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The Occurrence of Shiga Toxin-Producing *E. coli* in Aquaponic and Hydroponic Systems

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Abstract: Food safety concerns have been raised over vegetables and herbs grown in aquaponics and hydroponics due to the reuse of wastewater and spent nutrient solutions. This study was conducted to determine the occurrence of foodborne pathogens in greenhouse-based aquaponic and hydroponic systems. Fish feces, recirculating water, roots, and the edible portions of lettuce, basil, and tomato were collected at harvest, and microbiological analyses were conducted for the bacterial pathogens Shiga toxin-producing *Escherichia coli* (STEC), *Listeria monocytogenes*, and *Salmonella* spp. Enrichments and selective media were used for the isolation, and presumptive positive colonies were confirmed by PCR. STEC was found in fish feces, in the water of both systems, and on the surface of the roots of lettuce, basil, and tomato regardless of the system. However, contaminated water did not lead to the internalization of STEC into the roots, leaves, and/or fruit of the plants. Meanwhile, *L. monocytogenes* and *Salmonella* spp. were not present in any samples examined. Our results demonstrated that there are potential food safety hazards for fresh produce grown in aquaponic and hydroponic production systems.

Keywords: food safety; *E. coli*; agricultural water; PCR detection; indoor farming

1. Introduction

Food safety has become an important issue in fresh produce production worldwide due to many factors such as importation of fresh produce from various countries, potential sources of bacterial pathogens from the growing environment, and inappropriate domestic food preparation [1,2]. Nearly 48% of foodborne outbreaks are linked to the consumption of fresh fruits and vegetables [3]. The apparent number of outbreaks associated with *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. has increased in recent years [4,5] largely due to improved detection methods and traceback procedures following an outbreak [6–8].

In the United States, the Centers for Disease Control and Prevention (CDC) estimates that one in six people experience a foodborne illness every year [9]. From 2009 to 2015, the Foodborne Disease Outbreak Surveillance System (FDOSS) received reports of 344 outbreaks (27%) and 2288 illnesses (9%) associated with aquatic animal consumption and 334 outbreaks (26%) and 9746 illnesses (37%) associated with vegetable consumption in the United States and Puerto Rico [10]. Outbreaks caused by *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. were responsible for 4241 hospitalizations (82%) and 117 deaths (82%) [10]. According to a recently released annual summary from FDOSS [11], fruit and vegetable crops were in the top five pathogen-category pairs resulting in outbreak-associated illnesses in 2017, and 32 outbreaks (14%), 919 illnesses (25%), and 205 hospitalizations (51%) were associated with vegetables. Shiga toxin-producing *E. coli* (STEC) were responsible for 110 illnesses

associated with vegetables, while *Salmonella* spp. were responsible for 178 illnesses associated with fruits and vegetable crops [11].

Fruit and vegetables grown in the field are typically at greater risk of contamination because of increased exposure to contamination sources, such as through manure application, wildlife activities, and polluted irrigation water [5]. Bacterial pathogens can survive for a prolonged period in animal feces and may serve as a potential inoculum onto plants [3]. Once plants are exposed to bacteria, it is often difficult to remove the contamination from the harvested fruit and vegetables [5,12]. Meanwhile, vegetables and herbs grown in aquaponics and hydroponics in controlled environment agriculture (CEA) are often thought to be at a lower risk of contamination than their field-grown counterparts due to less exposure to environmental contaminants [13]. However, spent nutrient solutions are collected and reused in recirculating hydroponic systems [14,15], which may be a source of contamination if not properly treated and managed. In addition, the potential for internalization of *E. coli* O157:H7 and *Salmonella* spp. has been demonstrated in lettuce, spinach, and tomato grown in an inoculated hydroponic system [16–19] where contaminated seeds and/or irrigation water could potentially be the source of contamination [4].

Aquaponics, integrated food production combining aquaculture and hydroponics, is a sustainable farming method that addresses many agricultural challenges, such as water scarcity, resource stewardship, and global food security [20,21]. Recirculating aquaponic systems are designed to increase nutrient use efficiency and decrease environmental wastes generated in agricultural food production [21–23]. Nitrifying bacteria convert ammonia into nitrate from aquaculture wastewater. This process not only provides a nitrogen source and other nutrients for plant growth but also cleans the water for the fish, which allows water recirculation and reduces wastewater disposal issues associated with aquaculture [24]. However, the use of waste products derived from fish cultivation raises food safety concerns because foodborne pathogens can be carried in fish intestines up to 7 days [25]. Although some evidence demonstrates that they are not permanent flora or correlated with the level of environmental contamination [26,27], fish feces can be a potential contamination source of bacterial pathogens in aquaponic systems.

Considering the recent expansion of indoor aquaponics and hydroponics [15], it is important to determine possible factors that can adversely affect the food safety of fresh produce. The objective of this study was to investigate the occurrence of bacterial pathogens in fish feces, recirculating water, roots, and edible portions of the crops grown in aquaponic and hydroponic systems. We targeted Shiga toxin-producing *E. coli*, *L. monocytogenes*, and *Salmonella* spp., the most common foodborne pathogens of concern associated with fresh produce [9,28–30], to assess the presence of bacterial pathogens and the likelihood of contamination of these systems.

2. Materials and Methods

2.1. System Design

Six experimental units were operated in a greenhouse in West Lafayette, IN (lat. 40° N, long. 86° W), which consisted of three aquaponic and three hydroponic systems. Each unit was equipped with a fish tank, or a nutrient reservoir (350 L), a clarifier (20 L), a two-stage biofilter (40 L) [21,31], and a deep-water hydroponic grow bed (350 L; 1.0 m^2). A month before the study, systems were filled with reverse osmosis water. Nile tilapia (*Oreochromis niloticus* L.) fish were obtained from the Animal Sciences Research and Education Center at Purdue University, which had been cultivated in a conventional aquaculture system for 4 months. Fish fresh weight was measured (an average of 300 g per fish) and evenly distributed to three different fish tanks with a stocking density of 20 kg/m^3 in each aquaponics system. The pH of the aquaponic systems was maintained at 6.5 to 7.0 using a combination of KOH and $Ca(OH)_2$. The biofilter was connected to a peristaltic pump (Masterflex, Cole-Parmer, Chicago, IL, USA) to recirculate nutrient solution within a system unit. In each hydroponic system, the nutrient solution reservoir and hydroponic culture unit were filled with reverse osmosis water blended

