College of Tropical Agriculture and Human Resources
University of Hawai'i at Mānoa

Food Safety and Technology
October 2012
FST-51

A Preliminary Study of Microbial Water Quality Related to Food Safety in Recirculating Aquaponic Fish and Vegetable Production Systems

Bradley K. Fox[1], Clyde S. Tamaru[1], James Hollyer[2], Luisa F. Castro[3], Jorge M. Fonseca[4], Michele Jay-Russell[5], and Todd Low[6]

[1]Department of Molecular Biosciences and Bioengineering, CTAHR; [2]Plant and Environmental Protection Sciences, CTAHR; [3]Department of Natural Resources and Environmental Management, CTAHR; [4]College of Agriculture and Life Sciences, University of Arizona; [5]Western Center for Food Safety, University of California, Davis; [6]Aquaculture Development Program, Hawai'i Department of Agriculture

Introduction

Aquaponics (the integration of aquaculture and hydroponics) is a developing technology that serves as a model of sustainable food production. In a recirculating aquaponic system (RAS), liquid effluent rich in plant nutrients derived from fish manure, decomposing organic matter, and nitrogenous waste excreted from fish fertilizes hydroponic beds, providing essential elements for plant growth (Rakocy et al. 2006). This form of agricultural production is extremely versatile, as both edible and ornamental plants can be grown in these systems. Being independent of the quality of or even need for soil to produce crops, this technology provides extraordinary freedom to farmers and gardeners alike as to where they may be able to grow their crops.

Food safety in agricultural systems is emerging as an increasingly critical component of agriculture both domestically and internationally (U.S. FDA Food Safety Modernization Act 2011). Aquaponics (Fig. 1), as one type of agricultural system, has gained popularity in the past 20 years, but due to its novelty, food safety and proper harvesting procedures have only recently begun to be addressed for aquaponic producers (Hollyer et al. 2009). There have also been several recent reports focusing on food safety and levels of food safety-indicator microorganisms both from produce and the water in commercial aquaponic systems (Rakocy 2003, Chalmers 2004, González-Alanis et al. 2011). One of the most prominently studied is *Escherichia coli*.

Fig. 1. Growbeds with lettuce on a commercial raised-bed aquaponic farm in Hawai'i.

E. coli is a bacterium typically found in the intestines of warm-blooded animals like pigs, deer, birds, and cattle, which has recently been used in developing human health-based regulatory standards as a common indicator of fecal contamination and microbial water quality in recreational and agricultural water systems.

Published by the College of Tropical Agriculture and Human Resources (CTAHR) and issued in furtherance of Cooperative Extension work, Acts of May 8 and June 30, 1914, in cooperation with the U.S. Department of Agriculture, under the Director/Dean, Cooperative Extension Service/CTAHR, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822.

Indicator microbes and pathogenic bacteria, such as *E. coli* O157:H7 and *Salmonella* spp., if present in aquaponic systems, most likely originate from warm-blooded animals such as birds, since these zoonotic enteric bacteria are transient in fish gut microflora (Cahill 1990, Sugita et al. 1996).

There are several aspects of food safety with regard to raw vegetable produce, including chemical (i.e., pesticides, herbicides, antibiotic use, etc.), physical (i.e., foreign objects, metal or bone fragments, large pieces of soil or rocks), and biological (i.e., bacteria, parasites, viruses, etc.). This publication will focus on the biological aspects, addressing the subject of indicator microbes in aquaponic water, as this emerging production system poses unique questions with regard to food safety. Though questions about food safety related to aquaponic vegetable production are understandable, chiefly due to the fact that large-scale commercialization of this technology is a fairly recent innovation, aquaponic produce and fish have been shown to be consistently safe (Rakocy 2003, Chalmers 2004). Increasing awareness of the state of the scientific issues regarding indicator organisms in an aquaponic setting allows all participants to make informed decisions. The desired outcome of this publication is to provide preliminary data that strongly indicate that recirculating aquaponics systems are far less risky with respect to indicator microbes and pathogenic bacteria than soil-based production systems that are typically exposed to warm-blooded animals and use non-potable surface water for irrigation, such as is found in the Colorado River (Table 1).

Food Safety and Water Quality

The subject of food safety with regard to fresh vegetable production, specifically related to biological water-quality indicators, is a vast and inherently complex field of study. A recent report by Scharff (2012) suggests the total health-related cost of foodborne illness is approximately $77 billion annually in the United States. In a previous study, the same author suggested that continued foodborne pathogenic outbreaks in the U.S. are due to a variety of causes, including a fundamentally poor understanding of the complexities of food contamination, specifically with respect to pathogen vectors. Other causes include the inadequate resources available to address these complicated issues and a limited knowledge base as to what would be appropriate and effective methods for the prevention of outbreaks (Scharff 2009).

