Microbial Communities and Food Safety in Recirculating Aquaponics

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US foodborne illnesses

- Fish is implicated in about 250,000 cases of foodborne illness in the US each year, while produce is linked to over 4 million illnesses.
- Of produce-linked illnesses:
  - Leafy vegetables → nearly 50%
  - Fruits and nuts → 25%

Estimated annual attributions of domestically acquired foodborne illnesses to fish and produce	
Commodity or commodity group	***# of illnesses (% of total illnesses)***
Fish	258,314 (2.7)
Produce†	4,423,310 (45.9)
Fruits & nuts	1,123,808 (11.7)
Vegetables†	3,299,501 (34.2)
Leafy	2,152,652 (22.3)
Vine-stalk	759,889 (7.9)
Root	349,715 (3.6)
Sprout	32,703 (0.3)
Fungi	4,542 (0.0)
† Indicates a commodity group.	
Source: Painter et al., 2013	

Recent outbreaks

Timing	Pathogen	People affected (deaths) [#]	Attributed commodity	Likely source
Fall 2018	*E. Coli* O157:H7	62 (0)	Romaine	Surface irrigation water
Spring 2019	*Salmonella* Carrau	250 (0)	Cut melon	???
Summer 2019	*Listeria monocytogenes*	24 (2)	???	???

Food Safety from Farm to Fork

- **Education and training** of workers and consumers is key!
- Contamination can occur at any link in the chain.
- More complex distribution systems create more chances for contamination.

