CONSERVATION INNOVATION GRANTS

Final Progress Report

March 2011

Grantee Name: Washington State University	
Project Title: Pathogen Reduction in a Community Based Anaerobic Digester	
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Project End Date: 12 31 2010	

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Livestock Nutrient Management

Project Team

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Groeneveld Dairy	Cooperator dairy	Monroe
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Table of Contents

A. Executive Summary

In the original proposal developed in 2006, this project's focus was to demonstrate the reduction of pathogen and nutrient transport to surface water and a reduced risk of farm-to-farm transmission of pathogens between farms participating in a community-based anaerobic digester (AD) with post-AD pasteurization.

The project was divided into 7 specific components:

1) surface water quality monitoring before and after the community AD began operation,

2) on-farm monitoring of pathogens before and after the community AD began operation,

3) monitoring of pathogens in manure streams before and after the community AD began operation,

4) Johnes surveillance of participating farms,

5) effect of pasteurization post AD on bacterial fate,

6) comparing AD origin vs. non-AD origin bacterial die-off on soil after lagoon manure application to grass fields,

7) microbial source tracking to determine the source (origin) of bacteria identified in surface water.

In 2008 we requested an extension to the project as the construction of the community AD had been delayed. At that time (2008) we had made good progress with components 1, 2, 4, and 6.

In 2009, after the community AD began operation it became apparent that several dairy farm cooperators were not going to contribute manure to the community AD due to the poor economic climate for the dairy industry and due to perceived financial risks from participation.

In 2009, the focus of the project shifted from that of a community anaerobic digester to that of a anaerobic digester that was operated on 70 % dairy manure and 30% pre-consumer food waste, or a co-digestion project. Due to the lack of participation by the additional dairies and due to the change in focus, in 2010 a no cost extension (to December 2010) was requested to focus on the microbial source tracking effort to determine if bacteria identified after AD were different than bacteria entering the AD, and to evaluate bacteria in surface water run-off after major rain events following land application of AD and non-AD lagoon manure to grass fields.

In the fall of 2010, an additional component was added:

8) compare fate of AD origin vs. non-AD origin bacteria when stored in manure lagoons.

This component was added in the fall of 2010 after data from this project was presented at the International ASABE Air and Manure Symposium in Dallas, TX, in September, 2010. A question was asked by NRCS staff at the conference about "what was known about the fate of bacteria from AD manure that had been subsequently stored in a lagoon, would re-growth of bacteria counts occur?"

The format for the executive summary is presented as a series of questions with short answers and supportive data in graphic or tabular form. When appropriate an Implications statement is made.

Q 1) Will AD Treatment of Manure Lessen the Risk of Negatively Effecting Surface Water Quality?

Answer – Yes, when surface water run-off was monitored from grass fields that had applications of AD or non-AD lagoon-origin manure, fewer surface water samples were positive for total coliforms from AD amended grass plots than from non-AD amended (see table 1).

Table 1.Summary of presence of bacteria in surface run-off water from manure amended grass plots.

Treatment	**Number Positive Samples (%)**				**Number Samples**
AD Manure		Total Coliforms	Fecal Coliforms	*E. coli*	
	Summer 2010	-	4 (31%)	2 (15%)	13
	Fall10-Spring11	27 (47%)	4 (7%)	25 (43%)	58
	March 2011	7 (41%)	3 (18%)	16 (94%)	17
	Combined	**34 (45%)**	**11 (12%)**	**43 (49%)**	**88**
NonAD Manure					
	Summer 2010	-	5 (50%)	3 (30%)	10
	Fall10-Spring11	35 (74%)	2 (4%)	17 (36%)	47
	March 2011	13 (87%)	0	12 (80%)	15
	Combined	**48 (77%)**	**7 (10%)**	**32 (44%)**	**72**
Control					
	Fall10-Spring11	19 (73%)	0	18 (69%)	26

Q 2) Will AD Treatment of Manure Result in Less Bacteria (pathogens) for Transport Back to Cooperating Dairy Farms in a Community AD?

Answer – Yes, AD treatment of manure and pre-consumer food wastes reduced bacteria (pathogens and indicator bacteria) in the liquid, solid, and composted solid fractions of post-AD manure. (see figures 1, 2, 3, and 4, and table 2).

AD treatment of manure and pre-consumer food wastes resulted in a greater than 90 % kill of generic *E. coli* and fecal Enterococcus bacteria.

Answer – Yes and No. Almost no samples from the post-AD composted solids tested positive for Campylobacter, Listeria, *Mycobacterim paratuberculosis*, and Salmonella. AD treatment of manure and pre-consumer food wastes resulted in little reduction in the proportions of post-AD samples testing positive for Campylobacter, Listeria, *Mycobacterim paratuberculosis*, and Salmonella compared to pre-AD samples (see table 2).

Implications for dairies considering becoming part of a community AD – When liquid streams of manure are returned to a cooperating farm, make sure that on-farm bio-security practices are in place to avoid exposure of sensitive classes of dairy animals to Listeria, *Mycobacterim paratuberculosis*, and Salmonella.