with the nutrient stock solution (CropKing, Lodi, OH, USA) at a 1:100 dilution rate which was used as initial and follow-up daily replenishment for leafy vegetable and herb crops and fruit vegetables. The electrical conductivity (EC) was maintained at 2.0 dS/m by adding and replenishing nutrition solution daily, and the pH of hydroponics was adjusted at around 6. The total water volume in both aquaponic and hydroponic systems was 700 L with a flow rate of 138 L/h, giving a water retention time [(surface area × water depth)/flow rate] of 5 h in the hydroponic culture unit of each system (Figure 1). Aquaponic solution or nutrient solution flowed through the hydroponic culture unit of each system and back to the fish tank or reservoir. Each aquaponic and hydroponic system had air stones to maintain dissolved oxygen (DO) concentrations at full saturation, and aquatic heaters were set in the aquaponic systems to maintain the temperature in the range of 25 to 28 °C. Water temperature, pH, EC, and DO were measured daily using the HQ40d Portable Water Quality Lab Package (HACH Corp., Loveland, CO, USA). Total ammonia nitrogen, nitrite, and nitrate concentrations were analyzed immediately using HACH reaction kits (Hach Co., Loveland, CO., USA), namely Ammonia Reagent Powder Pillows, Nitrite Reagent Powder Pillows, and Nitrate Reagent Powder Pillows, respectively. The same water samples were used to confirm the concentrations of nitrogen species by ion chromatography (Dionex ICS–5000, Thermo Scientific, Waltham, MA, USA).

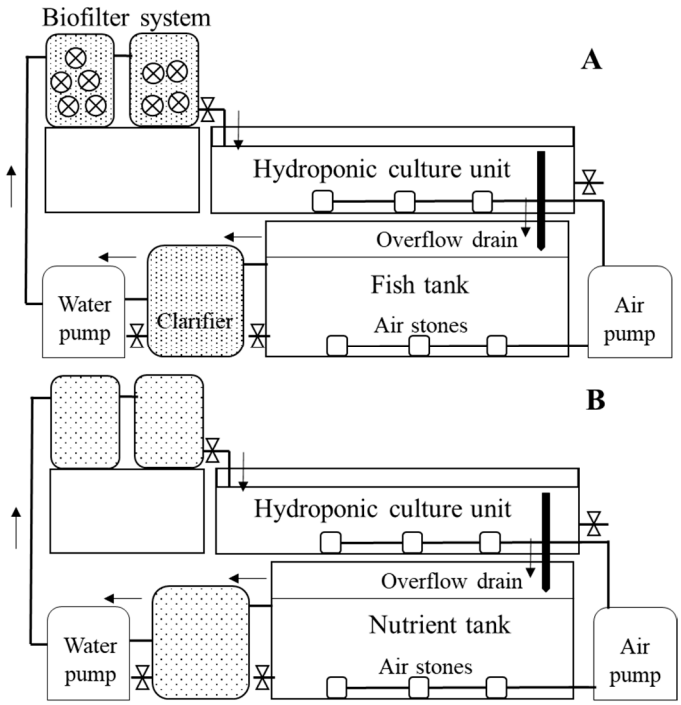

Figure 1. Schematic diagram of the experimental units (**A**) aquaponic system and (**B**) hydroponic system adapted from Yang and Kim (2019).

2.2. Plant Materials and Growing Conditions

Three crops were tested: lettuce (*Lactuca sativa*), basil (*Ocimum basilicum*), and tomato (*Solanum lycopersicum*). Seeds were purchased from a commercial source (Johnny's Selected Seeds, Winslow, ME, USA) and sown in Agrifoam soilless plugs (SteadyGROWpro, Syndicate Sales, Kokomo, IN, USA) with a few day intervals to ensure uniform seedling size at the time of transplanting. Seeds were germinated as described by Kim et al. (2018), and 2 to 3 weeks old seedlings were transplanted to aquaponic and hydroponic systems. Planting densities were 24 plants per m^2 for lettuce and basil and 8 plants per m^2 for tomato. During the study period, the fish were fed 60 g of a complete diet (41% protein, 1.1% phosphorus) with 4.8-mm floating pellets (AquaMax Sport Fish 500, Purina Mills, St. Louis, MO, USA) at 9:00 a.m. daily. The experiment was conducted between December 2017 and

February 2018. The photoperiod was 14-h (8:00 a.m. to 10:00 p.m.) consisting of natural daylight with supplemental lighting using high-pressure sodium (HPS) lamps (600-W, P.L. Light Systems Inc., Beamsville, ON, Canada). The supplemental photosynthetic photon flux (PPF) of the greenhouse was measured using a quantum sensor (LI-250A light meter; LI-COR Biosciences, Lincoln, NE, USA), and maximum photosynthetically-active radiation in the greenhouse was averaged at 168 μmol/m^2/s. Day (8:00 a.m. to 10:00 p.m.) and night (10:00 p.m. to 8:00 a.m.) temperatures were set at 24 and 18 °C, respectively.

2.3. Plant Sample Collection and Microbial Isolation

Leaf and root samples of lettuce and basil were collected at 30 and 60 days after transplanting, respectively, while fully ripe tomato fruit and root samples were collected at 60 days after transplanting. A total of six biological replicates (each with three technical replicates) were collected from each plant source in both the aquaponic and hydroponic systems. To investigate surface contamination, plant samples were blended with peptone water for 15 s without any surface-sterilized procedure. To examine if the bacteria of interest were able to internalize within the plant tissue, plant samples were surface-sterilized to remove surface-located bacteria using 0.1% sodium hypochlorite for 10 s followed by sterile water for 30 s. This procedure was repeated 3 times for each sample. A total of 5 g of fresh produce was blended with 45 mL of buffered peptone water (PW; Difco, Detroit, MI, USA) in a 50 mL centrifuge tube, allowing the sample to be completely homogenized [32–34]. The homogenized samples were then incubated at 37 °C for 6 h to allow for the recovery of the bacteria and potential enrichment. The samples were then serially diluted, and 0.1 mL was spread plated in duplicate on MacConkey agar with sorbitol, cefixime, and tellurite (CT-SMAC) agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), Oxford Listeria-selective agar (Becton, Dickinson and Company), and Xylose Lysine Tetrathionate 4 (XLT4) agar with supplement (Becton, Dickinson and Company) for the selection of *E. coli* O157, *L. monocytogenes*, and *Salmonella* spp., respectively [33,34]. Plates were incubated for 24 h at 37 °C for the isolations of *E. coli* O157 and *Salmonella* and for 48 h at 30 °C for the isolation of *L. monocytogenes* (Figure S1).

2.4. Microbial Detection in Water and Fish Feces Samples

Water samples were collected immediately after crop harvest. Each sample was randomly collected from two duplicate systems with a total of six biological replicates (each with three technical replicates) from each system. Fish feces were collected from the clarifier tank where most solid waste was found, and excess water was carefully drained.