Currently, government and industry entities estimate food safety risk factors in agricultural system irrigation waters by measuring levels of waterborne indicator microbes such as generic (commensal) *E. coli*, which are not necessarily pathogenic to humans. In the absence of any national or state irrigation water-quality standards, water-quality standards for agriculture are currently based on those set by the U.S. Environmental Protection Agency (EPA) for recreational uses, which apply to any body of water where human activity occurs (EPA 1986). The science behind the recreational water criteria was intended to maintain a risk of gastrointestinal illness lower than 8 cases per 1,000 swimmers at freshwater beaches based on exposure to a point-source, untreated human wastewater discharge or spill (Dufour 1984, EPA 1986). However, several recent studies have found these microbes to be poor predictors of foodborne illness because of a lack of accepted and compelling predictive value with regard to pathogenic bacteria (Suslow 2009, Pachepsky et al. 2011a).

Indeed, in most cases both domestically and internationally, the quality of surface water with respect to indicator microbes used for irrigation is not known because it is not tested at any meaningful frequency or with any scientifically justified, consistent, or standardized protocols (Suslow 2009, Pachepsky et al. 2011a, Pachepsky et al. 2011b). Based on a recent study by Fonseca et al. (2011), which represents one of the few exceptions to this information deficit, indicator microbes such as generic *E. coli* were regularly observed in surface irrigation water in the lower Colorado River basin, and these levels can be highly variable. Though the observed levels of indicator microbes occasionally violated the EPA threshold for single water samples (240 CFU/100 mL), the geometric mean remained below the accepted 126 CFU/100 mL, and no pathogenic bacteria were detected throughout the course of the study (Fonseca et al. 2011). Understandably, this general scarcity of science-based data is due in large part to the essentially prohibitive monetary cost of monitoring appropriate water-quality endpoints for all commercial vegetable producers. It is primarily due to this lack of actual risk-based data that the California Leafy Green Products Handler Marketing Agreement (LGMA) and other regional Community Supported Gardens (CSGs) have adopted EPA recreational water quality criteria for establishing microbiological action

Table 1. Comparison of the relative food safety risks on a soil-based farm and an elevated, soilless aquaponics farm.

Potential risk factors	Typical soil-based produce farm	Netted, elevated, soilless aquaponic produce farm
Soil	Soil-based risks are plot-by-plot, farm-by-farm. Soil cannot be sanitized.	Soil not used. Growing surface elevated above ground. Potential growing surfaces include gravel and synthetic materials such as Styrofoam and plastic—all can be sanitized as necessary.
Irrigation water	Water source could be river, ditch, well, lake, rooftop, municipal (potable), and/or recycled. Non-municipal water sources could contain pathogens, pesticides and other undesirable factors that could be applied to the edible portion of the crop.	The best practice is to use potable water in fish tanks and never to use open source water from rivers, ditches, wells, lakes, etc.
Warm-blooded animal intrusion	Cattle, deer, swine, birds, rats, etc., may be able to access ground-level growing areas.	The best practice is to net all production systems. Netted, elevated growing systems severely restrict any warm-blooded animal access.
Fish feces (feces from cold-blooded animals)	Fish wastes could be in open-source irrigation water (and/or in fish meal). Could be applied on the edible portion of the crop or on soil via irrigation water.	Fish waste-based nutrients are kept well under the edible portion of the crop and never applied directly to the edible portion of the crop. Fish wastes must first be converted to nitrate, by symbiotic bacteria, for plant uptake and use.
Worker hygiene	Same as for any produce farm.	Same as for any produce farm.
Packing facility safety	Same as for any produce farm.	Same as for any produce farm.
		

thresholds for irrigation water (LGMA 2007, Suslow 2009). Nevertheless, LGMA standards allow overhead irrigation of vegetable produce wherein water is sprayed or otherwise applied directly onto the edible portion of the crop using raw surface or well water, so long as indicator microbes remain within the acceptable levels. Coversely, overhead irrigation is not utilized in commercial aquaponic farms, and system water remains below the plants, in contact with the roots, in a variety of subsurface irrigation techniques, thus reducing the chance of direct contamination of the edible plant portion.

Foodborne Pathogens and Aquaculture

The utilization of waste products excreted by warm-blooded animals in vegetable production raises further concerns with respect to food safety. Integrated agricultural systems like aquaculture (fertigation) and aquaponics (recirculation), wherein uncomposted cold-blooded animal (fish) wastes (i.e., those that have not undergone a heating process high enough to kill pathogens) are utilized as fertilizer to grow vegetable crops either in-ground or hydroponically, present a novel set of challenges with respect to irrigation water quality. In

general the average body temperature of poikilothermic, or "cold-blooded," animals like fish is considered too low for optimal proliferation of most enteric bacteria likely to infect humans, though some cold-blooded land-based tetrapods like amphibians and reptiles have been shown to harbor *Salmonella* (Rio-Rodriguez et al. 1997, Chalmers 2004). Furthermore, fish are not considered by the Center for Disease Control (CDC) to be "animals of significant risk" of carrying *E. coli* O157:H7 (Commodity Specific Food Safety Guidelines for the Production and Harvest of Leafy Greens 2009). However, it has been well documented by a variety of studies that freshwater fish can harbor zoonotic and pathogenic bacteria (Sugita et al. 1996, Al-Harbi et al. 2005). Certainly, fish ingest a multiplicity of bacterial genera and species via water, sediment, and food; essentially the gut microflora of fish reflects the aquatic environment in which they live (Cahill 1990, Al-Harbi 2003, Chalmers 2004, El-Shafai et al. 2004).