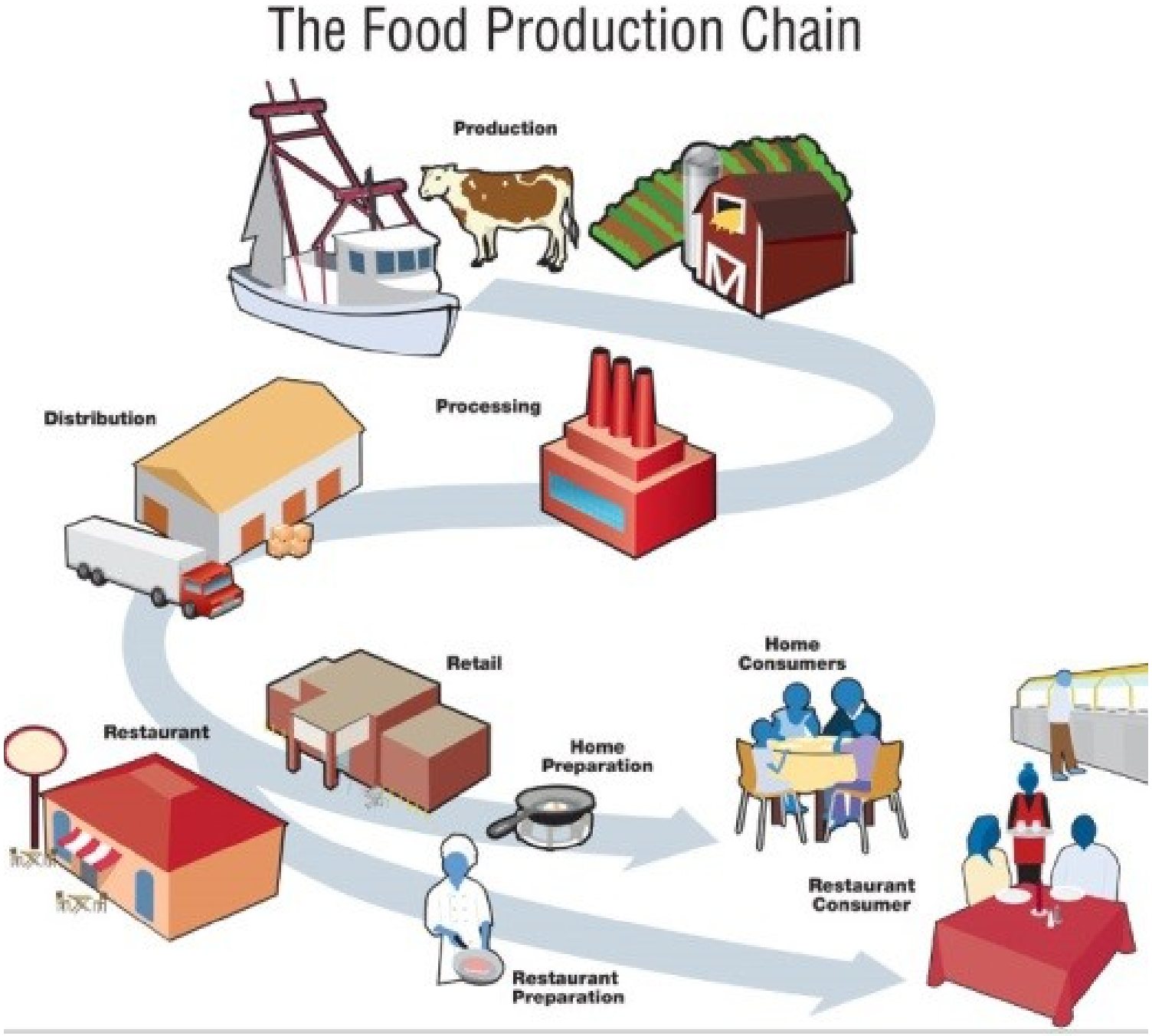


Image: https://blogs.cdc.gov/global/2013/06/10/when-food-bite-back-globglobal-networks-make-food-safer/

Shorter supply chains = safer food

Aquaponics will play a role in this, but…

Image: https://fairfoodnetwork.org/from-the-field/if-you-build-it-growing-farmer-grocer-pipeline/

Food safety concerns in aquaponics

- Shared culture water between fish and plants drastically restricts the use of sanitizing agents in the system.
- Organic carbon from fish waste in the culture water might serve as a resource for pathogen proliferation, as it does in other animal wastes like manure.
- Human pathogen risks associated with fish culture water coming into contact with produce have not been assessed.
- FSMA classification of culture water – “surface water,” “agricultural tea,” “biological soil amendment of animal origin,” or something else?
- Final FSMA water quality rules expected in 2022 (or 2024, or 2026…?)

Aquaponics Association GAPs

1. General hygiene to minimize introductions of foodborne pathogens into the system: handwashing, excluding vermin, etc.
2. Creating environments that discourage foodborne pathogens from persisting in the system if and when they are introduced.
3. Preventing edible parts of the plant from coming into contact with system water,

   OR

   Sanitizing water via heating, ultraviolet light, and/or ozone.

Aquaponic crop choice and assumed risk

Risk level	Unsanitized culture water contact?	Typically eaten raw?	Example
High	Intentional	Yes	Radish
	Incidental	Yes	Lettuce (poor design)
	None	Yes	Lettuce (better design)
	Intentional	No	Carrots for cooking/canning
	Incidental	No	Collard greens (poor design)
Low	None	No	Tomatoes for cooking/canning

Working Hypothesis

Culture water in a **well-managed** recirculating aquaponic system will not sustain human pathogen populations.

- **Well-managed** (by AA standards) =
  - Avoiding warm, wet, low-oxygen environments with high organic carbon through system design and maintenance
  - Good aeration and solids removal to reduce pathogen survival in fish
- Worker and farm hygiene are still essential!

