death in high concentrations. Nitrate is relatively harmless in comparison, and is the form of nitrogen commonly used to grow plants.

As time passes, the amount of nitrate, organic matter, and non-toxic nutrients build up as the water is continuously recycled. These dissolved nutrients, especially nitrates, can be utilized by plants as a type of fertilizer that promotes the growth of the plant. Certain species of plant can

uptake more nitrogen from the water than other species, and are more well suited to a hydroponic or aquaponics setup such as tomatoes (Hu, 2015).

The basic aquaponics system consists of a rearing tank where the fish are located attached to a biofilter and a method of solids removal, such as a settling tank (Buzby, 2014). The hydroponic system is fed water that has gone through both the solids removal and biofilter, and is physically separated from the fish rearing tank. Water is then collected in a sump at the end of the hydroponic system, and may or may not be recirculated back to the fish rearing tank.

Aquaponic system design varies among different systems, and is commonly influenced by factors such as location, climate, and types/species of crop(s) grown (Love, 2014). Different crops have various requirements depending on how they are grown within a system, and modifications may be made to the basic system design in order to accommodate the plants for maximum production. Some federal regulations may also determine certain components of the design (Diver, 2006). The capacity is also very important when designing an aquaponics system, as the uptake of nutrients by the plants must be balanced with the output from the fish (Buzby, 2014). A survey of commercial aquaculture producers in 2013 found that frequently grown plants in aquaponics include salad greens, tomatoes, cucumbers, peppers, head lettuce, and kale. It also found that most producers used tilapia as their fish species, and that most of them had their operations located in some sort of a greenhouse, or at least partially in one (Love, 2014). In 2015, a study at Auburn University compared the economics of using fertilizer versus the effluent from an aquaponics system in growing cherry tomatoes. It was found that the water use index and nitrogen conversion ratio were reduced when the aquaponics system was compared to the fertilizer. Fertilizer savings also resulted in a higher net return (Pickens, 2015).

A drawback of the recirculating model of aquaponics where water is returned to the fish culture tank after use by the plants is the growing conditions for plants and fish must be adjusted to meet the requirements of both, and this may not be the optimal growing condition for either the fish or the plants. Some nutrients needed for plants are naturally lacking in aquaponics systems, including potassium, calcium, and iron which need to be added as a supplement to the system for proper plant growth (Rakocy, 2006). Plants often suffer poor growth in alkaline conditions, and this issue is typically remedied by injecting acid into the water used for irrigation in greenhouse setups with alkaline irrigation water (Pickens, 2015). However, this is not ideal for aquaculture, as changes in acidity can change pH and be detrimental to both fish and nitrifying bacteria (Pickens, 2015; Rakocy, 2006). A decoupled aquaponics system does not recirculate the water back to the fish culture tank, so it is a linear flow where only some water is taken out at a time and sent to the plants, and another source of water is used to replenish the fish tank as the water is lost to the hydroponic unit. The advantage of the decoupled unit is that the hydroponic component can be managed separately from the fish component, and conditions can be maximized for plant growth within the hydroponic component without affecting the fish (Goddek, 2016).

## 2.2 Food Safety Concerns

Information on safety of produce grown in aquaponics systems is lacking, which has raised the concerns of food safety. There are some foodborne pathogens that might live in aquaponics systems, fish, or produce (Novoslavskij, 2016). These include *Salmonella* spp., a cause of gastroenteritis, and *Listeria monocytogenes,* the agent that causes listeriosis, which are commonly found in food (Novoslavskij, 2016). *Escherichia coli,* with some strains that can produce the Shiga toxin, is also present, usually from fecal matter from the fish that is deposited

directly into the water, or from water that is introduced to the system to replace water lost to evaporation (Fox, 2012).

There is a great concern that these pathogens may be transmitted via the water into the plants, especially in produce such as tomatoes or leafy greens that are consumed raw, which have a higher chance of impacting human health. Plants are able to internalize bacteria, especially *E. coli* and *Salmonella* spp. via water (Lopez-Galvez, 2014). There are recorded outbreaks of salmonellosis in the United States due to the irrigation of produce with contaminated water where the *Salmonella* was internalized by the plants after watering (Hollyer, 2009).

Schmautz et. al. (2016) determined microbial diversity located at different parts of an aquaponics system. The model system consisted of tilapia grown in a tank with water that was fed to a hydroponic unit with lettuce on floating rafts, and then the water was returned to the fish tank. DNA was extracted from different parts of the aquaponics system and 16s rRNA sequencing via Illumina was performed. Microbial communities in the biofilter, plant roots, and periphyton (biofilm from the side of the fish tank) were all found to be very similar to each other. Proteobacteria, such as *Escherichia* and *Salmonella*, are the dominate genera in all three groups. Firmicutes, which includes *Listeria* spp., was also present in all three groups. When the feces of the fish were examined, both Firmicutes and Proteobacteria were present; however, Fusobacteria (which includes *Cetobacterium,* a common fish gut inhabitant) made up about 75% of the total genera.

A study from the University of Hawai'i at Manoa (2012) examined microbial numbers in various aquaponics systems in both water and fish fillets from these systems. Standard methods recommended by the Environmental Protection Agency (EPA) were used to search for *E. coli*, while simultaneously using polymerase chain reaction (PCR) to examine samples for *E. coli* and

*Salmonella.* Tests on tilapia fillets harvested from the systems produced neither *E. coli* nor *Salmonella* despite coliforms and *E. coli* being detected in system water. It was concluded that no significant risk was posed to the consumer from the fish raised in the system, as most of the indicator organisms and pathogens that would be of a concern are commonly found in the tracts of endothermic animals, such as birds and mammals, and not exothermic animals such as fish. It was also noted that out of eleven of the sites they sampled at, two farmers used surface water (from irrigation canals) to irrigate their plants and tanks instead of potable city water. Both of these farms had higher instances of circulating coliforms than other farms in the study, and the investigators therefore recommended using potable water sources for aquaponic production.

A previous publication by the same university from the extension office echoes the same recommendation. It also indicates the biggest issues for safety besides water sources are human contamination, safe harvest of produce, and zoonoses such as *Salmonella* and *E. coli.* They also recommended that *E. coli* populations shouldn't exceed more than 126 per 100 mL of water (Hollyer, 2009).

A study conducted by the University of Minnesota in 2015 examined the presence of several groups of microorganisms in leafy greens (such as lettuce) produced in aquaponic systems and sold locally at grocery stores (Barnhart, 2015). The investigators examined the numbers and presence of non-pathogenic *E. coli, Enterobacteriaceae* (members of this family include *E. coli* and *Salmonella*), and *Listeria monocytogenes* on leafy greens. The study found relatively high levels of aerobic bacteria on the leafy greens detected by aerobic plate count method. Coliforms and *Enterobacteriaceae* were found to be present in all treatments, but there were low values for non-pathogenic *E. coli*, less than 10 CFU/g. *L. monocytogenes* was tested for using a genus

specific test, which indicated that *Listeria* spp. were present, but was unable to determine if it was specifically *Listeria monocytogenes*.