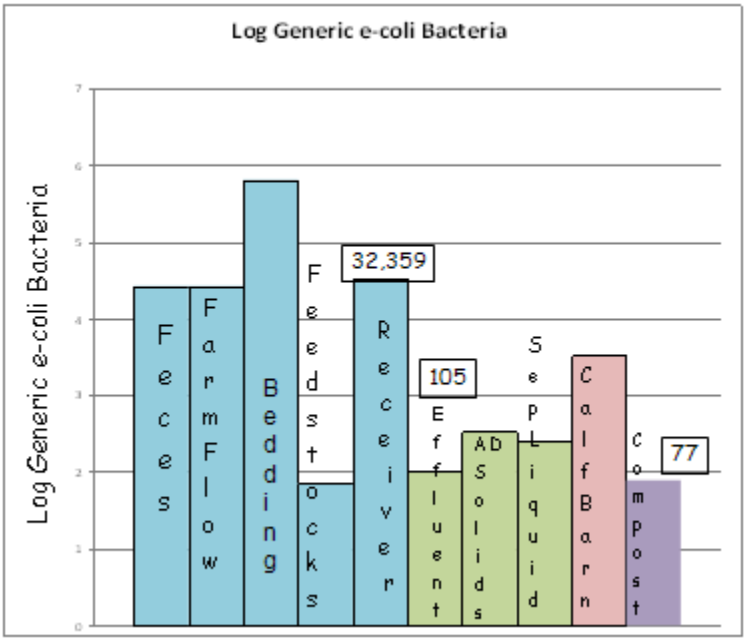


Figure 1. Log_{10} of generic *E.coli* / gram in inputs (blue) and outputs of the digester (green, pink, and purple).

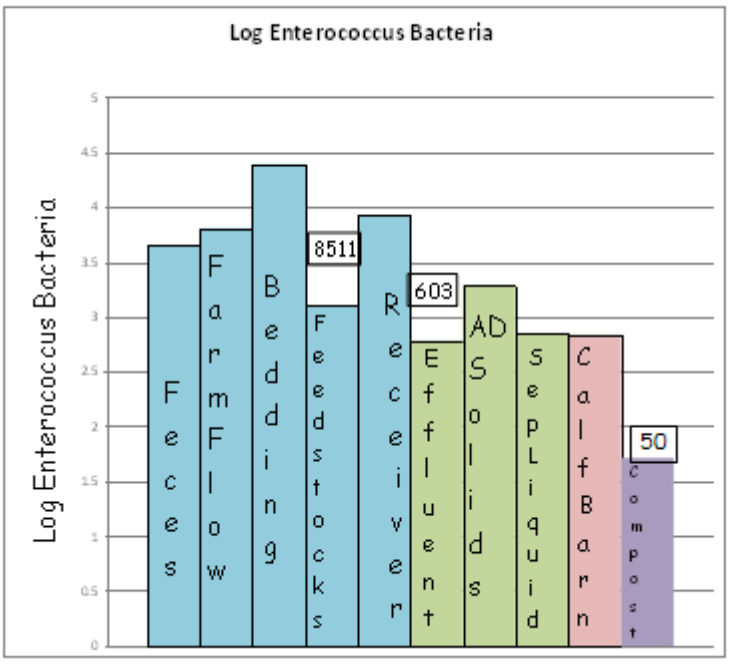


Figure 2. Log_{10} of fecal Enterococcus bacteria / gram in inputs (blue) and outputs of the digester (green, pink, and purple).

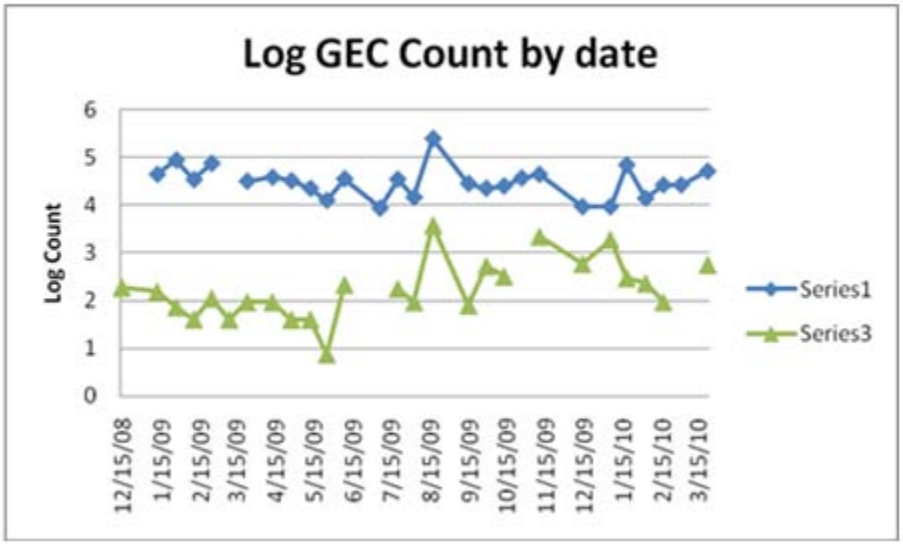


Figure 3. Log_{10} of generic *E. coli* / gram of inflow (blue) and outflow of the digester (green).