Major foodborne pathogens

Estimated annual number of hospitalization and deaths due to domestically acquired foodborne illnesses caused by major foodborne pathogens (those with >2,000 hospitalizations or >50 deaths per year)			
Pathogen	*Pathogen type*	*Number of hospitalizations*	*Number of deaths*
***Salmonella* spp., nontyphoidal**	Bacteria	19,336	378
Toxoplasma gondii	Parasite	4,428	327
Listeria monocytogenes	Bacteria	1,455	255
Norovirus	Virus	14,663	149
***Campylobacter* spp.**	Bacteria	8,463	76
Shiga toxin-producing *E. coli* (STEC) O-157	Bacteria	2,138	20
			Source: Scallan et al., 2011

Fox et al., 2012 (U of Hawai'i)

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.

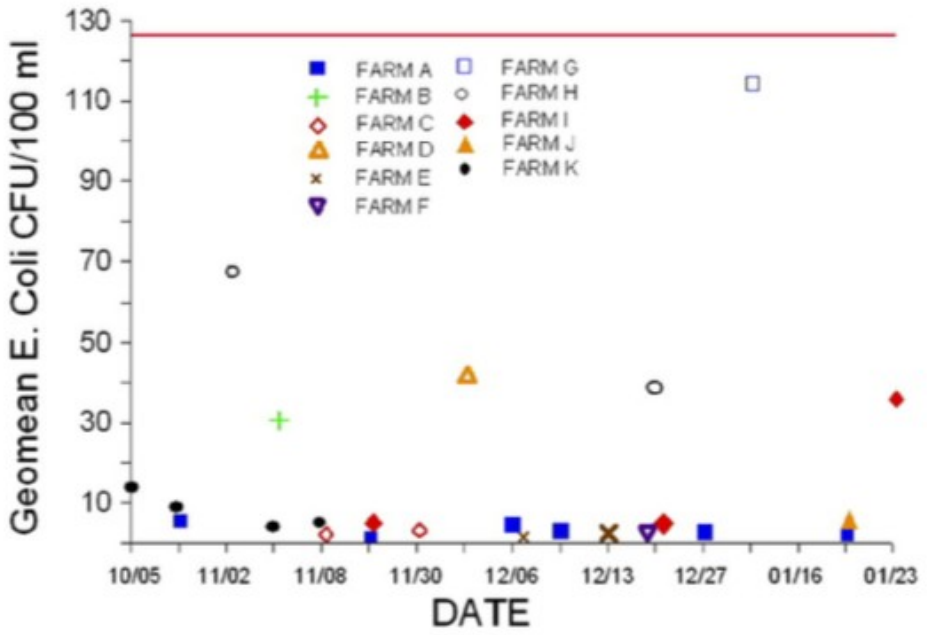


Table 2. A summary of 48 tissue samples of aquaponic produce originating from 11 different farms around the state of Hawai'i. All tissue samples analyzed were shown to have low levels of generic *E. coli*, or undetectable pathogenic *E. coli* O157:H7 and *Salmonella*.

Date	Sample	Farm	*E. coli* MPN/25 g*	*E. coli* O157:H7	*Salmo-nella*
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.

Microbial Community Analysis

Schmautz et al., 2017

- Single experimental coupled aquaponic system (10.8 m^3)
- 16S – bacterial communities
- Four solid biological components:
  - Fish feces
  - Plant roots
  - Biofilter biofilm
  - Periphyton