### 2.3 Food Safety Regulations on Aquaponically Grown Produce

Food safety in aquaponics is an unclear subject, with not much knowledge or research having been done. Few, if any, regulations exist, and the ones that do are minimal at best. The World Health Organization has recommendations for the use of wastewater in aquaculture, but they are mostly associated with using wastewater from human sources and storm runoff, not in the context of an aquaponics setup (WHO, 2006). Currently, the FDA is the regulatory agency in charge of regulating food safety both for seafood (excluding Siluriformes species) and for produce grown on interstate commerce. On the state level, multiple agencies may be sharing regulatory oversight of aquaponics operations, and many of these agencies have little to no experience in regulating aquaponics operations of any kind. Confusion may arise between different regulatory bodies, and views on the safety of the water may vary greatly-some regulatory agencies may consider the water to be equivalent to raw manure and may prohibit its use, while others consider it to be agricultural water that must be held to microbial water testing standards (Stivers, 2016). The FDA currently classifies the water as being close to agricultural water, but in a separate category, as the water does not directly come in contact with the edible portions of the plant (leafy greens or fruit) (FDA, 2015). The current requirements under the Produce Safety Rule for agricultural irrigation water include a geometric mean of less than 126 CFU per 100 mL of water and a statistical threshold value of less than 410 CFU per 100 mL water for generic *E. coli* (FDA, 2015).

The World Health Organization does have some guidelines regarding the use of waste-fed aquaculture. They recommend less than $10^3$ CFU of fecal coliforms per 100 milliliters of water

in fish pond water, and the edible muscle in the fish is typically contaminated when populations of fecal coliforms and salmonellae reach $10^4$ and $10^5$ CFU/100 mL water, respectively (WHO, 2006). However, most of the guidelines are more directed towards the use of wastewater and excreta used to fertilize fish ponds, and not specifically towards aquaponics operations.

In 2015, the FDA specifically addressed aquaponics and hydroponics operations under the Food Safety Modernization Act (FSMA) in the final rule on the handling of produce (FDA, 2015). Under the new FSMA rules, Subpart E, which concerns agricultural water and standards, only applies if the edible parts of the produce come in contact with or are likely to come in contact with the water from the fish. Subpart F, which concerns biological soil amendments (i.e. manure) only applies if plants are grown in a semi-solid or solid matrix; if they are grown in a liquid only matrix, then Subpart F does not apply. Subpart I, which concerns potential contamination and control of domestic and wild animals only applies to operations if fish are housed outside or in partially enclosed buildings; greenhouses and indoor facilities are not affected by this rule. Subpart I also does not apply to fish raised in an aquaculture operation. The FDA considers Subpart E to be better suited to regulate the concerns that are raised by the possible contamination of fish rather than Subpart I (FDA, 2015; Stivers, 2016).

There is also no standard for food auditing specifically tailored for unique needs of the aquaponics industry. This can present a problem for growers attempting to grow on a commercial scale and sell their products to larger buyers, as many big box retailers and buyers require third party audits for food safety before they commit to purchasing a product (Aquaponics Association, 2015). The lack of this effectively means that many growers are unable to enter the market easily. Some industry efforts have been made, such as the Aquaponics Association having issued a Good Agricultural Practices in Aquaponics. They recommend

general hygiene such as hand washing and vermin exclusion, the construction of facilities and environments that are discouraging to the colonization by foodborne pathogens to a system, and either preventing edible parts of the plant from contacting system water or sanitizing the water with methods such as ultraviolet or ozone.

# Chapter 3 Materials and Methods

## 3.1 Sample Collection

Sample collection was performed every 14 days (2 weeks). Collection of water, soil medium, and produce (if present) samples from the aquaponics system were performed simultaneously. The aquaponics system consisted of two large fish tanks containing Nile tilapia (*Oreochromis niloticus)*, with water removed at the filter and drip-irrigated onto plants held in plastic planters with two plants per pot, and excess water collected in tanks after irrigation (Figure 1).

Samples of water were collected at both the effluent point for the water from the fish tanks, before it entered the irrigation system for the greenhouse, and the effluent at the end of the irrigation system that passed through each set of plants. The water was collected in sterile plastic bottles and transported to the lab for immediate processing.

Samples of the soil medium from each pot in each treatment (pine bark for cucumber, perlite for tomato, perlite for cucumber, and pine bark for tomato) were collected, approximately 100 g, using a premeasured marker cup. All cups were sterilized before use, and a different cup was used for each treatment. The soil media were placed in sterile plastic bags and transported back to the lab.

If cucumbers and tomatoes were available, approximately three cucumbers and six tomatoes were collected from each pot. They were placed in sterile sample bags and the bags were transported to the lab and refrigerated before processing.

### 3.2 *Escherichia coli* and coliform detection in water

The methods described in both the EPA method 1103.1 and the 3M Petrifilm Interpretation Guide: *E. coli*/Coliform Count Plate were followed with modification (EPA, 2010; 3M Food Safety, 2017). Ten milliliters of sample were placed into a dilution bottle with 90 mL of sterile phosphate buffered saline (PBS) for a 1:10 dilution and a ten-fold dilution made two more times. One milliliter was removed from the original sample and the $10^{-1}$ and $10^{-2}$ dilutions, and placed on 3M *E. coli*/coliform Petrifilm plates. The Petrifilm plates were incubated for 24 h at 37 °C.

For the filtration method, 100 mL of a water sample from each dilution was through a sterile 0.45 um filter with a 47 mm diameter grid side up in a sterile apparatus. The filter was then transferred to a membrane-Thermotolerant *Escherichia coli* agar (mTEC) plate with the bacteria side facing up (grid side). The filter was rolled onto the agar to avoid bubbles between the filter membrane and medium. This process was repeated for all water samples.

The plates were allowed to sit at room temperature for 1 h to ensure proper contact between the filter and the agar. Then, plates were inverted and incubated at 37 °C for 2 h. Afterward, the plates were transferred to 44.5 °C for 22 h incubation.

After incubation, *E. coli* colonies on the Petrifilm plates and mTEC plates were recorded for analysis. The coliform colonies on the Petrifilm plates were also recorded.

### 3.3 *Escherichia coli* detection in produce growth media

The methods described in the 3M Petrifilm Interpretation Guide: *E. coli*/Coliform Count Plate were followed with modification (3M Food Safety, 2017). Approximately 25 g of sample were placed in a sterile bag and diluted with sterile PBS at a 1:10 ratio using an automatic dilution instrument. Then, the samples were blended by a stomacher at 260 rpm for 2 minutes. A 10-fold serial dilution was made for each sample up to $10^2$ dilution.

One mL of sample from each dilution was plated in duplicate on 3M *E. coli*/coliform Petrifilm plates. Another 1 mL of sample was spread plated in duplicate on VRBA agar with MUG plates. The spread plating was performed in duplicated for each dilution sample. All Petrifilm and spread plates were incubated at 37 °C for 18-24 h. The *E. coli* and coliform colonies were then recorded for analysis.