A separate fish-growing system was constructed to confirm if the fish feces were the source of contamination in aquaponics. Tilapia (2.5 kg per tank) were placed in a fish tank (125 L) filled with 50 L reverse osmosis water and grown for two weeks. Approximately 20% of the water was replaced daily to maintain water quality. Fish feces were collected from the fish-growing system.

A 20 mg fish feces sample from each system was mixed with 180 μL water and vortexed for 15 s. Water samples and fish feces samples were plated after collection without enrichment. The water samples and fish feces samples were serially diluted and spread plated as described above in duplicate on CT-SMAC agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), Oxford Listeria-selective agar (Becton, Dickinson and Company), and XLT4 agar with supplement (Becton, Dickinson and Company) for the selection of *E. coli* O157, *L. monocytogenes*, and *Salmonella* spp., respectively.

2.5. PCR Assay for Detection of Virulence Genes

A total of 90 isolated colonies each from presumptive positive STEC and *Salmonella* spp. cells (no presumptive *L. monocytogenes* positive colonies were isolated) were picked into 20 μL of distilled water and then heated to 90 °C using a dry heat bath (Benchmark Scientific Inc., Edison, NJ, USA) for 5 min. PCR was then performed to amplify the *stx1* gene from *E. coli* O157 [35] and the *iro*B gene for *Salmonella* spp. [36] (Table 1). A total of 5 μL samples of the PCR product was mixed with 1 μL of

6× DNA safe stain loading dye (Bullseye DNA Safe Stain C138, MIDSCI, St. Louis, MO, USA) and loaded into a 1% agarose gel (H26855 Agarose D1-LE, Thermo scientific, Co. Ltd., USA) in 50 mL of 1× TAE buffer (Tris-acetate-EDTA, Thermo scientific, Co. Ltd., USA). The gel was run at 110 V for 35 min. using a PowerPac™ Basic Power Supply (Bio-Rad, Hercules, CA, USA). The gel was then imaged using a Gel Doc EZ Imager (Bio-Rad, Hercules, CA, USA).

Table 1. PCR primers and program for **STEC** and *Salmonella* spp.

	Sequence	PCR Program	Reference
Shiga Toxin-Producing *E. coli* (STEC)			
stx1-F	CAGTTAATGTGGTGGCGAAG	95 °C for 3 min, 95 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min, repeat steps 2–4 30 times, 72 °C for 10 min	[35]
stx1-R	CACCAGACAATGTAACCGCTG		
Salmonella spp.			
*iro*B-F	TGCGTATTCTGTTTGTCGGTCC	95 °C for 3 min, 95 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min, repeat steps 2–4 30 times, 72 °C for 10 min	[36]
*iro*B-R	TACGTTCCCACCATTCTTCCC		

2.6. Experimental Design and Data Analysis

Treatments consisted of two production systems and three plant species and were arranged in a completely randomized design. Six plants per system were randomly chosen as biological replicates, and three subsamples (technical replicates) per plant were taken for the analysis of bacterial pathogens. Mean separation within each measured parameter was performed by Tukey's honestly significant difference (HSD) test at $p = 0.05$ using R 3.6.1 (R, Comprehensive R Archive Network, USA, http://cran.us.r-project.org/).

3. Results and Discussion

3.1. Water Conditions for Aquaponic and Hydroponic Systems

The water quality parameters in the aquaponic and hydroponic systems are shown in Table 2. The aquaponics water temperatures averaged 27.5 ± 1.4, 25.9 ± 1.2, and 27.5 ± 1.7 °C for lettuce, basil, and tomato, respectively, and the hydroponics averaged 20.3 ± 1.2 °C. Tilapia is a tropical fish that can grow in a wide range of temperature from 22 to 34 °C; however, the feed conversion ratio, fish weight gain in relation to feed consumption, and daily weight gain of tilapia are known to be better at temperatures between 26 and 30 °C [37]. The pH values averaged 6.7 ± 0.5 and 5.7 ± 0.5 in the aquaponics and hydroponic systems, respectively (Table 2). There were no significant differences between aquaponic and hydroponic systems in EC levels and nitrogen species concentrations, which averaged 1.2 ± 0.5 and 1.6 ± 0.1 and 72.9 and 76.6 mg/L, respectively. The average DO level was significantly lower in aquaponics (6 mg/L) than in hydroponics (10 mg/L), although it was maintained at full saturation in both systems.

It is well-documented that environmental factors, such as temperature, pH, nutrient availability, and DO, affect bacterial populations [2,3,38,39]. Bacterial pathogens survived for a longer time (up to 91 days) at cold (4–8 °C) and freezing (−4 °C) temperatures than at warmer (20–30 °C) temperatures (up to 84 days) when river water and sterilized soil were inoculated [40]. *E. coli*, *L. monocytogenes*, and *Salmonella* sv. Typhimurium can survive in animal waste at 28 °C during anaerobic digestion [41], and the population can be affected by the interaction between temperature and pH [42]. Environmental factors in our study were similar between aquaponics and hydroponics, and, therefore, the differences in temperature and pH between aquaponic and hydroponic systems are not likely to have affected the population of bacteria between the systems. For example, the pH values in aquaponic and hydroponic systems were 6 and 7 which are in the optimal ranges of 6 to 9 for enteric pathogens [43–45], and at pH 6, the population densities of *E. coli* O157:H7 have been reported to be the same at 20 and 30 °C [46].

EC is a common indicator of soluble salts dissolved in a nutrient solution because soluble salts carry an electrical charge, and their presence affects the EC of the solution. The availability of nutrients (e.g., nitrogen) and energy sources is a key factor affecting the survival of bacteria in the environment [3]. It has been demonstrated that the viability of *E. coli* O157:H7 is increased in nutrient-rich soils [47]. Since the high level of nutrients in the hydroponic solution is also ideal for the growth of bacteria, the irrigation water containing a high concentration of nutrients poses the biggest contamination risk in soilless culture systems [48]. In fact, it was reported that the nutrient reservoirs of hydroponic systems can be a source of contamination [49,50]. Enteric pathogens are facultative anaerobic bacteria [44,51]. The average DO levels observed in our study were sufficient to allow the growth of *E. coli* O157:H7, *Listeria* spp., and *Salmonella* spp. The implication of these environmental conditions is that pathogenic bacteria can grow in greenhouse-based aquaponic and hydroponic systems if they are introduced by any means.

Table 2. Water quality parameters in lettuce-, basil-, and tomato-based aquaponic and hydroponic systems during the experimental period.