This latter point is key in understanding the presence of indicator microbes in aquaculture/aquaponic water systems within the context of the present discussion concerning food safety. Though it is possible that enteric and even potentially pathogenic bacteria can be detected in fish intestines, it is unlikely that the pathogens themselves originate or proliferate in aquaculture/aquaponic food production systems (Cahill 1990, Al-Harbi 2003, Chalmers 2004). Indeed, the cause of fecal indicator bacteria observed in pond- and tank-based aquaculture/aquaponic operations is predominantly due to the quality of the source water (i.e., non-potable or poorly treated municipal wastewater, contaminated well or surface ditch water, etc.) and/or fecal inputs from wildlife, primarily avian in origin (Hussong et al. 1979, Al-Harbi 2003, Chalmers 2004, El-Shafai et al. 2004). Of note is the fact that the edible portion of the plant is never exposed to fish feces in an aquaponic system. If foodborne pathogens entered the system, pre-harvest transfer to the plant would require root uptake, which is unlikely to occur or persist through harvest under natural conditions (Erickson 2012). Thus, if potable water is used in the system, the human health risk from contaminated fish feces followed by root uptake should be negligible.

Recently, an increasing number of commercial produce buyers are requiring their supplying farms to undergo annual third-party audits to reduce the chance of foodborne outbreaks from the food they sell. Many dozens of audit questions cover commercial farmer hygiene, pesticide use, post-harvest handling, and the use of various soil amendments, such as manures. The audit questions are known beforehand, and there are a number of "automatic failures" to protect against high-risk growing and handling conditions. Growing produce in direct contact with uncomposted animal feces is one of the automatic failures. Since the audits do not differentiate the types of feces, for example warm-blooded animal feces from cows or pigs versus cold-blooded animal feces from fish, aquaponic systems trigger an automatic failure regardless of all other conditions on the farm. The audits assume that all types of feces are potentially harmful, regardless of their source; however, this simply is not the case.

Methods

In response to questions arising from the lack of in-the-field knowledge about the quality of the production water in a well-run aquaponics system, the College of Tropical Agriculture and Human Resources (CTAHR) Farm Food Safety and Aquaculture Extension Projects solicited funding from the Hawaii Department of Agriculture's (HDOA) Agribusiness Development Corporation and leveraged resources in collaboration with the HDOA's Aquaculture and Livestock Support Services to conduct onsite product and water sampling in an effort to obtain real data in a real-world commercial aquaponics setting (Tamaru et al. 2012). Within the period of approximately one year, from January 2011 to January 2012, approximately 150 samples (including fish and vegetable tissue, as well as system water) were amassed from commercial and backyard-scale aquaponic producers located on three Hawaiian islands according to the protocol established by Tamaru et al. (2012). Briefly, water, plant and fish tissue, and supplemental aquaponic input samples were collected aseptically with gloves and immediately placed in sterile plastic containers (Fig. 2). Chain of custody was adhered to, as the collected samples were immediately submitted to an accredited private testing laboratory for analyses. The following data and discussion outline the results from this preliminary study. The concentration of indicator *E. coli* (CFU/100ml) was measured using standard protocols. Indicator microbes were estimated from water samples, and the data were analyzed graphi-

Fig. 2. A research scientist taking a water sample from an aquaponic farm using sterile techniques.

cally to illustrate the results within the framework of the EPA-recommended recreational water-quality standards for *E. coli* that are used by the produce industry in absence of other irrigation water-quality standards. These standards are 1) geometric mean of <126 colony-forming units (CFU)/100 ml based, or 2) <235 CFU/100 ml for any single water sample (EPA 1986). Subsets of samples were also analyzed by Polymerase Chain Reaction for the presence or absence of *E. coli* O157:H7 and *Salmonella*.

Results and Discussion

The first set of sampling for the current study was conducted on a single commercial aquaponic farm over the course of one year, and the data represent 8 individual sampling events. Data presented in Figure 3A summarize the single-point determinations for generic *E. coli* in CFU/100ml, and while there is some variation in the values, none exceeded the 235 CFU/100 ml action threshold which would trigger an automatic retesting by LGMA audit standards (LGMA 2007).