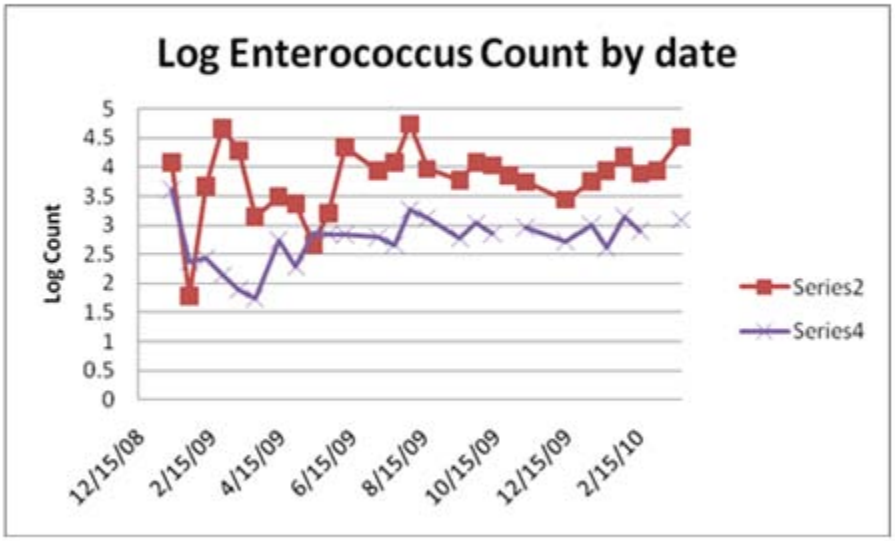


Figure 4. Log_{10} of Enterococcus bacteria / gram of inflow (red) and outflow of the digester (blue).

Presence-absence of bacteria in pre- and post-AD materials

Sampling Location	*Campylobacter*	Listeria	*Mycobacterium paratuberculosis*	Salmonella
On-farm Feces	56% (14/25)	12% (3/25)	84% (21/25)	44% (11/25)
Farm Flow	35% (9/26)	4% (1/26)	78% (21/27)	77% (20/26)
Bedding	0% (0/22)	0% (0/22)	9.5% (2/21)	27% (6/22)
Feedstocks	0% (0/4)	0% (0/4)	33% (0/6)	17% (1/6)*
Receiving Tank	28% (7/25)	0% (0/25)	63% (17/27)	89% (24/27)
Effluent after anaerobic digestion	28% (8/29)	7% (2/29)	71% (22/31)	90% (28/31)
AD Solids	0% (0/23)	9 % (2/23)	32% (8/25)	84% (21/25)
SepLiquid	7 % (3/43)	5 % (2/43)	54 % (24/44)	79% (35/44)
Compost	0% (0/20)	0% (0/20)	0% (0/19)	0% (0/20)
Calf Barn	50% (4/8)	0% (0/8)	33% (2/6)	50% (4/8)

Table 2. Presence-absence of bacteria in pre- and post-AD materials.

Q 3) Will Bacteria of Manure Origin re-grow on Soil in Grass Fields after AD Manure has been Applied?

Answer – No, the limited bacteria present in AD manure do not re-grow after land application. In contrast, non-AD manure amended soils were observed to have a rapid re-growth of bacteria before a period of die-off (see figure 5 and 6).