Production System	Vegetable	DO [z] (mg/L)	pH	Temperature (°C)	EC (dS/m)	NH_4^+ (mg/L)	NO_2^- (mg/L)	NO_3^- (mg/L)
Aquaponics	Lettuce	6.1 ± 0.7 [y]	6.9 ± 0.6	27.5 ± 1.4	1.1 ± 0.4	1.8 ± 0.4	0.8 ± 0.2	74.7 ± 10.8
	Basil	6.2 ± 0.7	6.5 ± 0.5	25.9 ± 1.2	1.3 ± 0.5	3.0 ± 1.1	0.3 ± 0.1	50.8 ± 4.9
	Tomato	6.1 ± 0.7	6.8 ± 0.5	27.5 ± 1.7	1.2 ± 0.5	2.9 ± 0.7	1.2 ± 0.5	83.1 ± 13.8
Hydroponics	Lettuce	10.0 ± 0.4	5.7 ± 0.5	20.3 ± 1.4	1.7 ± 0.1	0.8 ± 0.3	nd [x]	77.5 ± 7.8
	Basil	10.0 ± 0.4	5.7 ± 0.5	20.2 ± 1.2	1.6 ± 0.1	0.9 ± 0.2	nd	73.9 ± 7.0
	Tomato	9.9 ± 0.5	5.8 ± 0.6	20.5 ± 1.2	1.5 ± 0.2	1.2 ± 0.4	nd	95.5 ± 9.4
Significance								
System		*** [w]	***	***	***	***	***	***
Vegetable		ns	ns	ns	ns	ns	ns	ns
System × Vegetable		ns	ns	ns	ns	***	ns	ns

[z] Abbreviations: dissolved oxygen (DO); electrical conductivity (EC). [y] Each value is the mean of four replicates (2 sample replicates per system) ± SD. [x] nd means not detected. [w] ns, *, **, *** mean no significant or significant at $p \leq 0.05$, 0.01, or 0.001, respectively.

3.2. The occurrence of Shiga Toxin-Producing E. coli (STEC), Listeria Monocytogenes, and Salmonella spp.

We tested the presence of STECs in fish feces in the aquaponic and aquaculture systems and found STECs in fish feces and the water regardless of the system (Table 3). The colonies were confirmed after incubation at 37 °C for 20 h and detected by PCR targeting of the *stx1* gene (Figure S2). STEC were also detected on the root surfaces but were not found to internalize into the roots or the edible parts of lettuce, basil, and tomato grown in the aquaponic system (Table 3; Figure S2). These results indicated that water contaminated by fish feces is likely the primary source of root surface contamination.

Irrigation water is often a major source of contamination in outbreaks associated with bacterial pathogens, and this can be particularly true for the field-grown vegetables due to overhead irrigation with contaminated water, damaged roots and shoots by herbivores, and/or groundwater contamination by a plume of wastewater [49,52–54]. However, our study suggests that there is a potential risk associated with aquaponic produce even when the solutions are directly applied to the roots due to water contamination. Our separate aquaculture system confirmed that fish feces were a major source of contamination with STEC in the aquaponic system. These results indicate that introducing contaminated fish can be a source of foodborne pathogens in aquaponics. Previous work has shown that, when fish were reared in ponds where the concentration of coliforms was low, a small number of *E. coli* O157:H7 cells were recovered from fish intestines but not fish muscle [55,56]. The bacterial intestinal flora of fish can survive up to 84 days in water at 20-30 °C [57,58]. Therefore, Tilapia fish used in this study may have been contaminated before receipt, and the STEC could have been introduced by the fish to the tanks, contaminating the water in the aquaponic systems. Importantly, the contaminated water did not lead to the internalization of STEC into the roots and edible parts of lettuce, basil, and tomato grown in the aquaponic systems suggesting the main source of contamination during

production would be from accidental splash of the water to the edible portions of the plant during harvest (Table 3; Figure S2).

Table 3. Occurrence of *STEC*, *L. monocytogenes*, and *Salmonella* spp. in recirculating water and the roots and leaves, and/or fruit of lettuce and basil, and the roots, and fruit of tomato grown in aquaponic and hydroponic systems.

Vegetable	Tissue Type	Shiga Toxin-Producing *E. coli*		*L. monocytogenes*		*Salmonella* spp.	
		Aquaponics	Hydroponics	Aquaponics	Hydroponics	Aquaponics	Hydroponics
Lettuce	Internal leaf	–[z]	–	–	–	–	–
	Leaf surface	–	–	–	–	–	–
	Internal root	–	–	–	–	–	–
	Root surface	+	+	–	–	–	–
	Water	+	+	–	–	–	–
	Fish feces	+	NA	–	NA	–	NA
Basil	Internal leaf	–	–	–	–	–	–
	Leaf surface	–	–	–	–	–	–
	Internal root	–	–	–	–	–	–
	Root surface	+	+	–	–	–	–
	Water	+	+	–	–	–	–
	Fish feces	+	NA	–	NA	–	NA
Tomato	Fruit	–	–	–	–	–	–
	Internal root	–	–	–	–	–	–
	Root surface	+	+	–	–	–	–
	Water	+	+	–	–	–	–
	Fish feces	+	NA	–	NA	–	NA

[z] The symbols, + and –, indicate presence and absence, respectively. NA means no fish feces in hydroponics. Each symbol in the table is the result of 18 samples (six biological replicates × three technical replicates). Ten isolates per positive plate were examined for PCR confirmation.

STEC were also detected in the water of the hydroponic systems. The bacteria may have been introduced accidentally to the hydroponic systems during experimental setup or handling, possibly allowing the formation of biofilms on the surfaces of the hydroponic culture unit, nutrient reservoir, and/or irrigation tubing. A variety of factors, such as water temperature, pH, nutrient availability, solar radiation, the presence of other microorganisms, and the ability to form biofilms, influence both survival and proliferation of STEC in water [57,59,60]. STEC concentrations in irrigation water are also affected by diurnal and seasonal variations [61]. In fact, the biofilm of *E. coli* O157:H7 can grow rapidly and have a high number of adherent cells, even at low nutrient availability and low temperature [62,63]. Once formed, it is hard to remove them from the system because the biofilm increases the survival rate of *E. coli* O157:H7 even if exposed to hydrogen peroxide, quaternary ammonium sanitizer, or citric acid [64]. Therefore, we speculate that the hydroponic systems in this study may have been contaminated with STEC due to incomplete sanitation before cultivating plants.

Human activities can also increase the risk of contamination. In a study to determine the source of contamination in hydroponic tomato greenhouses, work and personal shoes were identified as a vehicle for transmission of *E. coli* O157:H7 [65]. In this study, there is the possibility that the bacteria may have been introduced from the aquaponic system to the hydroponic systems from visitors to the greenhouse, during the feeding of fish, during sample collection, and/or by cross-contamination from other human activities [15,66]. Contrary to our results, very low levels of generic *E. coli* and undetectable pathogenic *E. coli* O157:H7 and *Salmonella* spp. were found in water and plant samples of aquaponic produce originated from outdoor aquaponic farms located in a tropical climate [67]. In their study, solar radiation and heat might have played a key role in controlling the bacterial levels in the system [68,69]. However, the results cannot be translated into the indoor aquaponic and hydroponic systems located in a temperate climate since solar radiation is often limited, and the environmental conditions are very different from the situation in a tropical climate.