### 3.4 Preparation of produce for microbial isolation

Samples of cucumbers and tomatoes harvested from the aquaponics system and refrigerated until processing. Cucumbers and tomatoes were sliced, using an ethanol sterilized cutting board and knife. Then, 25 g of each sample were placed into a sterile stomacher bag, and two bags of each sample were prepared. Sample bags were separated into two sets in numerical order, with one being for *Listeria monocytogenes* and *Salmonella* isolation, respectively.

### 3.5 Isolation of *Listeria monocytogenes* from produce

Standard methods for the detection and enumeration of *L. monocytogenes* in food by the FDA with modifications were followed (FDA, 2016). One hundred milliliters of buffered *Listeria* enrichment broth (BLEB) were added to each bag in the set of samples for the *L. monocytogenes* isolation. All bags were stomached at 260 rpm for 2 min, and then incubated at 30 °C for 4 h. After 4 h incubation, three filter sterilized selective agents (acriflavine, cycloheximide, and nalidixic acid) were aseptically added to each sample bag at the concentrations of 10 mg/L acriflavine, 40 mg/L cycloheximide, and 50 mg/L nalidixic acid. The bags were closed and incubated at 30 °C for another 20 h. Then, each sample was streaked on modified Oxford agar (MOX), and incubated another 24 h. After 24 h, each sample was streaked again on MOX plates. The MOX plates were incubated at 37 °C for 48 hours, and bacterial growth was examined at both 24 and 48 h. Black colonies surrounded by a black halo were recorded as *L. monocytogenes* for analysis.

### 3.6 Isolation of *Salmonella* from produce

Standard methods for the detection *Salmonella* in food by the FDA with modifications were followed (FDA, 2016). One hundred milliliters of buffered peptone water (BPW) were added to each stomacher bag from the set for *Salmonella* isolation. Each bag was stomached for 2 min at 260 rpm and then incubated at room temperature for 1 h. After 1 h, the pH of each sample was measured and adjusted to 6.8±0.2 if necessary. The tops of the bags were left loose and the samples were incubated 24 h at 35 °C. After incubation, 0.1 mL and 1mL of each sample were added to 10 mL Rappaport-Vassiliadis (RV) broth and Tetrathionate (TT) broth, respectively. All samples were incubated for 24 h, with the RV at 44.5 °C and the TT at 37 °C. After 24 h incubation, each RV and TT sample was streaked onto Bismuth Sulfate (BS), Hekotoen Enteric

(HE), and Xylose Lysine Desoxycholate (XLD) agar plates and the plates were incubated at 37 °C for 24 h. Plates were examined for *Salmonella* positives and recorded.

### 3.7 PCR Verification of *L. monocytogenes* and *Salmonella* positive samples

Presumptive *Listeria monocytogenes* colonies were picked off the modified Oxford agar and suspended in sterile water. After vortexing, the samples were transferred to a 96-well PCR plate, where the positive and negative controls (*L. monocytogenes* and *L. innocula* ATCC33090 respectively) were included in the plate. A mixture of 4 µL dNTPs (2.5 mM each), 5 µM of 10x PCR buffer, 3 µL of $MgCl_2$ (25 mM), 36.5 µL sterile water, 0.5 µM of Taq enzyme, and 1µM of primer mix (0.5 µM each) were added to each well. PCR conditions were programmed at 95 °C denaturation step for 5 min, followed by 40 cycles of heat denaturation at 95 °C for 30 s, an annealing step at 54 °C for 1 min, extension for 1 min at 72 °C, and the final extension time 5 min at 72 °C. PCR products were analyzed using 1.5% agarose gel electrophoresis stained with ethidium bromide and visualized under a UV light (Khan, 2013).

Presumptive *Salmonella* colonies were chosen from samples that had shown *Salmonella* positive colonies on all Hekoten Enteric agar, Bismuth Sulfate agar, and Xylose Lysine Desoxycholate agar plates. The colonies were picked off the agar and suspended in sterile water, then vortexed briefly to ensure even suspension and transferred to a 96-well PCR plate, including the positive controls of *Salmonella enterica and Salmonella heidelberg* and a negative control of *L. monocytogenes,* respectively. A mixture of 4 µL of dNTPs (2.5 mM each), 5 µM of 10x PCR buffer, 3 µL of $MgCl_2$ (25 mM), 36.5 µL sterile water, 0.5 µL of Taq enzyme, and 1 µL of primer mix (0.5 µM each) were added to each well. PCR conditions were programmed with a 95 °C denaturation step for 5 min, followed by 40 cycles of heat denaturation at 95 °C for 30 s, 40 cycles at 59 °C for 30 s, 40 cycles at 72 °C for 1 min, and extension for 5 min at 72 °C. PCR

products were analyzed using 1.5% agarose gel electrophoresis stained with ethidium bromide and visualized under a UV light (Kawasaki, 2005).

# Chapter 4 Results

## 4.1 Bacterial populations collected in water samples

Bacterial populations of both *E. coli* and coliforms in the water effluents are summarized in Tables 1 and 2, and the population trends over time summarized in Figures 2 and 3, respectively. Water temperatures corresponding to each sampling date are summarized in Figure 1. Populations of both *E. coli* and coliforms showed a decrease from initial numbers in December as the temperature fell, with the lowest numbers (0 for all *E. coli*) on the samples taken on January 9, 2017, with the lowest temperature of 17.5 °C over the sampling period. Populations remained very low for *E. coli* before increasing again in May 2017, when populations remained high until the temperature began to cool off at the end of November. Coliform populations remained low after January 2017, with numbers leveling off. Populations of coliforms were more varied and higher than *E. coli*, with significantly higher numbers (the highest at 2940 CFU/100mL) on April 3, 2017. No significant differences in populations of *E. coli* were found between the fish tank effluent and the effluents of the tomato plants, or between the perlite and pine bark treatments. However, the populations of *E. coli* in cucumber effluents were significantly higher than that of the fish tank effluent. There was no significant difference between the pine bark and perlite treatments. Coliform populations from the fish tank effluent were significantly higher than the populations in both effluents of the tomato growth media and the cucumber perlite treatment, but not for the effluent of the cucumber pine bark. Significant

differences were also found between coliform populations in the pine bark and perlite treatments for both tomatoes and cucumbers.

### 4.2 Bacterial populations in produce growth media

The populations of both *E. coli* and coliform bacteria in the soil media are summarized in Tables 3 and 4, and the population trends over time in Figures 4 and 5, respectively. Both *E. coli* and coliform populations showed a decrease starting in December as temperatures decreased. Populations of coliforms continued to decrease throughout January and February, and remained low. *E. coli* populations also fell and remained low until the end of August. There was little difference over time in *E. coli* populations; however, coliform populations exhibited more variation. No significant difference was found between the cucumber pine bark and perlite treatments when compared to each the tomato treatments for any *E. coli* populations. When coliform populations in each treatment were compared, only those in the cucumber perlite and tomato perlite populations showed any significant difference from each other.