It is well known that a variety of factors influence both the survival and proliferation of pathogenic bacteria in water, including temperature, bacterial predators, competing microbes, pH, nutrient quality and availability, and solar radiation, among other factors (Pachepsky et al. 2011a). Additionally, not only does the concentration of fecal indicator microbes follow diurnal variations throughout the water column; it is not uncommon for researchers to observe differences in minimum and maximum values that vary between several orders of magnitude for indicator species sampled at the same location in surface irrigation water (Pachepsky et al. 2011a and 2011b). It is partially due to this intrinsic sampling error when estimating fecal indicator microbes from irrigation water that the data are typically transformed and expressed as rolling geometric means. The EPA's standard for calculating this number in recreational water quality for *E. coli* is a geometric mean of 126 organisms/100 ml based on several samples collected; generally not less than five samples equally spaced over a 30-day period (Tamaru et al. 2012). Thus, for the current study, the geometric means for the same data set were also calculated and expressed graphically (Figure 3B) to illustrate the tightening of the data. It is clear that the levels of *E. coli* recorded during this initial sampling period were very low, and accordingly the values are compliant with EPA standards for recreational use of water. The values observed fall well within the threshold values of the LGMA and thus should be considered adequate to pass a GAP audit by food safety auditors.

As stated previously, additional aquaponic system water samples were collected from producers in multiple locations around the state of Hawai'i and analyzed in a similar manner. Figure 4 illustrates the results of these samplings at 11 different farms expressed as geometric means. The data show that, as with the system water sampled from a single farm in Figure 3B, all values of indicator microbes (*E. coli*) are very low, and accordingly the values are compliant with EPA and LGMA standards. Furthermore, in the same study, 48 samples of aquaponic produce originating from these 11 farm locations were submitted to the laboratory for analyses (Table 2). As observed with the water samples, all the produce tissue samples analyzed were shown to have very low levels of generic *E. coli*, or undetectable pathogenic *E. coli* O157:H7 and *Salmonella*.

The nutritional complement of fish food is lacking in key micronutrients for hydroponic vegetable production in aquaponic systems, specifically with respect to leafy greens (Rakocy et al. 2006). Thus, commercial aqua-

Fig 3A: A summary of the single-point determinations of water samples taken for generic *E. coli* in CFU/100ml, from a single farm over the course of one year. This figure illustrates some variation in the values; however, none exceeded the 235 CFU/100 ml action thresholds that would trigger an automatic retesting by LGMA audit standards.

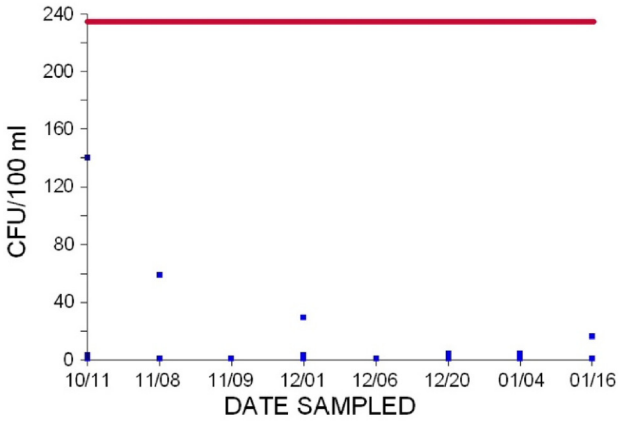


Fig 3B: A summary of the rolling geometric means of the data expressed in Fig. 3A for generic *E. coli* in CFU/100ml, from a single farm over the course of one year. Once transformed, all values of fecal indicators (*E. coli*) are very low, and accordingly the values are compliant with EPA recreational water use and LGMA standards.

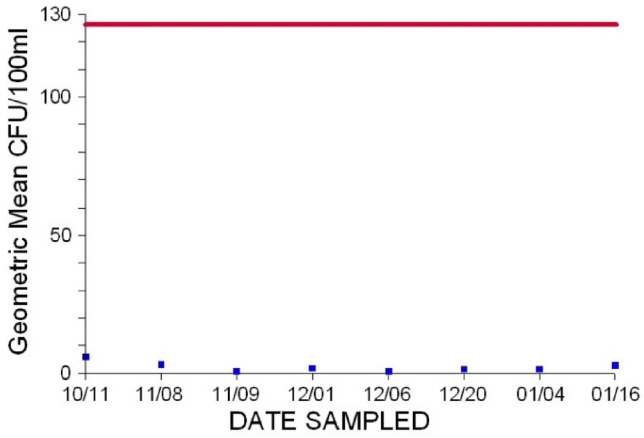
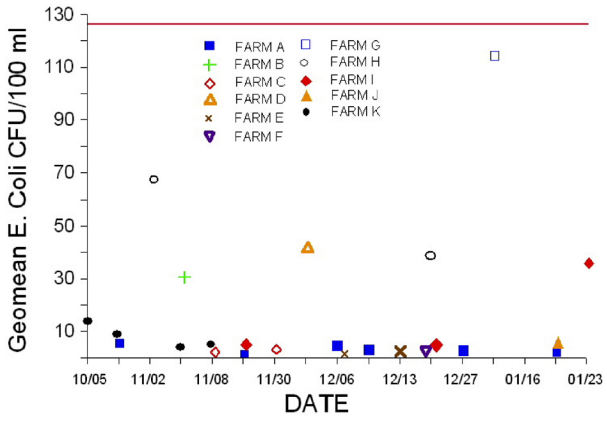