Our results indicated that contamination with bacterial pathogens could likely be reduced in aquaponic and hydroponic systems if the entire systems were thoroughly sanitized before each use

and pathogen-free fish were used for the operation. One of the major routes of enteric pathogen internalization is through sites of biological or physical damage and/or through natural openings on the plant surface, such as stomata, lenticels, and sites of lateral root emergence [4]. The contaminated irrigation water in our study did not have direct contact with the edible aerial parts of the plants as there was a foam board between the water and the plants, and the plants were not disturbed until harvest. It should be noted that damaged roots during handling would create entry points for the bacterial pathogens, and, therefore, the risk of contamination can be avoided if the plant tissues, particularly the roots, are carefully handled during production and harvest. Similarly, previous reports found that foodborne illness was controlled in aquaponics when the following practices were performed: cleaning and sanitation of reusable plastic containers, environmental controls, handwashing, and the use of clean irrigation water [70,71].

E. coli can also be internalized if contaminated seeds or water are used during seed germination [4,72]. That no internalization was observed in the present study suggested that the seeds and seedlings were free of foodborne pathogens. Several studies demonstrated that bacterial pathogens can contaminate fresh produce in the greenhouse if it is not protected from wild animals or heavy rain causing a flood [65]; however, this possibility can be eliminated as the source of contamination in this study because the plants were grown in a well-protected greenhouse.

While the optimal water temperature and the pH pose a potential contamination concern with *L. monocytogenes* in aquaponic and hydroponic systems (Table 1), our selective growth medium and colony PCR assay did not detect the occurrence of *L. monocytogenes* in fish feces, recirculating water, and plants grown in the systems. These results suggest that there was no contamination source of *L. monocytogenes* in either system (Table 3; Figure S2). One source of *L. monocytogenes* is from the soil, and there was no soil in the greenhouse during this experiment. Other potential sources of *L. monocytogenes* are human activities and contaminated seeds. In the event of pathogen introduction to the systems, *L. monocytogenes* can internalize through natural openings on the plant surface or through the sites of biological or physical damage [4]. Although the fish feces or human activities were demonstrated as potential sources of contamination with STEC, our results showed that these were not the major sources of contamination for *L. monocytogenes,* and the production practices employed in this study were not associated with the risk of contamination with *L. monocytogenes* in either system.

Similarly, various environmental factors, such as nutrient, pH, and water temperature, affect the population of *Salmonella* spp. [43]. The optimal pH range for the growth of *Salmonella* spp. is between 6.5 and 7.5, and the temperature range is between 20 and 30 °C in the soil [40]. Our results showed that there were no *Salmonella* spp. in water samples collected from fish tanks, nutrient reservoirs, or hydroponic culture units, although the pH and temperature were within the optimal range for their growth and the nutrient level was sufficient to allow the growth of *Salmonella* spp. in both systems (Table 1). Further, *Salmonella* spp. were not present in fish feces or the roots and edible portions of plants (Table 3; Figure S2). Outbreaks associated with fruits and vegetables contaminated with *Salmonella* spp. have also been attributed to contaminated irrigation water sources [73]. It should be noted that we used reverse osmosis water to fill and replenish the systems. It is apparent that the plants in the aquaponic and hydroponic systems did not have contact with contamination sources of *Salmonella* spp. A previous study reported that tomato fruit was not contaminated with *Salmonella* spp. even when the nutrient solution was inoculated with an avirulent strain of S. Typhimurium at a level of 10^5 colony-forming units (CFU)/mL in a hydroponic system [50] and when the plants were irrigated with 350 mL of 10^7 CFU/mL of S. Montevideo every 2 weeks for 10 weeks [74].

4. Conclusions

This study demonstrated that there is a potential risk of pathogen contamination in fresh produce grown in indoor aquaponic and hydroponic systems. STEC was found in fish feces, the recirculating water, and on the plant root surfaces in both aquaponic and hydroponic systems. Importantly, the bacteria did not internalize in the roots or the edible parts of plants regardless of the production

system. Fish feces were considered the major source of the pathogenic bacteria in the aquaponic system, possibly due to the introduction of contaminated fish. Therefore, it is essential to follow proper handling, cleaning, and sanitizing practices to minimize the risk of contamination of fresh fruit and vegetables grown in an aquaponic system.

Supplementary Materials: The following are available online at http://www.mdpi.com/2311-7524/6/1/1/s1, Figure S1: Bacterial isolation using a selective medium for *E. coli* O157 by plating on CT-SMAC agar with supplement. The red colonies on the plate are presumptive positive *E. coli* O157. A total of 10 colonies from each sample were confirmed for the presence of *stx1* gene using colony PCR., Figure S2: Colony PCR of selected colonies of (A) STEC in aquaponics and (B) STEC in hydroponics. M: 100bp Marker, P: Positive control (*E. coil* O157:H7), N: Negative control (H_2O), 1: Recycled water, 2: Fish feces, 3: Lettuce root surface, 4: Lettuce internal roots, 5: Lettuce leaves, 6: Basil root surface, 7: Basil internal roots, 8: Basil leaves, 9: Tomato root surface, 10: Tomato internal roots, and 11: Tomato leaves; Colony PCR of selected colonies of (C) *L. monocytogenes* and (D) *Salmonella* spp. in aquaponics and hydroponics. M: 100bp Marker, P: Positive control (*L. monocytogenes* or *Salmonella*), N: Negative control (H_2O), 1: Recycled water in aquaponics, 2: Recycled water in hydroponics, and 3: Fish feces.