### 4.3 Comparison of generic *E. coli* populations in aquaponics water to federal standards under the Produce Safety Rule

The standards for generic *E. coli* populations under the FDA's Produce Safety Rule for irrigation water are a geometric mean (GM) of less than 126 CFU/100 mL in water and a statistical threshold value (STV) of less than 410 CFU/100 mL in water. Our results showed that the microbial populations of generic *E. coli* in all water samples met the requirements, with all geometric means and statistical threshold values falling below the limits. Fish tank effluent water had a GM of 2 CFU/100 mL and a STV of 98 CFU/100 mL. The cucumber perlite effluent had a GM of 5 CFU/100 mL and a STV of 50 CFU/100 mL, while the cucumber pine bark effluent had

a GM of 3 CFU/100 mL and a STV of 46 CFU/100 mL. Tomato pine bark effluent had a GM of 11 CFU/100 mL and a STV of 66 CFU/100 mL, and tomato perlite effluent had a GM of 4 CFU/100 mL and a STV of 76 CFU/100 mL.

### 4.4 Presence of *Salmonella* and *Listeria monocytogenes* in cucumbers and tomatoes

A total of 310 cucumber samples and 270 tomato samples were tested for the presence of both *Salmonella* and *L. monocytogenes*, which were then confirmed via PCR. No *Salmonella* was detected in all tomato and cucumber samples. There were 5 cucumber samples and 1 tomato sample found *L. monocytogenes* positive.

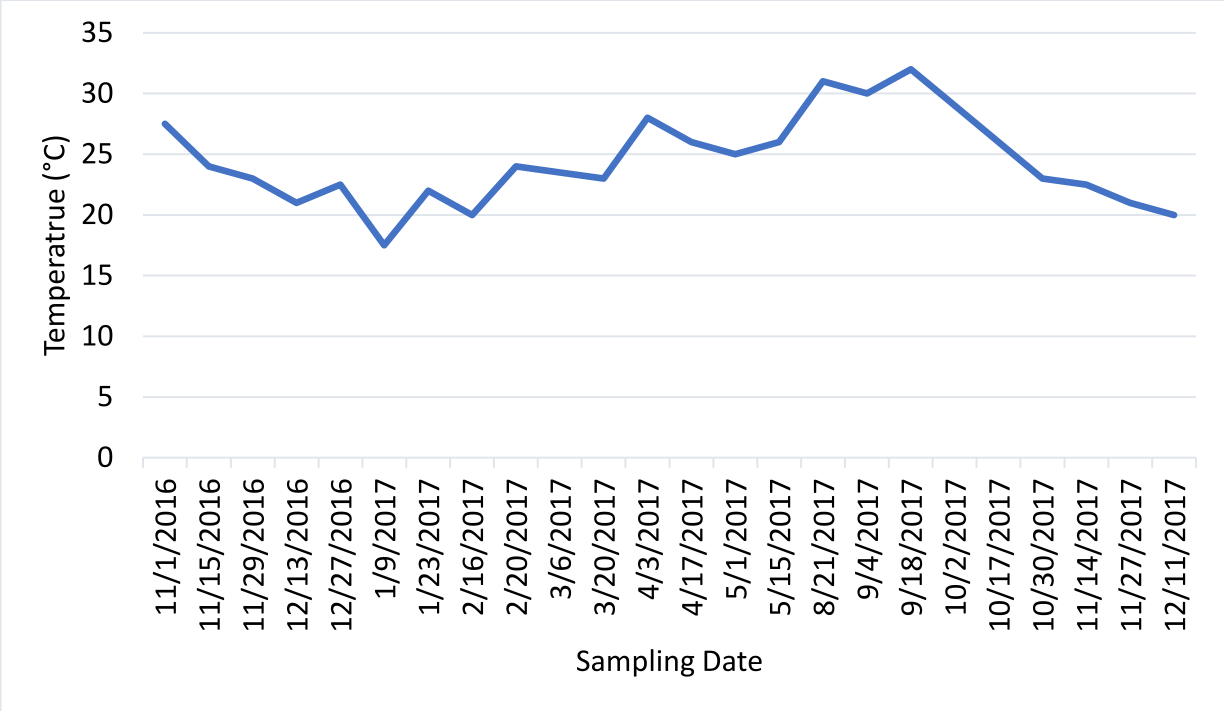


Figure 1. Water temperatures of sampling dates.

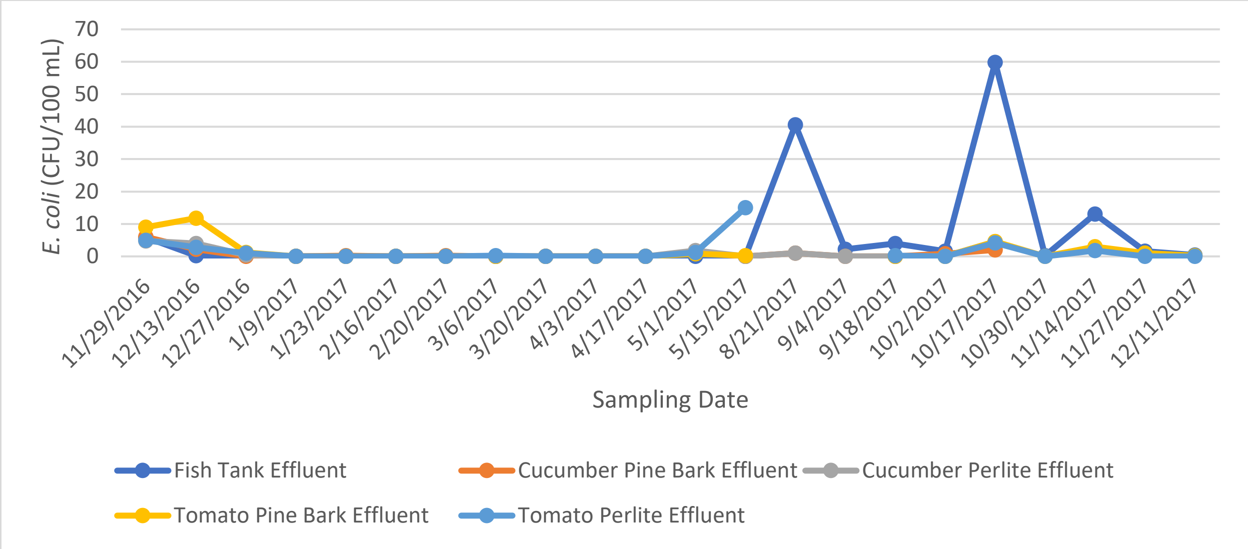


Figure 2. *E. coli* populations in water effluents (CFU/100 mL).