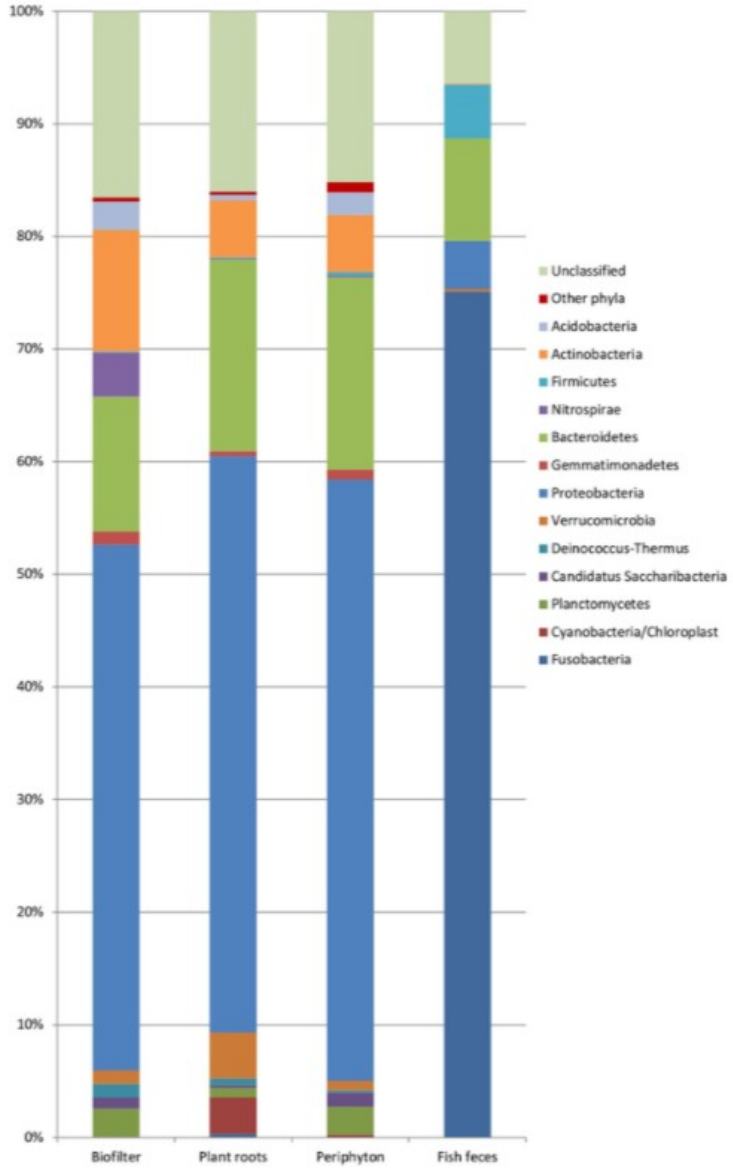
Eck et al., 2019

- Eight commercial coupled aquaponic, decoupled aquaponic, and RAS
- 16S – bacterial communities
- One solid and one liquid component:
  - Biofilter biofilm
  - Sump water

Both studies were a <u>single snapshot in time,</u>
<u>looking at bacterial communities.</u>

Schmautz et al., 2017

Fig. 3 Classification of reads from biofilter, plant roots, periphyton, or fish feces to level phylum indicated as percentage of population. Phyla that were represented in all samples below 0.5% of total community (Table S1) are displayed under "other phyla", which contain the phyla Armatimonadetes, Parcubacteria, Chloroflexi, Spirochaetes, Hydrogenedentes, Chlamidiae, candidate division WPS-2, and SR1