Author Contributions: Y.-J.W. conducted the experiment, collected data, undertook data analysis and interpretation, and drafted the manuscript. A.J.D. supervised the microbial analysis and was involved in manuscript production, and made critical revisions. H.-J.K. coordinated and supervised the research, made critical revisions, and completed the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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References

1. Redmond, E.C.; Griffith, C.J. Consumer perceptions of food safety risk, control and responsibility. *Appetite* **2004**, *43*, 309–313. [CrossRef]
2. Tirado, M.C.; Clarke, R.; Jaykus, L.A.; McQuatters-Gollop, A.; Frank, J.M. Climate change and food safety: A review. *Food Res. Int.* **2010**, *43*, 1745–1765. [CrossRef]
3. Hoagland, L.; Ximenes, E.; Seockmo, K.; Ladisch, M. Foodborne pathogens in horticultural production systems: Ecology and mitigation. *Sci. Hortic.* **2018**, *236*, 192–206. [CrossRef]
4. Deering, A.J.; Mauer, L.J.; Pruitt, R.E. Internalization of E. coli O157:H7 and Salmonella spp. in plants: A review. *Food Res. Int.* **2012**, *45*, 567–575. [CrossRef]
5. Strawn, L.K.; Gröhn, Y.T.; Warchocki, S.; Worobo, R.W.; Bihn, E.A.; Wiedmann, M. Risk factors associated with salmonella and listeria monocytogenes contamination of produce fields. *Appl. Environ. Microbiol.* **2013**, *79*, 7618–7627. [CrossRef]
6. Abadias, M.; Alegre, I.; Oliveira, M.; Altisent, R.; Viñas, I. Growth potential of Escherichia coli O157:H7 on fresh-cut fruits (melon and pineapple) and vegetables (carrot and escarole) stored under different conditions. *Food Control* **2012**, *27*, 37–44. [CrossRef]
7. Brashears, M.M.; Durre, W.A. Antagonistic Action of Lactobacillus lactis toward Salmonella spp. and Escherichia coli O157:H7 during Growth and Refrigerated Storage. *J. Food Prot.* **1999**, *62*, 1336–1340. [CrossRef]
8. Walker, S.J.; Archer, P.; Banks, J.G. Growth of Listeria monocytogenes at refrigeration temperatures. *J. Appl. Bacteriol.* **1990**, *68*, 157–162. [CrossRef]
9. Painter, J.A.; Hoekstra, R.M.; Ayers, T.; Tauxe, R.V.; Braden, C.R.; Angulo, F.J.; Griffin, P.M. attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerg. Infect. Dis.* **2013**, *19*, 407–415. [CrossRef]
10. Dewey-Mattia, D.; Manikonda, K.; Hall, A.J.; Wise, M.E.; Crowe, S.J. Surveillance for foodborne disease outbreaks—United States, 2009–2015. *MMWR Surveill Summ* **2018**, *67*, 1–11. [CrossRef]

11. Dewey-Mattia, D.; Kisselburgh, H.; Manikonda, K.; Silver, R.; Subramhanya, S.; Sundararaman, P.; Whitham, H.; Crowe, S. *Surveillance for Foodborne Disease Outbreaks. Annual Report 2017*; Centers for Disease Control and Prevention (CDC): Atlanta, GA, USA, 2017. Available online: https://www.cdc.gov/fdoss/pdf/2017_FoodBorneOutbreaks_508.pdf (accessed on 10 August 2019).
12. Alegbeleye, O.O.; Singleton, I.; Sant'Ana, A.S. Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: A review. *Food Microbiol.* **2018**, *73*, 177–208. [CrossRef] [PubMed]
13. Despommier, D. The vertical farm: Controlled environment agriculture carried out in tall buildings would create greater food safety and security for large urban populations. *J. Verbr. Lebensm.* **2011**, *6*, 233–236. [CrossRef]
14. Kim, H.-J.; Yang, T.; Lin, M.-Y.; Langenhoven, P. Plant propagation for successful hydroponic production. *Acta Hortic.* **2018**, *1212*, 109–116. [CrossRef]
15. Gómez, C.; Currey, C.J.; Dickson, R.W.; Kim, H.-J.; Hernández, R.; Sabeh, N.C.; Raudales, R.E.; Brumfield, R.G.; Laury-Shaw, A.; Wilke, A.K.; et al. Controlled environment food production for urban agriculture. *HortScience* **2019**, *54*, 1448–1458. [CrossRef]
16. Guo, X.; van Iersel, M.W.; Chen, J.; Brackett, R.E.; Beuchat, L.R. Evidence of association of Salmonellae with tomato plants grown hydroponically in inoculated nutrient solution. *Appl. Environ. Microbiol.* **2002**, *68*, 3639–3643. [CrossRef]
17. Warriner, K.; Ibrahim, F.; Dickinson, M.; Wright, C.; Waites, W.M. Interaction of *Escherichia coli* with Growing Salad Spinach Plants. *J. Food Prot.* **2003**, *66*, 1790–1797. [CrossRef]
18. Franz, E.; Visser, A.; Vandiepeningen, A.; Klerks, M.; Termorshuizen, A.; Vanbruggen, A. Quantification of contamination of lettuce by GFP-expressing *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium. *Food Microbiol.* **2007**, *24*, 106–112. [CrossRef]
19. Macarisin, D.; Patel, J.; Sharma, V.K. Role of curli and plant cultivation conditions on *Escherichia coli* O157:H7 internalization into spinach grown on hydroponics and in soil. *Int. J. Food Microbiol.* **2014**, *173*, 48–53. [CrossRef]
20. Quagrainie, K.K.; Flores, R.M.V.; Kim, H.-J.; McClain, V. Economic analysis of aquaponics and hydroponics production in the U.S. Midwest. *J. Appl. Aquac.* **2018**, *30*, 1–14. [CrossRef]
21. Yang, T.; Kim, H.-J. Nutrient management regime affects water quality, crop growth, and nitrogen use efficiency of aquaponic systems. *Sci. Hortic.* **2019**, *256*, 108619. [CrossRef]
22. Chalmers, G.A. Aquaponics and Food Safety. 2004. Available online: http://www.backyardaquaponics.com/Travis/Aquaponics-andFood-Safety.pdf (accessed on 10 August 2019).
23. Rakocy, J.E. Aquaponics—Integrating Fish and Plant Culture. In *Aquaculture Production Systems*; Wiley-Blackwell: Hoboken, NJ, USA, 2012; pp. 344–386.
24. Enduta, A.; Jusoh, A.; Ali, N.; Wan Nik, W.B. Nutrient removal from aquaculture wastewater by vegetable production in aquaponics recirculation system. *Desalin. Water Treat* **2011**, *32*, 422–430. [CrossRef]
25. Geldreich, E.E.; Clarke, N.A. Bacterial Pollution Indicators in the Intestinal Tract of Freshwater Fish. *Appl. Microbiol.* **1966**, *14*, 429–437. [CrossRef]
26. WHO. *Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture*; (WHO Technical Report Series No. 778), Report of a World Health Organization, (WHO) Scientific Group; WHO: Geneva, Switzerland, 1989.
27. Pillay, T.V.R. *Aquaculture and the Environment*, 2nd ed.; Blackwell Pub: Oxford, UK; Malden, MA, USA, 2004; ISBN 978-1-4051-0167-7.
28. Rangel, J.M.; Sparling, P.H.; Crowe, C.; Griffin, P.M.; Swerdlow, D.L. Epidemiology of *Escherichia coli* O157:H7 Outbreaks, United States, 1982–2002. *Emerg. Infect. Dis.* **2005**, *11*, 603–609. [CrossRef]
29. Silk, B.J.; Mahon, B.E.; Griffin, P.M.; Gould, L.H.; Tauxe, R.V.; Crim, S.M.; Jackson, K.A.; Gerner-Smidt, P.; Herman, K.M.; Henao, O.L. Vital Signs: Listeria Illnesses, Deaths, and Outbreaks—United States, 2009–2011. *MMWR Morb. Mortal. Wkly. Rep.* **2013**, *62*, 448–452.
30. Jackson, B.R.; Griffin, P.M.; Cole, D.; Walsh, K.A.; Chai, S.J. Outbreak-associated *Salmonella enterica* Serotypes and Food Commodities, United States, 1998–2008. *Emerg. Infect. Dis.* **2013**, *19*, 1239–1244. [CrossRef]
31. Wongkiew, S.; Popp, B.N.; Kim, H.-J.; Khanal, S.K. Fate of nitrogen in floating-raft aquaponic systems using natural abundance nitrogen isotopic compositions. *Int. Biodeterior. Biodegrad.* **2017**, *125*, 24–32. [CrossRef]