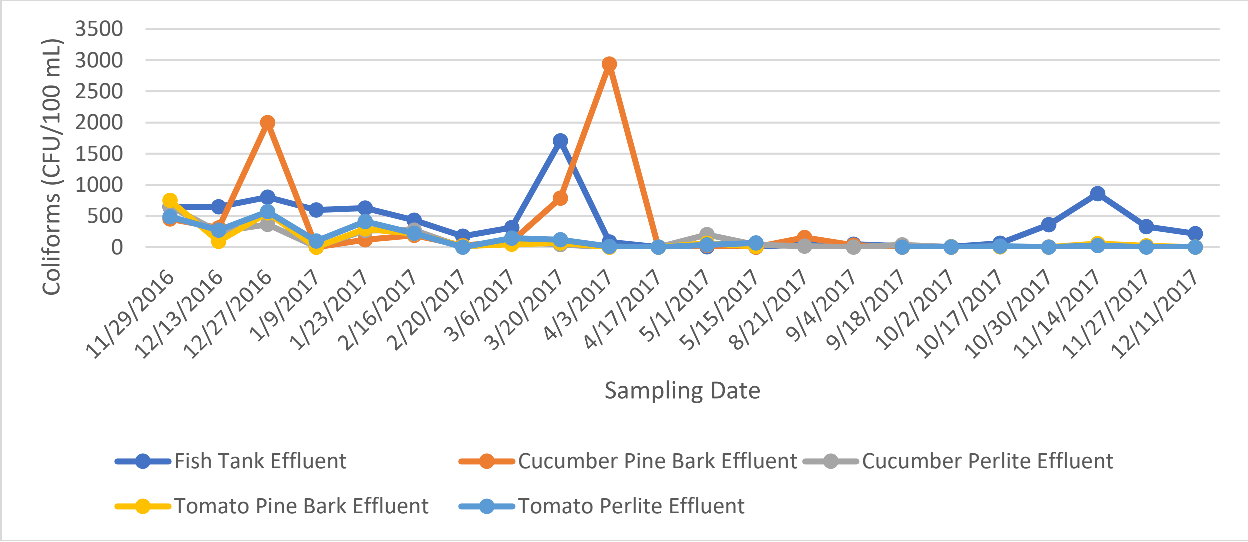


Figure 3. Coliform populations in water effluents (CFU/100 mL).

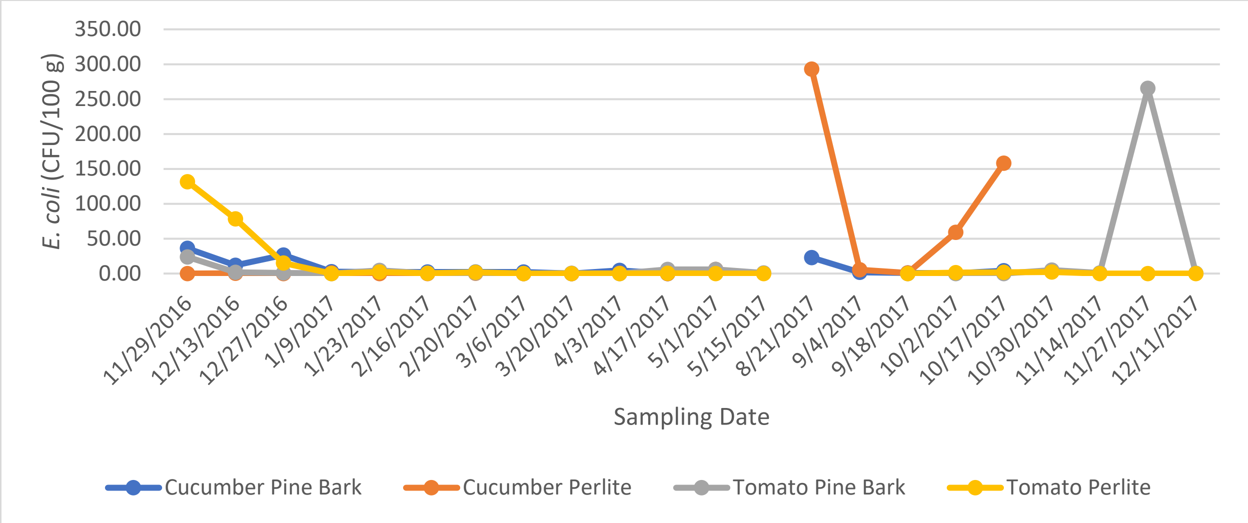


Figure 4. *E. coli* populations in soil medium (CFU/100 g).

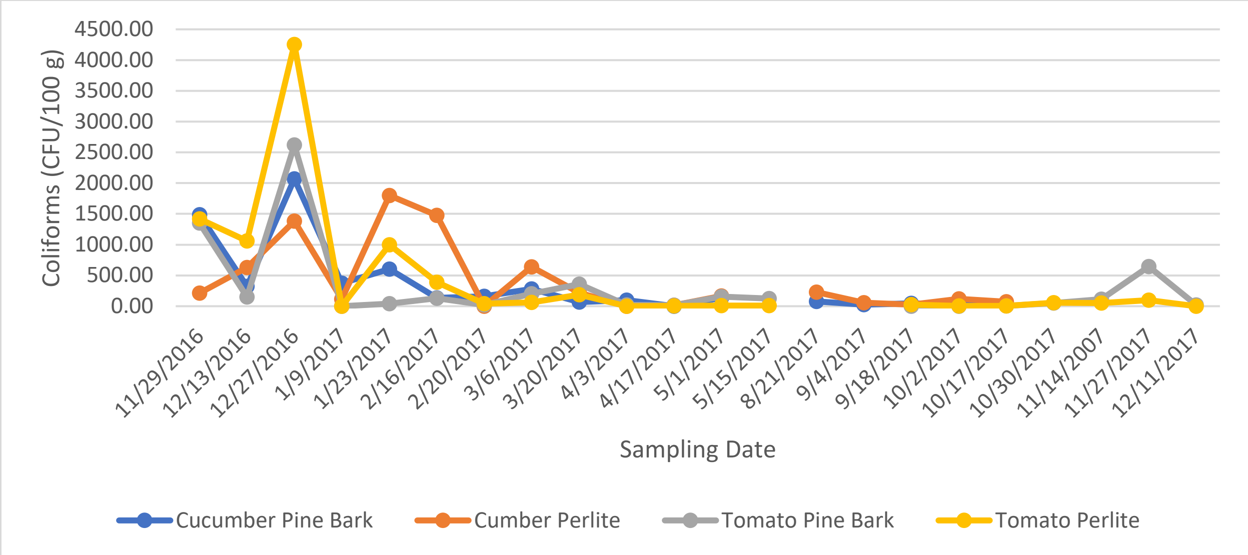

Figure 5. Coliform populations in soil medium (CFU/100 g).