Fig 4: A summary of the rolling geometric means for generic *E. coli* in CFU/100ml, of water samples collected from producers in 11 different farms around the state of Hawai'i. All the values of fecal indicators (*E. coli*) are low, and accordingly the values are compliant with EPA recreational water use and LGMA standards.



ponic producers typically rely on a variety of synthetic and/or organic inputs to supplement common nutritional deficiencies and water-quality endpoints in their produce. These inputs represent an additional potential vector for the introduction of pathogenic bacteria into aquaponic systems. Accordingly, during the course of the current study, samples of some of these amendments were submitted for microbial analyses. Table 3 illustrates the results of the aquaponic supplements tested for the pathogenic bacteria *E. coli* O157:H7 and *Salmonella*. In agreement with the aquaponic system water tested, the results were negative or below the detection limit of the assay in all cases.

It is important to note that throughout the course of the current study, the majority of aquaponic producers (backyard and commercial alike) utilize potable water as the initial source of the water for their systems. However, in two specific instances, source water used by commercial farmers for their aquaponic systems originated from surface irrigation; that is, water derived from open earthen or channelized irrigation canals, which resulted in extremely high circulating indicator microbe levels within the system (>2000 fecal coliform MF/100mL and >200 generic *E. coli*, unpublished results). Therefore,

GAPs for aquaponic fish and vegetable production should always include using potable water as the source water for initial startup and recharge to offset evaporation, as suggested by Hollyer et al. (2009).

The final sampling set in the current study consists of a comparison of the indicator microbes present in traditional aquaculture (semi-static green water, or algae-based system) versus aquaponic (closed, bacteria-based recirculating system). Additionally, the microbiological quality of the edible livestock component of these systems (i.e., the fish muscle tissue) was also examined. Raising fish for food in poor water-quality environments, such as recycling domestic human sewage for aquaculture source water, is a common practice worldwide (though not in Hawai'i), especially in developing nations (El-Shafai et al. 2004). This trend has prompted the World Health Organization (WHO 1989) to recommend guidelines for fecal coliforms in aquaculture pond water above which it can be reasonably assumed the edible portion of the fish (i.e., the muscle) will be contaminated by pathogens. The most common zoonotic incidences in aquaculture originate from either puncture wounds that are not properly washed during the cleaning or handling of fish, or ingestion of raw unprocessed fish or shellfish (Chalmers 2004). Many species of bacteria, including coliforms, are inherently present in aquaponic systems due to the nature of the recirculating biofiltration component of the system (Rakocy et al. 2006, Hollyer et al. 2009). These levels, however, are consistently well below the recommended WHO-initiated guidelines of 10^3 MF/100 mL (WHO 1989).

Nevertheless, in the current study levels of indicator microbes and pathogenic bacteria were estimated from system water and the raw flesh of freshly harvested tilapia fish originating from both aquaculture (containing high-density single-cell algae in suspension) and aquaponic tanks (Table 3). Of the 12 fish fillets aseptically collected, no *E. coli* O157:H7 or *Salmonella* were detected, even though fecal coliforms

Table 2. A summary of 48 tissue samples of aquaponic produce originating from 11 different farms around the state of Hawai'i. All tissue samples analyzed were shown to have low levels of generic *E. coli*, or undetectable pathogenic *E. coli* O157:H7 and *Salmonella*.

Date	Sample	Farm	*E. coli* MPN/25 g*	*E. coli* O157:H7	*Salmonella*
1/31/2011	Cucumbers	A	< 3.0	Neg.	Neg.
1/31/2011	Lettuce	A	< 3.0	Neg.	Neg.
1/31/2011	Beets	A	< 3.0	Neg.	Neg.
1/31/2011	Lettuce	A	< 3.0	Neg.	Neg.
1/31/2011	Tomatoes	A	< 3.0	Neg.	Neg.
10/05/2011	Lettuce	K	-	Neg.	Neg.
10/05/2011	Lettuce	K	-	Neg.	Neg.
10/11/2011	Lettuce	A	-	Neg.	Neg.
10/11/2011	Lettuce	K	-	Neg.	Neg.
10/11/2011	Lettuce	A	-	Neg.	Neg.
10/11/2011	Lettuce	K	-	Neg.	Neg.
10/20/2011	Lettuce	A	-	Neg.	Neg.
10/20/2011	Lettuce	A	-	Neg.	Neg.
11/02/2011	Lettuce	K	-	Neg.	Neg.
11/02/2011	Lettuce	K	-	Neg.	Neg.
11/03/2011	Lettuce	K	-	Neg.	Neg.
11/03/2011	Lettuce	C	-	Neg.	Neg.
11/03/2011	Lettuce	C	-	Neg.	Neg.
11/03/2011	Lettuce	K	-	Neg.	Neg.
11/08/2011	Lettuce	A	-	Neg.	Neg.
11/08/2011	Lettuce	I	< 0.5		
11/08/2011	Lettuce	I	< 0.5		
11/08/2011	Lettuce	A	-	Neg.	Neg.
11/23/2011	Lettuce	C	-	Neg.	Neg.
11/23/2011	Lettuce	C	-	Neg.	Neg.
11/30/2011	Watercress	D	-	Neg.	Neg.
11/30/2011	Watercress	D	-	Neg.	Neg.
12/01/2011	Lettuce	E	-	Neg.	Neg.
12/01/2011	Lettuce	A	-	Neg.	Neg.
12/01/2011	Lettuce	E	-	Neg.	Neg.
12/01/2011	Lettuce	A	-	Neg.	Neg.