Eck et al., 2019

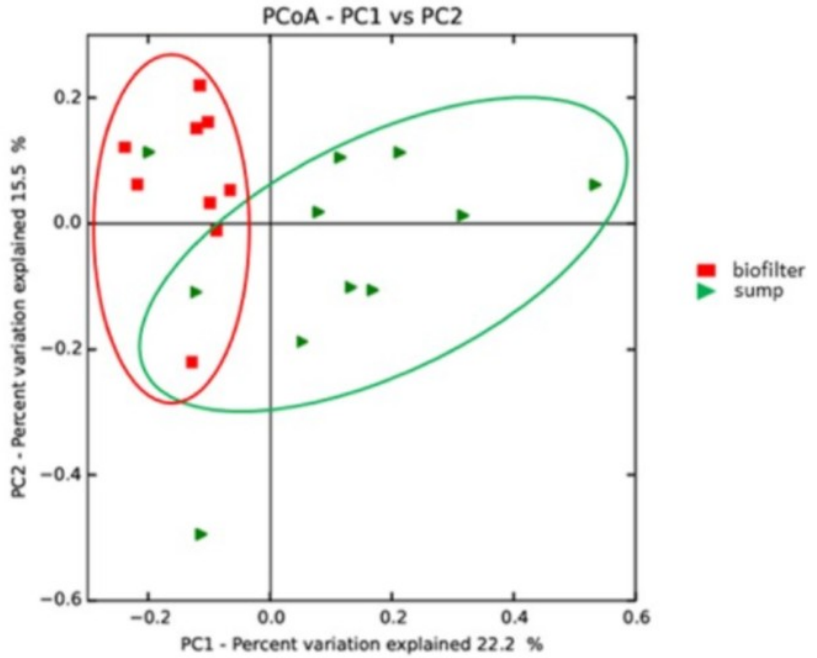


Figure 4. Weighted UniFrac principal coordinates analysis presenting the separation between the sumps and the biofilter samples. Axis 1 and 2 explain 37.8% of the total variability.

Research questions

In a “well-managed” recirculating aquaponic system:

1. Are there human pathogens or fecal indicator organisms in the culture water? (i.e. *Salmonella*, *Listeria*, *E. coli*)
2. How do microbial community composition and pathogen incidence differ among aquaponic unit processes?
3. Is the composition of microbial communities in each system component stable over time?

UNH aquaponic greenhouses

- Nile *Tilapia* + butterhead lettuce
- 3 replicated systems
  - 10 m^3 volume
- In operation since Sept 2018
- 650 heads/week

Photo: Guerdat

UNH aquaponic greenhouses

Photo: Fogarty

Photo: Fogarty

UNH aquaponic greenhouses

Photo: Fogarty

Photo: Fogarty

UNH recirculating aquaponics process flow

- Effluent from fish and plants (red) flows to mechanical drum filter before being redistributed
- Solid waste (>54µm) flows to external storage tank (not shown)
- Side loop from sump supplies biofilter