Table 1. Populations of *E. coli* in water effluents

| Sampling Date | Fish Tank Effluent | Cucumber | | Tomato | |
|---|---|---|---|---|---|
| | | Pine Bark Effluent | Perlite Effluent | Pine Bark Effluent | Perlite Effluent |
| 11/29/2016 | 6* | 6 | 5 | 9 | 5 |
| 12/13/2016 | 1 | 3 | 4 | 12 | 3 |
| 12/27/2016 | 1 | 0 | 1 | 2 | 1 |
| 01/09/2017 | 0 | 0 | 0 | 0 | 0 |
| 01/23/2017 | 0 | 1 | 0 | 0 | 0 |
| 02/16/2017 | 0 | 0 | 0 | 0 | 0 |
| 02/20/2017 | 0 | 1 | 0 | 0 | 0 |
| 03/06/2017 | 0 | 0 | 0 | 0 | 1 |
| 03/20/2017 | 0 | 0 | 0 | 0 | 0 |
| 04/03/2017 | 0 | 0 | 0 | 0 | 0 |
| 04/17/2017 | 0 | 0 | 0 | 0 | 0 |
| 05/01/2017 | 0 | 2 | 2 | 1 | 2 |
| 05/15/2017 | 0 | 0 | 0 | 1 | 15 |
| 08/21/2017 | 41 | 1 | 1 | | |
| 09/04/2017 | 3 | 0 | 0 | | |
| 09/18/2017 | 4 | 0 | 0 | 0 | 1 |
| 10/02/2017 | 2 | 2 | 0 | 0 | 0 |
| 10/17/2017 | 60 | 2 | 5 | 5 | 5 |
| 10/30/2017 | 1 | | | 0 | 0 |
| 11/14/2017 | 13 | | | 3 | 2 |
| 11/27/2017 | 2 | | | 1 | 0 |
| 12/11/2017 | 1 | | | 1 | 0 |

*CFU per 100 mL water sample.

Table 2. Populations of coliforms in water effluents

| | | Cucumber | | Tomato | |
|---|---|---|---|---|---|
| Sampling Date | Fish Tank Effluent | Pine Bark Effluent | Perlite Effluent | Pine Bark Effluent | Perlite Effluent |
| 11/29/2016 | 648* | 454 | 656 | 750 | 490 |
| 12/13/2016 | 648 | 312 | 246 | 94 | 272 |
| 12/27/2016 | 802 | 2000 | 368 | 552 | 570 |
| 01/09/2017 | 596 | 0 | 9 | 0 | 102 |
| 01/23/2017 | 630 | 120 | 270 | 294 | 412 |
| 02/16/2017 | 434 | 194 | 274 | 220 | 224 |
| 02/20/2017 | 178 | 30 | 0 | 16 | 2 |
| 03/06/2017 | 314 | 96 | 92 | 46 | 148 |
| 03/20/2017 | 1706 | 786 | 42 | 68 | 122 |
| 04/03/2017 | 82 | 2940 | 0 | 3 | 13 |
| 04/17/2017 | 4 | 0 | 0 | 0 | 0 |
| 05/01/2017 | 6 | 26 | 202 | 64 | 39 |
| 05/15/2017 | 3 | 1 | 38 | 12 | 69 |
| 08/21/2017 | 52 | 155 | 20 | | |
| 09/04/2017 | 48 | 34 | 4 | | |
| 09/18/2017 | 20 | 3 | 38 | 3 | 2 |
| 10/02/2017 | 8 | 2 | 1 | 3 | 2 |
| 10/17/2017 | 65 | 13 | 5 | 5 | 16 |
| 10/30/2017 | 362 | | | 3 | 1 |
| 11/14/2017 | 860 | | | 57 | 26 |
| 11/27/2017 | 332 | | | 23 | 0 |
| 12/11/2017 | 220 | | | 3 | 3 |

*CFU per 100 mL water sample.

Table 3. Populations of *E. coli* in soil mediums.

| Sampling Date | Cucumber | | Tomato | |
|---|---|---|---|---|
| | Pine Bark Effluent | Perlite Effluent | Pine Bark Effluent | Perlite Effluent |
| 11/29/2016 | 36* | 0 | 24 | 132 |
| 12/13/2016 | 12 | 1 | 2 | 79 |
| 12/27/2016 | 27 | 0 | 1 | 15 |
| 01/09/2017 | 3 | 1 | 0 | 0 |
| 01/23/2017 | 3 | 0 | 5 | 3 |
| 02/16/2017 | 2 | 0 | 1 | 1 |
| 02/20/2017 | 2 | 1 | 1 | 2 |
| 03/06/2017 | 2 | 0 | 0 | 0 |
| 03/20/2017 | 0 | 0 | 1 | 0 |
| 04/03/2017 | 5 | 1 | 0 | 0 |
| 04/17/2017 | 0 | 0 | 6 | 1 |
| 05/01/2017 | 1 | 6 | 6 | 0 |
| 05/15/2017 | | | 1 | 0 |
| 08/21/2017 | 24 | 293 | | |
| 09/04/2017 | 2 | 6 | | |
| 09/18/2017 | 1 | 1 | 0 | 0 |
| 10/02/2017 | 1 | 59 | 0 | 2 |
| 10/17/2017 | 4 | 159 | 0 | 2 |
| 10/30/2017 | | | 5 | 2 |
| 11/14/2017 | | | 0 | 0 |
| 11/27/2017 | | | 265 | 0 |
| 12/11/2017 | | | 1 | 0 |

*CFU per 100 g of produce growth medium.

Table 4. Populations of coliforms in soil mediums.

| Sampling Date | Cucumber | | Tomato | |
|---|---|---|---|---|
| | Pine Bark Effluent | Perlite Effluent | Pine Bark Effluent | Perlite Effluent |
| 11/29/2016 | 1488* | 211 | 1350 | 1422 |
| 12/13/2016 | 324 | 628 | 149 | 1061 |
| 12/27/2016 | 2070 | 1384 | 2619 | 4256 |
| 01/09/2017 | 375 | 115 | 0 | 0 |
| 01/23/2017 | 601 | 1801 | 41 | 999 |
| 02/16/2017 | 134 | 1478 | 129 | 392 |
| 02/20/2017 | 159 | 0 | 19 | 43 |
| 03/06/2017 | 279 | 637 | 200 | 62 |
| 03/20/2017 | 67 | 218 | 361 | 187 |
| 04/03/2017 | 97 | 1 | 19 | 0 |
| 04/17/2017 | 2 | 15 | 13 | 3 |
| 05/01/2017 | 135 | 166 | 157 | 10 |
| 05/15/2017 | | | 127 | 9 |
| 08/21/2017 | 76 | 230 | | |
| 09/04/2017 | 26 | 58 | | |
| 09/18/2017 | 47 | 24 | 0 | 24 |
| 10/02/2017 | 33 | 118 | 1 | 6 |
| 10/17/2017 | 35 | 72 | 5 | 7 |
| 10/30/2017 | | | 50 | 57 |
| 11/14/2017 | | | 114 | 51 |
| 11/27/2017 | | | 645 | 100 |
| 12/11/2017 | | | 20 | 0 |

*CFU per 100 g of produce growth medium.

# Chapter 5 Discussion

With aquaponics growing in popularity in recent decades, there has been a corresponding lack of information on foodborne pathogens that are possibly present in the systems (Stivers, 2016; Love, 2014). Aquaponics systems have become more widespread in part because of their versatility, since they can be adapted to either larger scale commercial operations or smaller systems that can be set up in backyards. However, with the rise in the number of aquaculture systems, there is an increased risk that outbreaks linked to an aquaponics system, in particular commercial growers, may occur. While some efforts have been made to standardize commercial aquaponics practices and address food safety concerns (FDA, 2015; Aquaponics Association, 2015), producers still face significant hurdles if they want to sell their products commercially. Many retailers such as grocery store chains, are reluctant to take on the risks of selling products that have not been certified by measures such as food safety audits (Aquaponics Association, 2015).