* - Indicates that test was not conducted

Table 2, cont'd.

Date	Sample	Farm	*E. coli* MPN/25 g*	*E. coli* O157:H7	*Salmonella*
12/01/2011	Lettuce	A	-	Neg.	Neg.
12/12/2011	Lettuce	E	-	Neg.	Neg.
12/12/2011	Green Onion	E	-	Neg.	Neg.
12/13/2011	Pak Choi	I	-	Neg.	Neg.
12/13/2011	Lettuce	F	-	Neg.	Neg.
12/13/2011	Lettuce	I	-	Neg.	Neg.
12/13/2011	Lettuce	H	-	Neg.	Neg.
12/13/2011	Lettuce	F	-	Neg.	Neg.
12/13/2011	Lettuce	G	-	Neg.	Neg.
12/27/2011	Lettuce	G	-	Neg.	Neg.
01/16/2012	Lettuce	A	-	Neg.	Neg.
01/16/2012	Tomatoes	A	-	Neg.	Neg.
01/16/2012	Blueberries	A	-	Neg.	Neg.
01/16/2012	Watercress	J	-	Neg.	Neg.
01/16/2012	Beets	A	-	Neg.	Neg.
01/16/2012	Cucumbers	A	-	Neg.	Neg.
01/16/2012	Lettuce	A	-	Neg.	Neg.
01/16/2012	Watercress	J	-	Neg.	Neg.
01/23/2012	Lettuce	I	-	Neg.	Neg.
01/23/2012	Pak Choi	I	-	Neg.	Neg.

* - Indicates that test was not conducted

and generic *E. coli* were detectable in all system waters (Table 4). Although more in-depth studies are certainly warranted, the initial findings presented in the current report certainly suggests that the presence of algae, fecal coliforms, and commensal *E. coli* in aquaculture/aquaponic system water does not necessarily correlate with the presence of pathogenic bacteria in fish flesh.

Summary

Aquaponic fish and vegetable production is gaining popularity and acceptance both domestically and abroad. As the industry matures, it must do so in accordance with Good Agricultual Practices and rapidly developing food safety guidelines. Similarly, with respect to food safety, third-party auditors must recognize aquaponics as a viable, sustainable, and safe method of vegetable production. This is especially true when elevated recirculating aquaponics systems that use potable source water are contrasted with thousands of soil-based vegetable farms that draw their overhead irrigation water from non-potable, highly exposed sources such as rivers, lakes, ditches, and hillside ponds. Many of these water sources contain cold-blooded fish, just like an aquaponics system. The concept of recirculating aquaponic system water being defined as irrigation water means it must be held to similar standards as water originating from surface or well sources. Likewise, the dual status of aquaponic system water as both irrigation water and the primary source of fertilizer in the growing system must be addressed by LGMA standards, as it is currently being held to an additional standard regarding unprocessed, uncomposted animal (fish) manure. Indeed, in the last 20 years there have been no confirmed reports of human illness due to contaminated aquaponic fish or raw produce (Rakocy 2003, Chalmers 2004, González-Alanis et al. 2011).

The field of food safety is rapidly evolving, and future national and international guidelines and restrictions are difficult to predict. Certainly it is possible that any animal or insect can potentially act as a vector to contaminate produce with pathogens (Crohn et al. 2008). The results presented in the current study represent some novel microbial data regarding food safety in commercial and backyard aquaponic production systems. Nevertheless, the data herein represent merely a preliminary glimpse into an otherwise complicated issue. Further rigorous, replicated scientific trials encompassing larger sample sizes, statistical correlation with farm management and environmental co-variates, and culture for pathogens (as the results from the private lab tests conducted in the current study were PCR only), are certainly needed to better elucidate food safety in aquaponic systems. In the future, a successful strategy to prevent foodborne illness from produce of all surfaces may be to monitor specific human pathogens directly on raw-consumed produce (Chalmers

Table 3. A summary of the aquaponic supplements tested for the pathogenic bacteria *E. coli* O157:H7 and *Salmonella* in the various aquaponic systems operating at Farm A. In agreement with the aquaponic system water tested, the results were negative in all cases.