32. Guzel, M.; Moreira, R.G.; Omac, B.; Castell-Perez, M.E. Quantifying the effectiveness of washing treatments on the microbial quality of fresh-cut romaine lettuce and cantaloupe. *LWT-Food Sci. Technol.* **2017**, *86*, 270–276. [CrossRef]
33. Andrews, W.H.; Wang, H.; Jacobson, A.; Hammack, T. *Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella*; FDA: Silver Spring, MD, USA, 2018.
34. Feng, P.; Weagant, S.D.; Grant, M.A.; Burkhardt, W. *BAM 4: Enumeration of Escherichia coli and the Coliform bacteria*; FDA: Silver Spring, MD, USA, 2002.
35. Fode-Vaughan, K.A.; Maki, J.S.; Benson, J.A.; Collins, M.L.P. Direct PCR detection of *Escherichia coli* O157:H7. *Lett. Appl. Microbiol.* **2003**, *37*, 239–243. [CrossRef]
36. Ziemer, C.J.; Steadham, S.R. Evaluation of the specificity of Salmonella PCR primers using various intestinal bacterial species*. *Lett. Appl. Microbiol.* **2003**, *37*, 463–469. [CrossRef]
37. Azaza, M.S.; Dhraïef, M.N.; Kraïem, M.M. Effects of water temperature on growth and sex ratio of juvenile Nile tilapia Oreochromis niloticus (Linnaeus) reared in geothermal waters in southern Tunisia. *J. Therm. Biol.* **2008**, *33*, 98–105. [CrossRef]
38. D'Souza, R.M.; Becker, N.G.; Hall, G.; Moodie, K.B.A. Does Ambient Temperature Affect Foodborne Disease? *Epidemiology* **2004**, *15*, 86. [CrossRef]
39. Shadbolt, C.; Ross, T.; McMeekin, T.A. Differentiation of the effects of lethal pH and water activity: Food safety implications. *Lett. Appl. Microbiol.* **2001**, *32*, 99–102. [CrossRef]
40. Guan, T.Y.; Holley, R.A. Pathogen survival in Swine manure environments and transmission of human enteric illness—A review. *J. Environ. Qual.* **2003**, *32*, 383–392. [CrossRef]
41. Kearney, T.E.; Larkin, M.J.; Frost, J.P.; Levett, P.N. Survival of pathogenic bacteria during mesophilic anaerobic digestion of animal waste. *J. Appl. Bacteriol.* **1993**, *75*, 215–219. [CrossRef]
42. Ellajosyula, K.R.; Doores, S.; Mills, E.W.; Wilson, R.A.; Anantheswaran, R.C.; Knabel, S.J. Destruction of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in Lebanon bologna by interaction of fermentaron pH, heating temperature, and time. *J. Food Prot.* **1998**, *61*, 152–157. [CrossRef]
43. Matches, J.R.; Liston, J. Effect of pH on low temperature growth of Samonella. *J. Milk Food Technol.* **1972**, *35*, 49–52. [CrossRef]
44. Lungu, B.; Ricke, S.C.; Johnson, M.G. Growth, survival, proliferation and pathogenesis of Listeria monocytogenes under low oxygen or anaerobic conditions: A review. *Anaerobe* **2009**, *15*, 7–17. [CrossRef]
45. Ukuku, D.O.; Zhang, H.; Huang, L. Growth parameters of *Escherichia coli* O157:H7, *Salmonella* spp., Listeria monocytogenes, and aerobic mesophilic bacteria of apple cider amended with Nisin–EDTA. *Foodborne Pathog. Dis.* **2009**, *6*, 487–494. [CrossRef]
46. Clavero, M.R.S.; Beuchat, L.R. Survival of *Escherichia coli* O157:H7 in broth and processed Salami as influenced by pH, water activity, and temperature and suitability of media for Its recovery. *Appl. Environ. Microbiol.* **1996**, *62*, 6.
47. Van Elsas, J.D.; Semenov, A.V.; Costa, R.; Trevors, J.T. Survival of *Escherichia coli* in the environment: Fundamental and public health aspects. *ISME J.* **2011**, *5*, 173–183. [CrossRef]
48. Settanni, L.; Miceli, A.; Francesca, N.; Cruciata, M.; Moschetti, G. Microbiological investigation of *Raphanus sativus* L. grown hydroponically in nutrient solutions contaminated with spoilage and pathogenic bacteria. *Int. J. Food Microbiol.* **2013**, *160*, 344–352. [CrossRef] [PubMed]
49. Allende, A.; Monaghan, J. Irrigation Water Quality for Leafy Crops: A Perspective of Risks and Potential Solutions. *Int. J. Environ. Res. Public Health* **2015**, *12*, 7457–7477. [CrossRef]
50. Coleman, S.M.; Bisha, B.; Newman, S.E.; Bunning, M.; Goodridge, L.D. Transmission and Persistence of Salmonella enterica in Nutrient Solution of Hydroponic Greenhouse Grown Tomatoes. *HortScience* **2017**, *52*, 713–718. [CrossRef]
51. Semenov, A.V.; van Overbeek, L.; Termorshuizen, A.J.; van Bruggen, A.H.C. Influence of aerobic and anaerobic conditions on survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in Luria–Bertani broth, farm-yard manure and slurry. *J. Environ. Manag.* **2011**, *92*, 780–787. [CrossRef] [PubMed]
52. Franz, E.; Bruggen, A.H.C. van Ecology of *Escherichia coli* O157:H7 and *Salmonella enterica* in the primary vegetable production chain. *Crit. Rev. Microbiol.* **2008**, *34*, 143–161. [CrossRef]