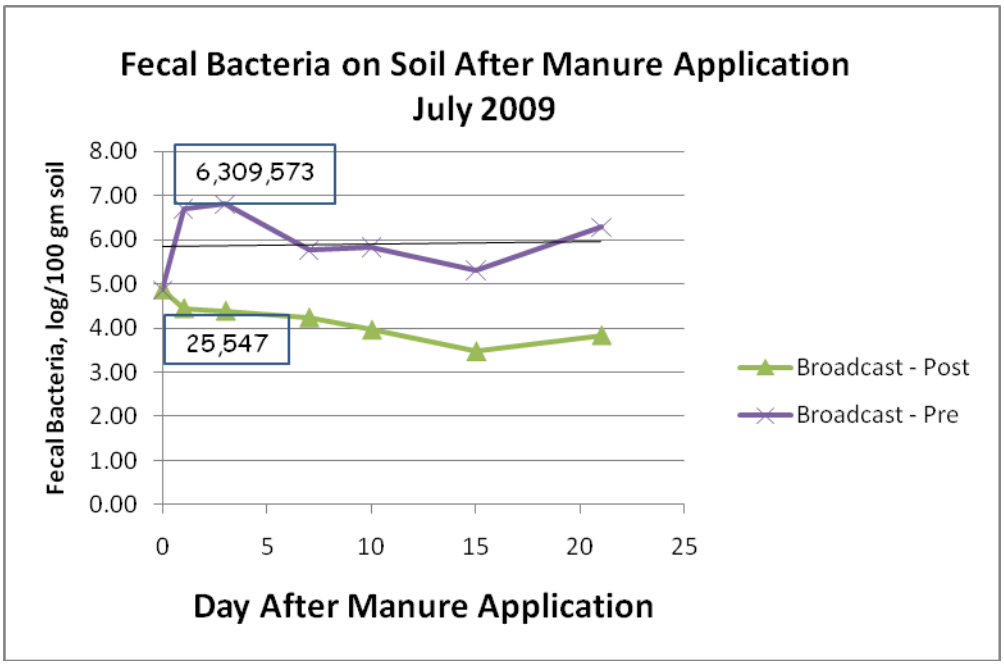


Figure 5. Fecal bacteria on soil after application of Pre (non-AD) and Post (AD) manure.

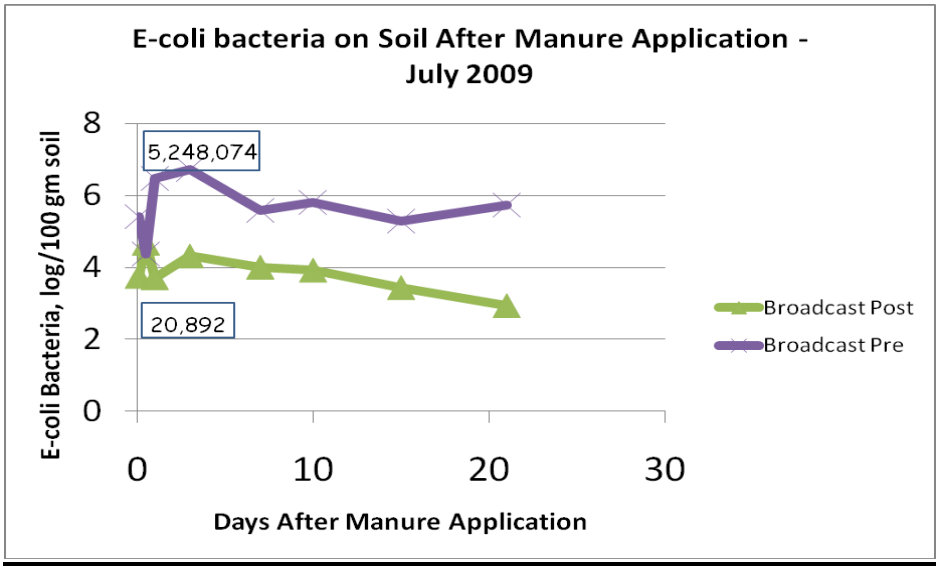


Figure 6. *E-coli* bacteria on soil after application of Pre (non-AD) and Post (AD) manure.

Q 4) – Are the bacteria exiting the AD different than those entering? (or are we creating super bugs during AD?)

Answer – No, the bacteria exiting the AD do not seem to be substantially different than those entering the AD when evaluated by four different methods.

When generic *E. coli* were evaluated in pre- and post AD manure, they did not ferment sugars differently.

When generic *E. coli* and fecal Enterococcus were evaluated for antibiotic resistance in pre- and post AD manure, there was no difference in antibiotic resistance.

When serotypes of Salmonella were evaluated in pre- and post AD manure, there was no difference in proportion of serotypes.

When genetic evaluation (REP PCR) was conducted on generic *E. coli* from pre- and post-AD manure, no indication that the AD selected for any specific generic *E. coli*.

Q 5) – Do bacteria re-grow when AD manure is stored in lagoons for months?

Answer – No, when AD manure and non-AD manure lagoons were monitored over a five month period of time, the AD manure had less bacteria (generic *E. coli* and fecal Enterococcus) (see figure 7)..