Date	Sample	*E. coli**	*E. coli* O157:H7*	*Salmonella*
12/06/2011	Fish Food	-	< 3	Neg.
12/06/2011	Sustane	-	< 3	Neg.
12/06/2011	Bone Meal	-	< 3	Neg.
12/06/2011	Kelp Meal	-	< 3	Neg.
1/16/2012	Source Water	< 1	-	Neg.

* - Indicates that test was not conducted.

2004, Suslow 2009, Pachepsky et al. 2011a). Finally, with respect to aquaponic GAP certification by third-party auditors, in aquaponics the simple and unavoidable fact is that raw animal (fish) manure is purposefully and persistently present on-farm and in intimate contact with the non-edible portion of a crop. Indeed, it is hard to imagine that fish and a variety of other aquatic and terrestrial animals are absent from the numerous irrigation systems around the United States that rely on surface water. Some California and Arizona leafy green growers are using water directly, unfiltered or treated, from the Colorado River for overhead irrigation, i.e., direct application of potentially hazardous water to the edible portion of the leafy greens. The Colorado River water, like aquaponic systems, has fish and thus fish feces in it, among other potential contaminates from agricultural, industrial, and

Table 4. A summary of indicator microbes and pathogenic bacteria estimated from system water and raw flesh of freshly harvested tilapia fish originating from both aquaculture and aquaponic tanks in aquaponic research systems at the University of Hawai'i. No *E. coli* O157:H7 or *Salmonella* were detected, even though fecal coliforms and generic *E. coli* were detectable in all system water samples.

Fish Tank	Sample	*E. coli* CFU/100mL*	Fecal Coliform MF/100mL*	*E. coli* O157:H71 25 g*	*Salmonella* 25 g or 25 mL
Source	Water	< 1	< 1	-	Negative
Aquaculture 1	Water	24	29	-	Negative
Aquaculture 2	Water	4	36	-	Negative
Aquaponics 1	Water	>200	340	-	Negative
Aquaponics 2	Water	62	26	-	Negative
Aquaculture 1	Fish Muscle	-	-	Negative	Negative
Aquaculture 1	Fish Muscle	-	-	Negative	Negative
Aquaculture 1	Fish Muscle	-	-	Negative	Negative
Aquaculture 2	Fish Muscle	-	-	Negative	Negative
Aquaculture 2	Fish Muscle	-	-	Negative	Negative
Aquaculture 2	Fish Muscle	-	-	Negative	Negative
Aquaponics 1	Fish Muscle	-	-	Negative	Negative
Aquaponics 1	Fish Muscle	-	-	Negative	Negative
Aquaponics 1	Fish Muscle	-	-	Negative	Negative
Aquaponics 2	Fish Muscle	-	-	Negative	Negative
Aquaponics 2	Fish Muscle	-	-	Negative	Negative
Aquaponics 2	Fish Muscle	-	-	Negative	Negative

* - Indicates that test was not conducted

residential sources of biological, chemical, or physical adulterants. Current GAP audits do not take notice that many leafy greens are now being grown in direct contact with fish feces in the irrigation water, or they do not see them as a risk. Based on these observations, criteria for a science-based variance to the "auto-failure" for raw manure should be established for aquaponic systems.

References

Al-Harbi, A.H. 2003. Fecal Coliforms in Pond Water, Sediments, and Hybrid Tilapia *Oreochromis niloticus* x *Oreochromis aureus* in Saudi Arabia. *Aquaculture Research* 34: 517–524.

Al-Harbi, A.H., and M.N. Uddin. 2005. Microbiological Quality Changes in the Intestine of Hybrid Tilapia (*Oreochromis niloticus* x *Oreochromis aureus*) in Fresh and Frozen Storage Condition. *Letters in Applied Microbiology* 40: 486–490.

Byappanahalli, M.N., R. Sawdey, S. Ishii, D.A. Shively, J.A. Ferguson, R.L. Whitman, and M.J. Sadowsky. 2009. Seasonal Stability of *Cladophora*-associated *Salmonella* in Lake Michigan Watersheds. *Water Research* 43: 806–814.

Cahill, M.M. 1990. Bacterial Flora of Fishes: A Review. *Microbial Ecology* 19: 21–41.

Chalmers, G.A. 2004. Aquaponics and Food Safety. Retrieved February 10, 2011, from http://www.backyardaquaponics.com/Travis/Aquaponics-and-Food-Safety.pdf.

Commodity Specific Food Safety Guidelines for the Production and Harvest of Leafy Greens. 2009. Retrieved February 25, 2012, from http://www.wga.com/DocumentLibrary/scienceandtech/California%20GAPs%20-%20metrics%20071009.pdf

Crohn, D.M., and M.L. Bianchi. 2008. Research Priorities for Coordinating Management of Food Safety and Water Quality. *Journal of Environmental Quality* 37: 1411–1418.