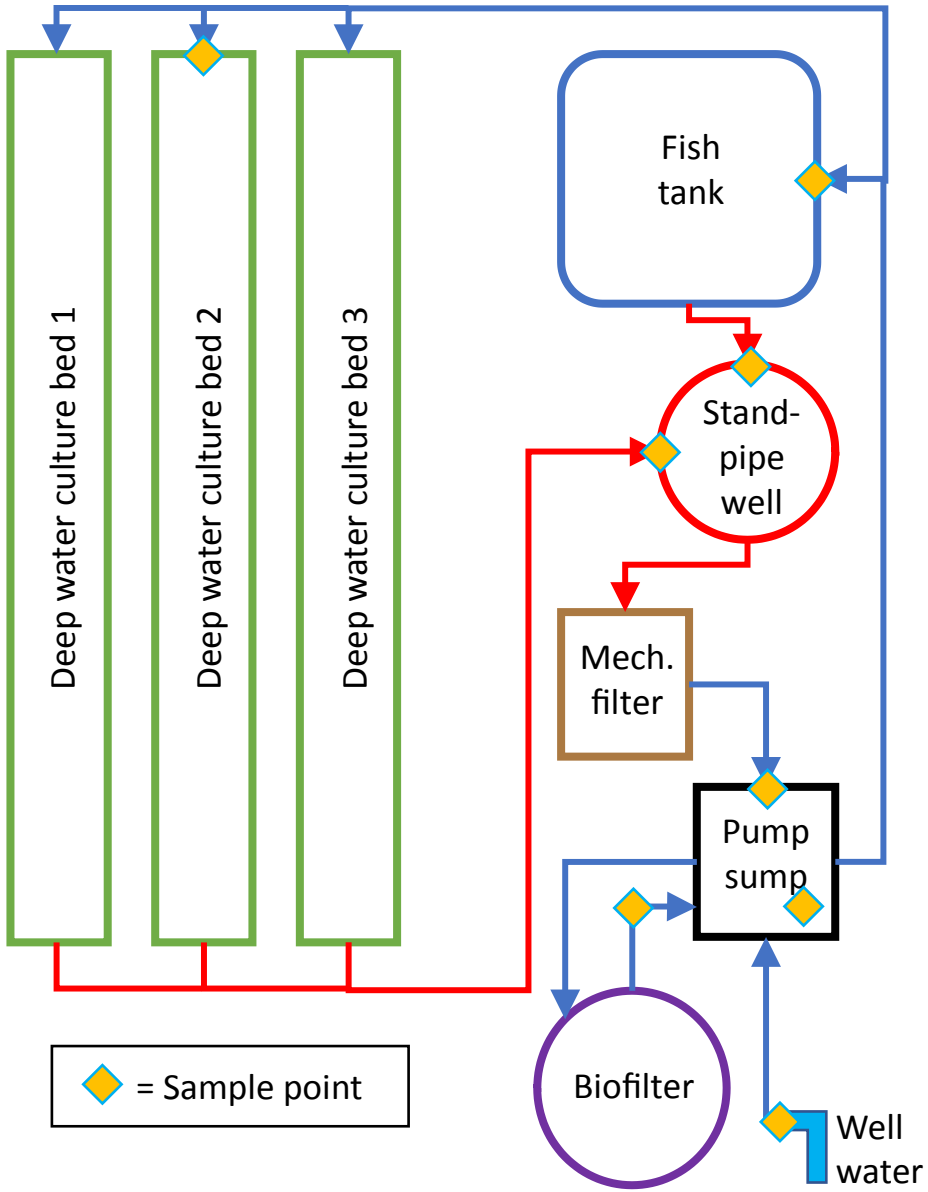
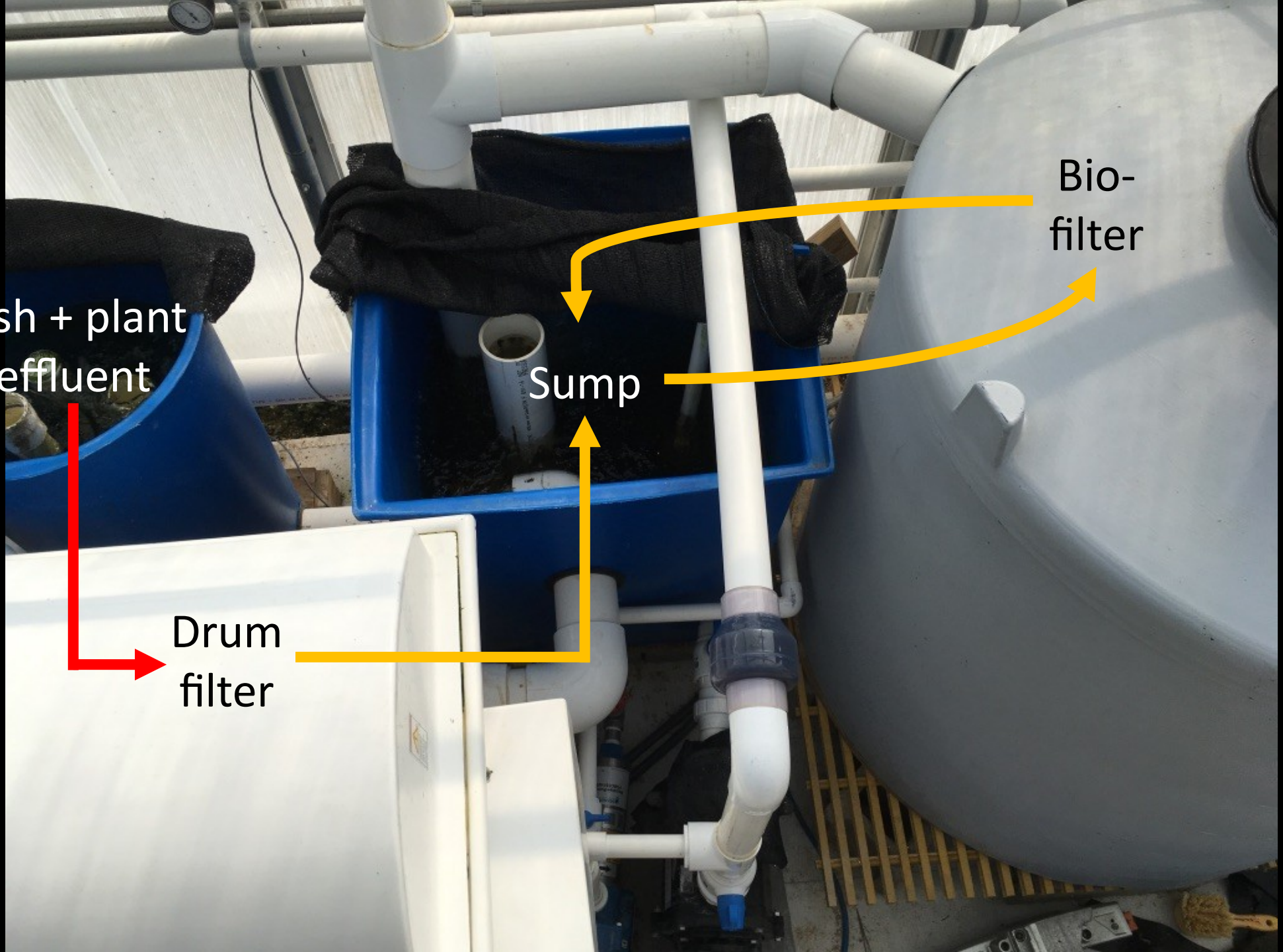