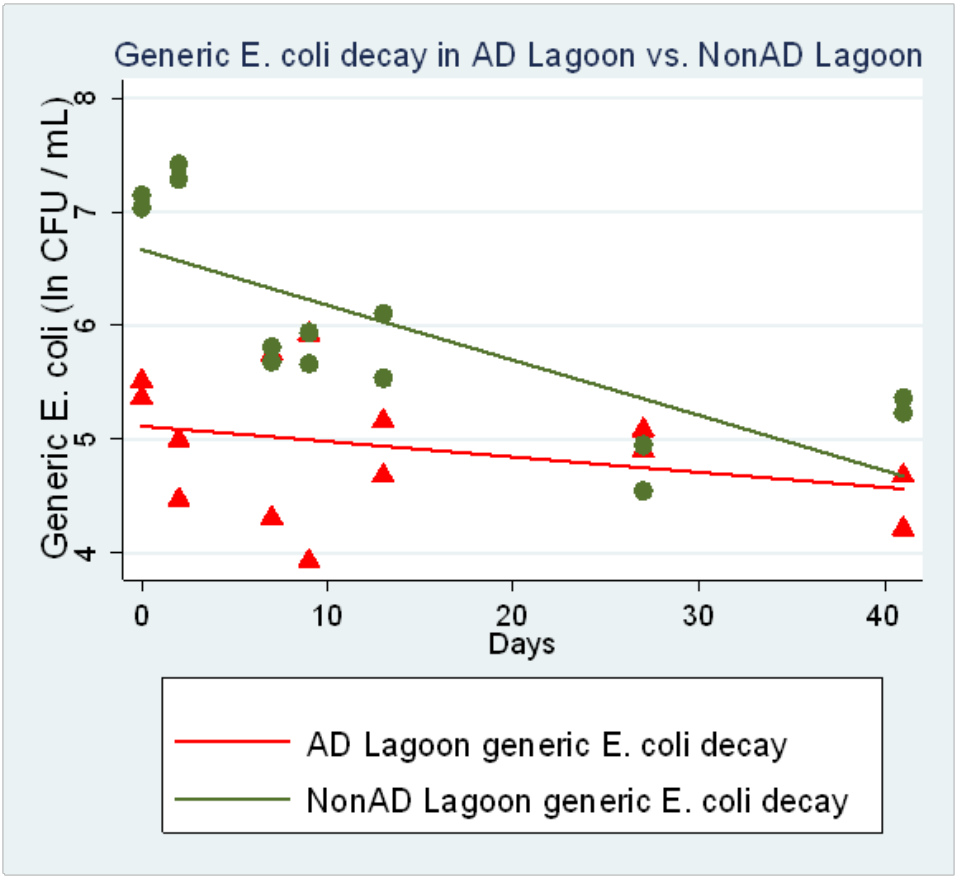


Figure 7. Generic *E.coli* decay in bucket storage of AD and non-AD manure.

B. General Methodology and Approach

2006 – In the original proposal, the focus of this project was to demonstrate a reduction in pathogen and nutrient transport to surface water and a reduced risk of farm to farm transmission of pathogens between farms that participate in a community based anaerobic digester with post AD pasteurization (see figure 8). A unique aspect of the original design to the project was that we would be able to obtain information on water quality and pathogen levels at individual farms before and after the adoption of the community AD. Water quality monitoring had been underway on portions of the watershed and historical baseline data was available. This project was conducted in a watershed that has seven dairy and beef producers that have a combined animal inventory of ~ 3000 animals. A community anaerobic digester (AD) that is composed of a plug-flow design with post AD pasteurization, liquid-solid separation, and solids composting would be the central technology to achieve pathogen reduction. Liquid and solids manure would be returned to participating dairies for use as a nutrient source for crop growth. We proposed to use water quality monitoring on and adjacent to study farms after land application of manure to document changes in water quality due to AD treatment of manure. Temporal (weekly) sampling of manure entering the community AD, and at each point of handling post AD (liquid, solid, and compost) would be used to demonstrate the reduction in pathogens or pathogen surrogates of salmonella, enterococci, generic e-coli, mycobacterium avium paratuberculosis (Map or Johnes), listeria, campylobacter, and enterovirus.

The project was divided into 7 specific components:
1) surface water quality monitoring,
2) on-farm monitoring of pathogens,
3) pre- post AD monitoring of pathogens
4) Johnes surveillance,
5) pasteurization
6) bacteria die-off on soil after manure application
7) microbial source tracking.

During the project period, an additional component was added:

8) fate of bacteria upon lagoon storage

List of deliverables – Two major outcomes were expected from this project utilizing a community based AD with pasteurization: 1) a reduction in transport of pathogens and nutrients to surface water from land applied manure that has been AD treated, and 2) demonstration of the reduction in pathogens that are of significance in farm to farm transmission, therefore reducing the risk (producer concern) of between farm movement of pathogens. These two goals were focused on achieving improved water quality and increasing the adoption of AD systems that are community based.