Various factors, including competing microbes, pH, nutrient availability, and solar radiation, can affect the populations of pathogenic bacteria in irrigation water, and how they spread throughout the water column (Pachepsky, 2011). Therefore, concern exists that the high temperatures and availability of nutrients found in the water of an aquaponics system might be more favorable towards the proliferation of pathogenic bacteria. Both generic *E. coli* and coliforms were found in both the effluents from the fish tank, and after excess water from the irrigation of plants. Like

Fox et al. (2012) and Barnhart et al. (2015), who found geometric means of less than 70 and 10 CFU/100 mL respectively, *E. coli* numbers in our study were low, at less than 11 CFU/100 mL.

Little significant difference was found between sampling dates in the changes of populations of both generic *E. coli* or coliforms, but this could be attributed to both the fish tank and plants being held inside a greenhouse under conditions that were usually warm inside despite outside weather conditions and temperatures. While Schmautz et al. (2016) found that the Proteobacteria, the phylum that includes *E. coli* and *Salmonella* spp., dominated most areas of the aquaponics system they sampled, this phylum also includes members of the genera *Nitrobacter* and *Nitrosomonas.* These bacteria are common and essential members of the fish tank microbial community, as they convert the toxic ammonia from fish waste to nitrate and help maintain proper water quality (Kowalchuk, 2001). The abundance of Proteobacteria could be attributed to species such as these rather than pathogens. With fish not being the preferred hosts of *E. coli* (Fox, 2012; Stivers, 2016; Hollyer, 2009), it is likely that fish would not significantly affect *E. coli* populations. It is also possible that the bacteria native to the recirculating fish culture system may also outcompete potential pathogens, leading to lower populations. Sirakov et al. (2016) examined the use of bacteria cultured from an aquaponics system as a form of biocontrol against the fungal fish pathogen *Saprolegnia parasitica* and found over 80% of their cultured bacteria were antagonistic to the fungal pathogen. This hostility may possibly be extended to other species, including pathogenic bacteria. For example, antagonistic effects by members of the genus *Pseudomonas,* phylum Proteobacteria, have been observed against fish pathogens such as *Aeromonas hydrophila, Vibrio* spp., and *Saprolegnia* spp. (Sirakov, 2016).

While bacterial trends in the growth media of the plants followed a similar trend to those found in water effluents, the growth media tended to have consistently higher numbers of both generic

*E. coli* and coliforms. *E. coli* can survive in both soil medium and water, and its survival is usually limited by factors such as availability of a carbon source, temperature, and water availability (Elsas, 2010). Given that the conditions in the greenhouse were a temperature range of 20-30 °C (with some variation), it is within the optimal growing range of *E. coli,* which possesses a tolerance for a large range of temperatures. The drip irrigation system also provided enough water that the growth media was consistently damp, water availability for bacterial growth is greater. These combined factors may have allowed for higher numbers of growth in the growth media when compared to the water effluent. Further examination of microbial populations in different growth media is recommended.

The FDA's current limits for agricultural water used for irrigation water are a GM of less than 126 CFU per 100 mL of water and a STV less than 410 CFU per 100 mL of water (FDA, 2015). As long as contact between water and edible portions of the plant is minimized, the FDA treats aquaponic water as a subset of agricultural water. Our results were similar to those that Fox et al. (2012) observed in their study of aquaponics farms that grew lettuce, the levels of *E. coli* found were below the regulation limits on agricultural water that are currently in place under the FDA's Produce Safety Rule. The most common sources of pathogens in aquaponics systems are usually either from irrigation water, or from workers (Hollyer, 2009).

Out of 580 total samples, only 6 were positive for *L. monocytogenes,* and none were positive for *Salmonella*. While Barnhart et al (2015) did find positive *Listeria* samples in leafy greens grown in an aquaponics system, they only tested at the genus level and the test was not species specific, so the samples they identified as *Listeria* may not have specifically been *L. monocytogenes.* With the presence of *L. monocytogenes* confirmed in produce grown within the aquaponics system used for this study and the zero-tolerance for this foodborne pathogen in ready-to-eat foods, there

is an issue for concern. *Salmonella* was not found in any of the samples in this study, but it has been found in tomatoes grown in hydroponics systems contained within greenhouses (Lopez-Galvez, 2014; Orozco, 2007). Fox et al. (2012) did not find any *Salmonella* spp. in any of the samples they examined, but only fillets from the tilapia grown in the system were sampled, not the produce.

While this study examines food safety in an aquaponics system, system design choices and handling of fish and produce may heighten the chances of contamination. The system that was used in this study was a decoupled system where plants were grown in a growth media and not directly in water, so may not be indicative of possible risks inherent in a coupled system, where water is continuously recycled between the fish and the plants. Since tomatoes and cucumbers were the only plants examined in this study, the microbial risks associated with them may also differ from other produce such as leafy greens. Future studies expanding on the microbial risks associated with various produce species or aquaponics system designs are ideal, as they could broaden the scope of knowledge on food safety for aquaponics. Studies examining and comparing sources of microbial contamination in aquaponics systems, as well as the population differences of microorganisms such as *E. coli* in irrigation water sources (i.e. surface water, well water, potable city water, etc.) would be recommended.

## Chapter 6 Summary

With an increase in the prevalence of aquaponics systems used for produce and the corresponding lack of information about potential food safety risks, there is concern about these safety issues. The FDA has classified the water as similar to agricultural water used for irrigation, and holds it to the standards in the Produce Safety Rule, where *E. coli* levels must not exceed a geometric mean of 126 CFU/100 mL and a statistical threshold value of 410 CFU/100 mL (FDA, 2015). Populations of *E. coli* and coliforms were examined every two weeks over the course of a year in both the water effluents and the growth media of the system. The possible presence of *L. monocytogenes* and *Salmonella* spp. was also examined. This research found that levels of generic *E. coli* in all effluent samples was below both the GM and STV standards set forth by the FDA, with the highest GM and STV at 11 and 98 CFU/100 mL, respectively. Both generic *E. coli* and coliforms were present in the growth media, with the highest values of 293 CFU/100 g generic *E. coli* and 2,940 CFU/100 g coliforms. Cucumber and tomato samples tested for *Salmonella* presence were all negative. Five cucumber samples and one tomato sample out of 310 cucumber samples and 270 tomato samples were *L. monocytogenes* positive.

# Chapter 7 Bibliography

Aquaponics Association. 2015. Aquaponics Association: Provisional GAPs. Available at: https://docs.google.com/document/d/1TykLMAyJRVysiOArokGYIAkFGVvDuosqfGF7-fYS_KA/edit. Accessed on July 13, 2018.