Dufour, A.P. 1984. Health effects criteria for fresh recreational waters. EPA-600/1-84-004. Office of Research and Development, USEPA, Washington, DC.

El-Shafai, S.A, H.J. Gijzen, F.A. Nasr, and F.A. El-Gohary. 2004. Microbial Quality of Tilapia Reared in Fecal-Contaminated Ponds. *Environmental Research* 95: 231–238.

EPA. 1986. Ambient Water Quality Criteria for Bacteria-1986. EPA/440/5-84-002. Office of Water Regulations and Standards. USEPA, Washington, DC.

Erickson, M.C. 2012. Internalization of fresh produce by foodborne pathogens. *Annual Review of Food Science and Technology* 3, 283–310.

Fonseca, J.M., S. Fallon, C.A. Sanchez, K. Nolte. 2011. *Escherichia coli* survival in lettuce fields following its introduction through different irrigation systems. *Journal of Applied Microbiology*. 110: 893–902.

Food Safety Modernization Act. 2011. United States Food and Drug Adminstration. Retrieved February 16, 2012, from http://www.gpo.gov/fdsys/pkg/PLAW-111publ353/pdf/PLAW-111publ353.pdf.

González-Alanis, P., J.I. Gutierrez-Olguín, I. Castro-Segura, H. Ezqueda-Palacios, M. Hernández Acosta, H.H. Gojon-Báez, G. Aguirre-Guzmán, F.M. Guzmán-Saénz, and K.M. Fitzsimmons. 2011. Food Safety Study of Leafy Green Irrigated with Tilapia Farm Effluents in Tamaulipas. Better Science, Better Fish, Better Life: Proceedings of the Ninth International Symposium on Tilapia in Aquaculture. Pages 121–122.

Hollyer, J., C. Tamaru, A. Riggs, R. Klinger-Bowen, R. Howerton, D. Okimoto, L. Castro, T. Ron, B.K., Fox, V. Troegner, and G. Martinez. 2009. On-Farm Food Safety: Aquaponics. College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa Food Safety and Technology FST-38: 1–7.

Hussong, D., J.M. Damare, R.J. Limpert, W.J. Slagen, R.M. Weiner, and R.R. Colwell. 1979. Microbial Impact of Canada Geese (*Branta Canadensis*) and Whistling Swans (*Cygnus columbianus columbanius*) on aquatic ecosystems. *Applied Environmental Microbiology* 37(1): 14–20.

LGMA. 2007. California Leafy Green Products Marketing Agreement. Retrieved February 10, 2012, from http://www.caleafygreens.ca.gov/.

Pachepsky, Y., D.R. Shelton, J.E.T. McLain, J. Patel, and R.E. Mandrell. 2011(a). Irrigation Waters as a Source of Pathogenic Microorganisms in Produce: A Review. *In*: Advances in Agronomy, Volume 113 pp. 73–138, Academic Press (San Diego, CA).

Pachepsky, Y.A. and D.R. Shelton. 2011(b). *Escherichia coli* and Fecal Coliforms in Freshwater and Estuarine Sediments. *Critical Reviews in Environmental Science and Technology* 41: 1067–1110.

Rakocy, J.E. 2003. Question and Answer. *Aquaponics Journal* 7: 29.

Rakocy, J.E., M.P. Masser and T.M. Losordo. 2006. Recirculating Aquaculture Tank Production Systems: Aquaponics—Integrating Fish and Plant Culture. *SRAC Publication* No. 454. 16 pp.

Rio-Rodriguez, R.E., V. Inglis, and S.D. Millar. 1997. Survival of *Escherichia coli* in the Intestine of Fish. *Aquaculture Research* 28: 257–264.

Scharff, R. 2009. Health-Related Costs from Foodborne Illness in the United States. Retrieved September 21, 2011, from http://www.producesafetyproject.org/media?id=0009.

Scharff, R. 2012. Economic Burden from Health Losses Due to Foodborne Illness in the United States. *Journal of Food Protection* 75(1): 123–131.

Sugita, H., K. Shibuya, H. Shimooka, and Y. Deguchi. 1996. Antibacterial Abilities of Intestinal Bacteria in Freshwater Cultured Fish. *Aquaculture* 145: 195–203.

Suslow, T. V. 2009. Safety Project Issue Brief: Standards for Irrigation and Foliar Contact Water. Produce Safety Project. Retrieved February 15, 2012, from http://www.producesafetyproject.org/admin/assets/files/Water-Suslow-1.pdf

Tamaru, C.S, B.K. Fox, J. Hollyer, L. Castro, and T. Low. Testing for Water Borne Pathogens at an Aquaponic Farm. 2012. College of Tropical Agriculture and Human Resources.

WHO. 1989. Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture. Technical Report Series, No. 778, World Health Organization, Geneva.