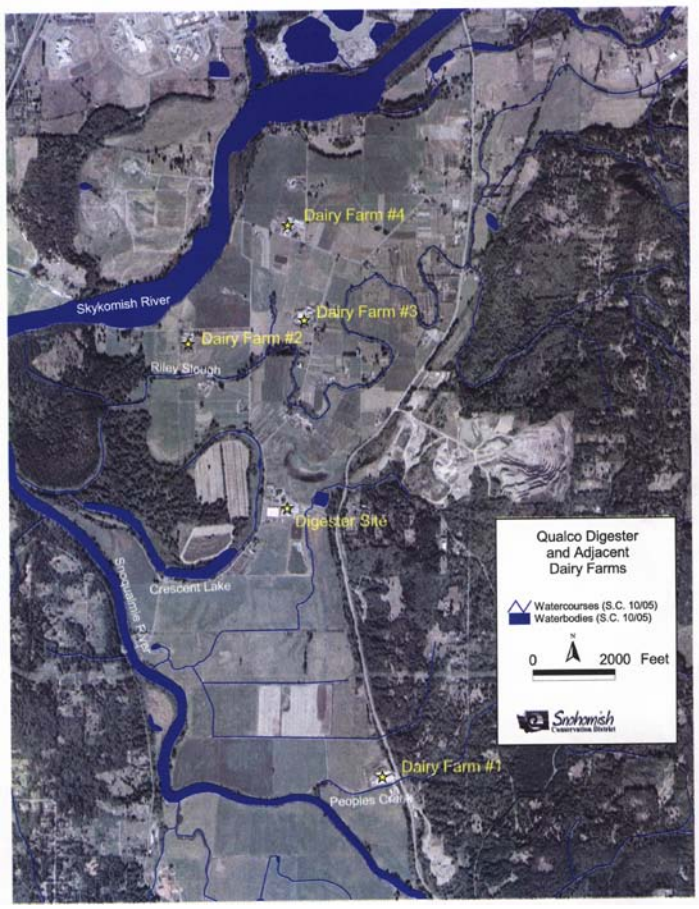


Figure 8 – Aerial map of digester study area.

Cooperator Farms

Cooperator farms were contacted for information relevant to the project objectives. A summary of the information on cow numbers, manure handling, and crop production follows.

Farm #1, : Birth to weaning: 100
Weaning to 1 yr: 300
1 yr to 1st calving: 300
Dry cows: 85
Lactating cows: 625
At 3-4 months calves are moved to old farm and return when ready to calve.

Current form(s) of manure collection: Flush with lagoon water and/or scrape, collect sand at end of barns. No separator right now.
Current method(s) of manure application: 98% Big gun, 2% Dry spreader. Grass fields are irrigated with big gun after each harvest during the growing season. Corn ground and grass fields are irrigated at end of growing season.
320 acres in production pasture.

Farm # 2, Birth to weaning 15
Weaning to 1 yr 60
1 yr to 1st calving 25
Dry cows 25
Lactating cows 130

Winter, heifers and dry cows moved to the honor farm (digester location). They are moved back to the farm spring and summer as long as pasture can sustain them.

Scrape alleys into pits. If there is enough liquid, manure is pumped straight to lagoon.

Manure is applied to grass fields and pasture with solids spreader or honey wagon.

250 acres in production and pasture.

Farm # 3, Birth to weaning: 70
Weaning to 1 yr: 120
1 yr to 1st calving: 200, 100 at Thomas's in Snohomish.
Dry cows: 60
Lactating cows: 500

100 heifers at the Thomas farm in Snohomish. When the grass can sustain them, they are moved back to pasture.

Barn alleys are scraped into underground pit and pumped to solids separator. Liquid stored in lagoon and solids stored on cement pad.
Solids applied to corn acres and rest is sold to DeJong's, with custom injection and big gun application of liquid to all ground.

400 acres in production and pasture.

Farm # 4, Birth to weaning 106
Weaning to 1 yr 294
1 yr to 1st calving 249
Dry cows 80
Lactating cows 740
Heifer's aged 6mo to 20 months housed at Ovenell's ranch.

Recycled liquid is flushed through alley when cows are in milking parlor. Flushed material goes to a sand settling basin. It is then pumped to a solids separator. Some solids are recovered for bedding, some is removed off farm. Liquid is run through a second settling basin before going to lagoon. From lagoon liquid is recycled for flush system or applied by injection to fields.

600 acres in production. I need to ask if this is still correct.

Farm # 5, Birth to weaning 35
Weaning to 1yr 45
1 yr to 1st calving
Dry cows 10 close up
Lactating cows 300

Heifers moved to Quincy. Rent some pasture in Snohomish for dry cows.

Barns and holding areas scraped into 30000 gallon storage tank. Milking parlor water goes in also. Manure is then pumped to lagoon. Custom injection of manure to corn ground.

95 acres in corn production.

1) Surface Water Quality Monitoring – **Snohomish Conservation District** - The water quality monitoring was lead by the Snohomish Conservation District. The goal of water quality monitoring was to document changes to water quality that may result from AD treatment of manure applied to farm fields. Samples were taken at several points along each of two streams (Riley Slough and Peoples Creek) that run through the project area. SCD staff measured in situ dissolved oxygen, pH, and temperature, and collect water samples for fecal coliform and turbidity analyses. The *Snohomish Conservation District Standard Operating Procedures for Water Quality Monitoring* outlines procedures for these tasks. In addition, samples were assayed for nitrogen and phosphorus (Edge Analytical Lab). Stream discharge was estimated when possible at each site. Replicate and blank samples will be collected and analyzed in order to quantify field sampling and sample handling procedures. Samples will be analyzed according to procedures outlined in *Edge Analytical Laboratory Fecal Coliform Procedures and Quality Control*.
The pre-AD monitoring was completed by the SCD, but due to the lack of participation of cooperating dairies, no post anaerobic digestion monitoring was conducted at the larger scale. However, it was decided in 2010 that a replicated surface water runoff study would be conducted to demonstrate the potential risk of AD and non-AD manure to result in movement of bacteria to surface water. AD and non-AD manure were applied to grass plots with a slope, and plots were surrounded with edging-border to contain and direct runoff to a central collection point. Manures were applied just prior to anticipated heavy rainfall events.