53. Pagadala, S.; Marine, S.C.; Micallef, S.A.; Wang, F.; Pahl, D.M.; Melendez, M.V.; Kline, W.L.; Oni, R.A.; Walsh, C.S.; Everts, K.L.; et al. Assessment of region, farming system, irrigation source and sampling time as food safety risk factors for tomatoes. *Int. J. Food Microbiol.* **2015**, *196*, 98–108. [CrossRef]
54. Kokkinos, P.; Kozyra, I.; Lazic, S.; Söderberg, K.; Vasickova, P.; Bouwknegt, M.; Rutjes, S.; Willems, K.; Moloney, R.; de Roda Husman, A.M.; et al. Virological Quality of Irrigation Water in Leafy Green Vegetables and Berry Fruits Production Chains. *Food Environ. Virol.* **2017**, *9*, 72–78. [CrossRef]
55. Buras, N.; Duek, L.; Niv, S.; Hepher, B.; Sandbank, E. Microbiological aspects of fish grown in treated wastewater. *Water Res.* **1987**, *21*, 1–10. [CrossRef]
56. Apun, K.; Yusof, A.M.; Jugang, K. Distribution of bacteria in tropical freshwater fish and ponds. *Int. J. Environ. Health Res.* **1999**, *9*, 285–292. [CrossRef]
57. Wang, G.; Doyle, M.P. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in water. *J. Food Prot.* **1998**, *61*, 662–667. [CrossRef]
58. Youssef, H.; El-Timawy, A.K.; Ahmed, S. Role of aerobic intestinal pathogens of fresh water fish in transmission of human diseases. *J. Food Prot.* **1992**, *55*, 739–740. [CrossRef] [PubMed]
59. Schuster, M.; Maertens, M. Do private standards benefit workers in horticultural export chains in Peru? *J. Clean. Prod.* **2016**, *112*, 2392–2406. [CrossRef]
60. Chen, Y.; Ji, H.; Chen, L.-J.; Jiang, R.; Wu, Y.-N. Food Safety Knowledge, Attitudes and Behavior among Dairy Plant Workers in Beijing, Northern China. *Int. J. Environ. Res. Public Health* **2018**, *15*, 63. [CrossRef]
61. Pachepsky, Y.; Shelton, D.R.; McLain, J.E.T.; Patel, J.; Mandrell, R.E. Irrigation Waters as a Source of Pathogenic Microorganisms in Produce: A Review. In *Advances in Agronomy*; Sparks, D.L., Ed.; Academic Press: Burlington, VT, USA, 2011; pp. 75–141.
62. Somers, E.B.; Schoeni, J.L.; Wong, A.C.L. Effect of trisodium phosphate on biofilm and planktonic cells of *Campylobacter jejuni*, *Escherichia coli* O157: H7, Listeria monocytogenes and *Salmonella typhimurium*. *Int. J. Food Microbiol.* **1994**, *22*, 269–276. [CrossRef]
63. Dewanti, R.; Wong, A.C.L. Influence of culture conditions on biofilm formation by *Escherichia coli* O157:H7. *Int. J. Food Microbiol.* **1995**, *26*, 147–164. [CrossRef]
64. Uhlich, G.A.; Cooke, P.H.; Solomon, E.B. Analyses of the Red-Dry-Rough Phenotype of an *Escherichia coli* O157:H7 Strain and Its Role in Biofilm Formation and Resistance to Antibacterial Agents. *Appl. Environ. Microbiol.* **2006**, *72*, 2564–2572. [CrossRef]
65. Orozco, R.; Iturriaga, M.H.; Tamplin, M.L.; Fratamico, P.M.; Call, J.E.; Luchansky, J.B.; Escartin, E.F. Animal and environmental impact on the presence and distribution of *Salmonella* and *Escherichia coli* in hydroponic tomato greenhouses. *J. Food Prot.* **2008**, *71*, 676–683.
66. Joyce, A.; Timmons, M.; Goddek, S.; Pentz, T. Bacterial Relationships in Aquaponics: New Research Directions. In *Aquaponics Food Production Systems: Combined Aquaculture and Hydroponic Production Technologies for the Future*; Goddek, S., Joyce, A., Kotzen, B., Burnell, G.M., Eds.; Springer International Publishing: Cham, Switzerland, 2019; pp. 145–161. ISBN 978-3-030-15943-6.
67. Fox, B.K.; Tamaru, C.S.; Hollyer, J.; Castro, L.F.; Fonseca, J.M.; Jay-Russell, M.; Low, T. A Preliminary Study of Microbial Water Quality Related to Food Safety in Recirculating Aquaponic Fish and Vegetable Production Systems. 2012. Available online: https://www.ctahr.hawaii.edu/oc/freepubs/pdf/FST-51.pdf (accessed on 10 August 2019).
68. Joyce, T.M.; Mcguigan, K.G.; Elmore-Meegan, M.; Conroy, R.M. Inactivation of fecal bacteria in drinking water by solar heating. *Appl. Environ. Microbiol.* **1996**, *62*, 399–402.
69. Crane, S.R.; Moore, J.A. Modeling enteric bacterial die-off: A review. *Water Air Soil Pollut* **1986**, *27*, 411–439. [CrossRef]
70. Barnhart, C.; Barnhart, C.; Ringle, D. Food Safety Hazards Associated with Smooth Textured Leafy Greens Produced in Aquaponic, Hydroponic, and Soil-Based Systems with and Without Roots in Retail. Minnesota Aquaponics Conference Posters, MN, USA. 2015. Available online: https://conservancy.umn.edu/handle/11299/172223 (accessed on 10 August 2019).
71. Saylor, L.R.; Turkon, P. *Escherichia Coli* and Salmonella Presence in Hydroponic and Aquaponic Systems: An Exploratory Case Study. 2018. Available online: https://digitalcommons.ithaca.edu/cgi/viewcontent.cgi?article=1397&context=whalen (accessed on 10 August 2019).
72. Wachtel, M.R.; Whitehand, L.C.; Mandrell, R.E. Association of Escherichia coli O157:H7 with preharvest leaf lettuce upon exposure to contaminated irrigation water. *J. Food Prot.* **2002**, *65*, 18–25. [CrossRef]

73. Gould, L.H.; Walsh, K.A.; Vieira, A.R.; Herman, K.; Williams, I.T.; Hall, A.J.; Cole, D. *Surveillance for Foodborne Disease Outbreaks—United States, 1998–2008*; U.S. Department of Health and Human Services: Washington, DC, USA, 2013.
74. Miles, J.M.; Sumner, S.S.; Boyer, R.R.; Williams, R.C.; Latimer, J.G.; McKinney, J.M. Internalization of Salmonella enterica serovar Montevideo into greenhouse tomato plants through contaminated irrigation water or seed stock. *J. Food Prot.* **2009**, *72*, 849–852. [CrossRef] [PubMed]