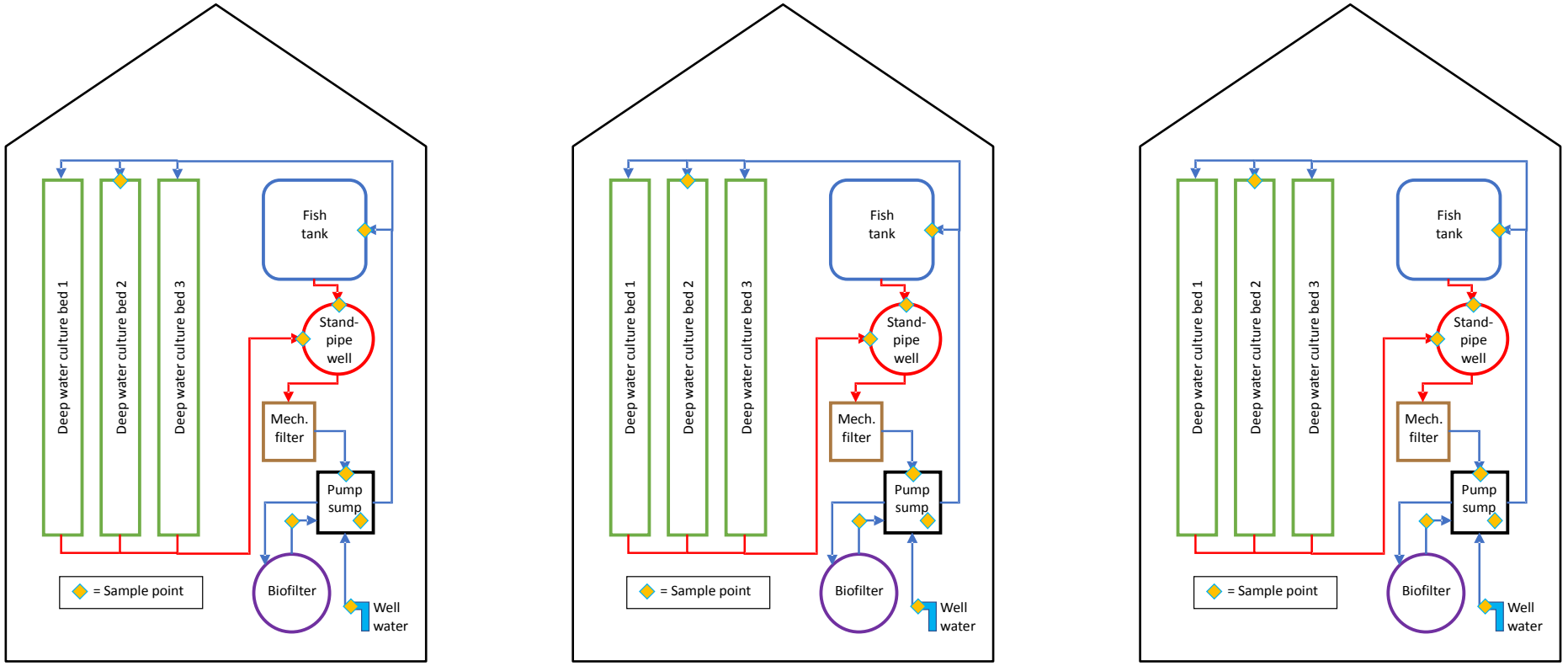