2) On Farm Pathogen Monitoring

Selection of organisms for the project: Generic *E. coli* was selected because high concentrations are dependably present in bovine fecal waste, and, because of its relatively low thermotolerance, survival of this organism in residues would indicate that a wide variety of biosecurity agents could likely survive. Enterococci were selected because they are dependably present in bovine fecal waste, and, because of their relatively high thermotolerance, survival of these organisms in residues would indicate that thermotolerant biosecurity agents could likely survive. Salmonella and

Mycobacterium paratuberculosis were selected because they are themselves important biosecurity agents, because they occur frequently enough in dairy herds that a good chance exists of finding them (at least in pre-digestion samples), and because they are environmentally resistant to a lesser (Salmonella) or greater (*Mycobacterium*) degree. Enteroviruses were selected because they occur ubiquitously in cattle populations at a high prevalence (Ley et al, 2002) and they have a similar level of environmental resistance as certain viruses with biosecurity implications. Importantly, enteroviruses and foot and mouth disease virus are members of the family Picornaviridae, hence the former is a good surrogate for the latter in a study such as that proposed.

On Farm Sampling – Farms were monitored for 18 months (2007 – 2008) prior to the start-up of the community AD, once each month a manure sample was obtained from each herd at their respective farm. Specific organisms evaluated were: Salmonella, Generic *E. coli* (including 0157:H7), enterococci, salmonella, *Mycobacterium paratuberculosis* (Johnes), listeria, campylobacter, and enterovirus. After the community AD had begun running, once each month (for ~ 1 year) a manure sample will be obtained from two herds that were willing and able to pump manure to the community AD. Specific organisms evaluated were the same.

3) Sampling at Community AD – The community AD is located centrally within the study area (see figure 1). Weekly samples were obtained of manure entering the community AD, and at each point of handling post AD (liquid, solid, and compost) and assayed for the same organisms as for on-farm samples. All samples from on-farm and the community AD were shipped via overnight transport to the WSU Field Disease Investigative Unit Lab and the WSU Animal Disease Diagnostic Lab.

4) Johnes surveillance - Special m*ycobacterium paratuberculosis* (Johnes) surveillance – The Washington State Department of Agriculture agreed to enroll all cooperating dairies farms in their Johnes surveillance program as a part of the original grant. They provided a financial contribution toward the project. The testing occurred in 2007.

5) Pasteurization - The original concept proposed by the Qualco Energy group when the project proposal was submitted in 2006 to include a pasteurization step post-AD. However, this technology was never adopted on-site and no evaluation was conducted. However, two proprietary technologies were evaluated for their merit for bacterial reduction.

6) Soil Bacteria Survival – Survival of bacteria of manure origin was monitored in soil cores post manure application on one farm. Soil samples will be taken using a 2.4 inch diameter soil probe at a depth of 1.5 inch. Soil cores will include grass and surface material in grass fields. Background levels of fecal coliform and *Escherichia coli* will be determined prior to manure application. Bacteria levels will be monitored immediately after manure application and until soil levels reach background levels. In

2007, baseline data was collected on the rate of bacteria die-off (BDO) on soil when undigested dairy manure was applied to grass cropland.
In 2009, and 2010, paired comparisons were made of two sources of manure, anaerobically digested and before-digestion dairy manure to replicated grass plots.

7) Microbial Source Tracking (MST) – The original project proposal planned for " Selected samples (~ 80/year) from the on-farm water quality monitoring and soil bacterial survival evaluation to be submitted to the Institute for Environmental Health (Seattle, WA) for identification of the source of the e-coli. Specific identification is expected as to source of cattle, avian, or wildlife." In February 2010, a change of General Methodology and Approach (see section C) was made due to the lack of participation of multiple farms to provide manure to the centralized AD. The plan for MST focused on determining if bacteria exiting the AD were different than those entering the AD.

8) Fate of Bacteria During Lagoon Storage – This component was added in the fall of 2010 after data from this project was presented at the International ASABE Air and Manure Symposium in Dallas, TX in September 2010. A question was asked by NRCS staff at the conference about "what was known about the fate of bacteria from AD manure that had been subsequently stored in a lagoon, would re-growth of bacteria counts occur". Two sets of lagoons located at two dairies that stored either anaerobically digested dairy manure or undigested dairy manure were utilized for this evaluation (2 lagoons with AD manure and 2 lagoons with undigested dairy manure). Samples were taken at ~ 2 week intervals for 7 samplings in the fall of 2010 and early 2011. When possible samples were obtained at 3 depths, bottom (6 ft), mid (3 ft), and top (12 inches) of each lagoon. In addition, 5 gallon buckets of manure were stored at ambient temperature and sampled twice per week for four samplings then every other week for three samplings to determine the fate of bacteria. The bacteria selected for evaluation were: generic e-coli, enterococcus, and salmonella for the lagoon samples; and, generic e-coli and enterococcus for the bucket study.