Barnhart, C., Hayes, L., and Ringle, D. 2015. Food safety hazards associated with smooth-textured leafy greens produced in aquaponic, hydroponic, and soil based systems with and without roots at retail. Available at: http://www.aquaponics.umn.edu/sites/g/files/pua2181/f/food-safety-hazards.pdf. Accessed on July 20, 2017.

Buzby, K.M, and Lin, L.S. 2014. Scaling aquaponics systems: balancing plant uptake with fish output. *Aquacultural Engineering*. 63:39-44.

Diver, S. Aquaponics-integration of hydroponics with aquaculture. 2006. *National Sustainable Agriculture Information Service.* Available at: https://attra.ncat.org/attra-pub/viewhtml.php?id=56. Accessed on February 2, 2017.

Elsas, J., Semenov, A. Costa, R., and Trevors, J. 2010. Survival of *Escherichia coli* in the environment: fundamental and public health aspects. *The ISME Journal: Multidisciplinary Journal of Microbial Ecology.* 5:173-183.

FDA. 2015. FSMA Final Rule on Produce Safety. “Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption.” Available at: https://www.federalregister.gov/documents/2015/11/27/2015-28159/standards-for-the-

growing-harvesting-packing-and-holding-of-produce-for-human-consumption. Accessed on July 13, 2018.

Food and Drug Administration. 2016. Bacterial Analytical Manual Chapter 5: *Salmonella*. Available at: https://web.archive.org/web/20161006130159/https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm. Accessed on October 21, 2016.

Food and Drug Administration. 2016. Bacterial Analytical Manual Chapter 10: Detection and Enumeration of *Listeria monocytogenes* in Foods and Environmental Samples. Available at: https://web.archive.org/web/20161021170922/https://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm071400.htm. Accessed on October 21, 2016.

Fox, B.K., Tamaru, C.S., Hollyer, J., Castro, L.F, Fonseca, J.M., Jay-Russell, M., and Low, T. 2012. A preliminary study of microbial water quality related to food safety in recirculating aquaponic fish and vegetable production systems. College of Tropical Agriculture and Human Resources, University of Hawai'i at Manoa. Food Safety and Technology. FST-51.

Goddek, S., Espinal, C.A., and Deliade, B. 2016. Navigating towards decoupled aquaponics systems: a system dynamics design approach. *Water*. 8:303.

Hanning, I.B., Nutt, J.D., and Ricke, S.C. 2009. Salmonellosis outbreaks in the United states due to fresh produce: sources and potential intervention measures. *Foodborne Pathogens and Disease*. 6:635-648.

Hollyer, J., Tamaru, C., Riggs, A., Klinger-Bowen, R., Howerton, R., Okimoto, D., Castro, L., Ron, T., Fox, B.K., Troegner, V., and Martinez, G. 2009. On-farm food safety-aquaponics. College of Tropical Agriculture and Human Resources, University of Hawai'i at Manoa. Food Safety and Technology. FST-38.

Hu, Z., Lee, J.W., and Chandran, K. 2015. Effect of plant species on nitrogen recovery in aquaponics. *Bioresource Technology.* 188:92-98.

Kawasaki, S., Horikoshi, N., Okada, Y., Takeshita, K., Sameshima, T., and Kawamoto, S. 2005. Multiplex PCR for simultaneous detection of *Salmonella* spp., *Listeria monocytogenes,* and *Escherichia coli* O157:H7 in meat samples. *Journal of Food Protection*. 68:551-556.

Khan, J., Rathore, R., Khan, S., and Ahmad, I. 2013. *In vitro* detection of pathogenic *Listeria monocytogenes* from food sources by conventional, molecular, and cell culture method. *Brazilian Journal of Microbiology.* 44:751-758.

Kowalchuk, G.A. and Stephen, J.R. 2001. Ammonia-oxidizing bacteria: a model for molecular microbial ecology. *Annual Review of Microbiology.* 55:485-529.

Lopez-Galvez, F. and Allende, A. 2014. Safety assessment of greenhouse hydroponic tomatoes irrigated with reclaimed and surface water. *International Journal of Food Microbiology.* 191: 97-102.

Love, D., Fry, J, Li, X., Hill, E., Genello, L, Semmens, K., and Thompson, R. 2015. Commercial aquaponics production and profitability: findings from an international survey. *Aquaculture.* 435:67-74

Novoslavskij, A. and Terentjeva, M. 2016. Major foodborne pathogens in fish and fish products: a review. *Annals of Microbiology.* 66:1-15.

Orozco, L., Rico-Romero, L., and Escartin, E. 2008. Microbial profile of greenhouses in a farm producing hydroponic tomatoes. *Journal of Food Protection.* 71:60-65.

Pachepsky, Y., Shelton, D., McLain, J., Patel, J., and Mandrell, R. 2011. Irrigation waters as a source of pathogenic microorganisms in produce: a review. *Advances in Agronomy.* 113:73-138.

Pickens, J. 2015. Integrating effluent from recirculating aquaculture systems with greenhouse cucumber and tomato production. Available at: https://eos.ucs.uri.edu/EOS_Linked_Documents/flsgp/R-LR-A-48%20-%20Pickens_J_%20Dissertation%202015.pdf. Accessed on July 12, 2017.

Rakocy, J.E., Masser, M.P, and Losordo, T.M. 2006. Recirculating aquaculture tank production systems: aquaponics-integrating fish and plant culture. *SRAC Publication No. 454.*

Schmautz, Z., Graer, A., Jaenicke, S., Groesmann, A., Junge, R., and Smits, T. 2016. Microbial diversity in different compartments of an aquaponics system. *Archives of Microbiology*. 199:613-620.

Sirakov, I., Lutz, M., Graber, A., Mathis, A., Staykov, Y., Smits, T., and Junge, R. 2016. Potential for combined biocontrol activity against fungal fish and plant pathogens by bacterial isolates from a model aquaponics system. *Water.* 8:518.

Stivers, T.L. 2016. Food safety and regulation of aquaponic operations. *Food Protection Trends.* 36:242-247.

U. S. Environmental Protection Agency. 2010. Method 1103.1: *Escherichia coli (E. coli)* in water by membrane filtration using membrane-thermotolerant *Escherichia coli* agar (mTEC). Available at: https://nepis.epa.gov/Exe/ZyNET.exe/P1003HT3.TXT?ZyActionD=ZyDocument&Client=EPA&Index=2000+Thru+2005&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C00thru05%5CTxt%5C00000019%5CP1003HT3.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-

&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL. Accessed on October 31, 2016.

World Health Organization. 1989. Guidelines for the use of wastewater in agriculture and aquaculture. Available at: http://apps.who.int/iris/bitstream/handle/10665/39401/WHO_TRS_778.pdf?sequence=1&isAllowed=y. Accessed on October 29, 2016.

3M Food Safety. 2017. 3M Petrifilm interpretation guide: *E. coli*/coliform count plate. Available at: http://multimedia.3m.com/mws/media/236246O/petrifilm-ecoli-coliform-interpretation-guide.pdf. Accessed on October 28, 2016.