Figure: Fogarty


Bio-
filter
Fish + plant
effluent
Sump
Drum
filter

Sample collection – Fall 2019



Weekly samples will be used to investigate microbial communities and pathogen presence simultaneously.

Methods – Microbial communities

1. Whole-genome sequencing (WGS) - metagenome
2. Sequence data QC and analysis with PALADIN.
   - What genes are there?
   - What can that tell us about which organisms are present?
   - Can we infer their functions by translating DNA into proteins?
3. Dissolved carbon/nitrogen analysis
   - Is C/N driving community composition?

Methods – Pathogen presence

1. Use traditional microbiological assays to screen for:
    1. *E. coli*
    2. *Salmonella*
    3. *Listeria*
2. Filter metagenomic sequence data to search for genes indicating presence of pathogenic organisms.
3. Dissolved carbon/nitrogen analysis
    - Is C/N driving pathogen presence?

Photo: Fogarty

Results to date

- White plates = ☺
- No confirmed *E. coli* in Winter 2019 and Fall 2019 screenings

In the pipeline

- *Salmonella* and *Listeria* assays begin next week.

- Metagenome DNA sequence data will be generated November 2019.

Next summer:

- Plant roots as biofilter – nitrification in the rhizosphere

Produce Safety Resources

- Produce Safety Alliance - https://producesafetyalliance.cornell.edu
  - General resources (e.g. water testing, recordkeeping reqs.) - https://producesafetyalliance.cornell.edu/resources/general-resource-listing/
  - University contacts - https://producesafetyalliance.cornell.edu/contact-us/university-contacts/
  - Farm Food Safety Plan - https://producesafetyalliance.cornell.edu/resources/farm-food-safety-plan-writing-resources/
  - Upcoming trainings - https://producesafetyalliance.cornell.edu/training/grower-training-courses/upcoming-grower-trainings/
  - Electronic Grower Manual - https://producesafetyalliance.cornell.edu/curriculum/english-grower-manual-electronic-public-release/

Produce Safety Resources (cont'd)

- Cornell Legal Information Institute - https://www.law.cornell.edu/cfr/text/21/part-112
- Aquaponics Association GAPs - https://docs.google.com/document/d/1TykLMAyJRVysiOArokGYIAkFGVvDuosqfGF7-fYS_KA/edit
- FDA
  - FSMA Technical Assistance Network - https://www.fda.gov/food/food-safety-modernization-act-fsma/fsma-technical-assistance-network-tan
  - Outbreak information - https://www.fda.gov/food/recalls-outbreaks-emergencies/outbreaks-foodborne-illness

Questions?

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