The anaerobic digester began operation in December of 2008. An aerial view of the AD is shown in figure 9. The AD was run at 100 degrees F (mesophilic), of plug flow design, with a 450 KW generator, liquids-solids separation post AD, and rotating drum composters (see figures 9 - 15). Figure 11 summarizes the flow and movement of liquids and solids in the system and points where samples were obtained for analyses.

Figure 9 – Aerial of anaerobic digester site.

'Genset"

Figure 10. 450 KW generator run on methane to produce electricity.

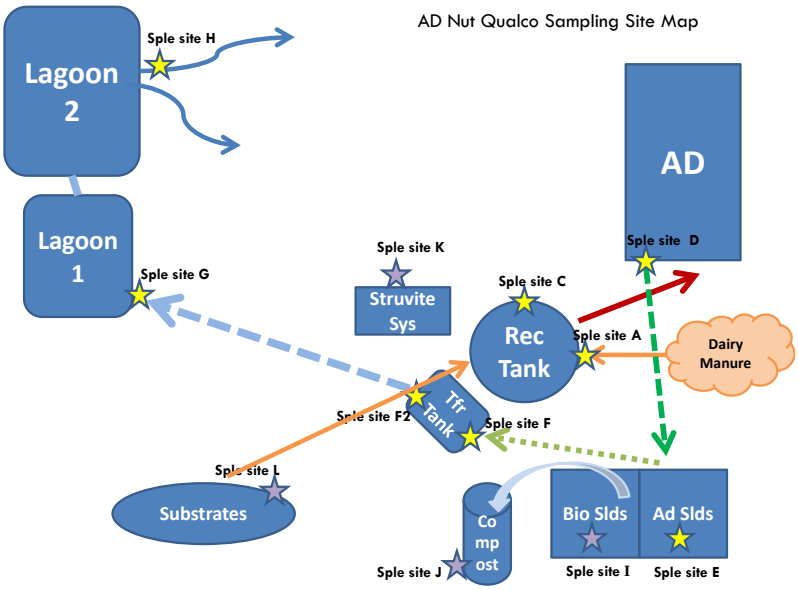


Figure 11 – Schematic of sampling sites at anaerobic digester.

Figure 12 – Rotating drum composters for solids after anaerobic digestion.

Figure 13. Sampling incoming manure and pre-consumer food-wastes at the receiving tank

Figure 14. Sampling incoming manure solids after liquid-solids separation of post AD material.

Figure 15. Sampling composted AD manure solids.

The original concept of the community anaerobic digester (manure from several dairies transported to a central AD) was modified in early 2009 due to the economic downturn in the dairy industry and a perceived risk by potential cooperating farms of acquiring any additional risk to their operation.

As a result, only manure from one dairy is pumped to the AD, with additional pre-consumer foodwastes comprising ~ 30% of total inputs since December 2008.

The inputs since December 2008 have included:

- Dairy cow manure from 1000 lactation dairy cows and 200 heifers and calves
- Liquid Whey
- Egg byproduct
- Fish stick byproduct
- Ruminant blood from slaughter facility
- Biodiesel byproduct
- Grease
- DAF (fat/grease product from poultry processing)
- Wood pulp

By permit (exemption), dairy ADs can utilize no more than 30% pre-consumer foodwastes.

C. Changes to General Methodology and Approach

2008 – In September 2008, it was determined that there was a need to request a no-cost extension to the project as the construction of the community anaerobic digester had been delayed.

No cost extension request for NRCS CIG project entitled: Pathogen Reduction in a Community Based Anaerobic Digester

Washington State University

Contract No. NRCS 68-3A75-6-134

a. The length of additional time required to complete the project and a justification for the extension.

We are requesting a no-cost extension of this project from the original term date of September 6, 2009 to a term date of May 6, 2010.

The project is focused on the evaluation of pathogen reduction of a set of farms associated with a community anaerobic digester (AD), and the AD itself. The plan has included a period of monitoring for 1 year prior to and 2 years after (pre and post AD) the completion of the AD. We have completed one year of pre- AD monitoring in the late spring of 2008. Due to construction delays related to permitting and acquisition of funding, the AD is scheduled to be functional at the end of 2008. The construction of the AD has not been funded by the CIG project. To accomplish our original intent, we would like to extend the project completion date to May 6, 2010 so that we can accomplish the post AD monitoring.

d. A projected timetable to complete the portions(s) of the project for which the extension is being required.

The remaining period of post-AD monitoring will occur between Sept 7, 2009 and May 6, 2010.

2010 – In February 2010, it was determined that there was a need to request a no-cost extension to the project to complete the following activities:

1) Microbial source tracking effort (comparative evaluation of the bacterial diversity of manure and substrates before and digested manure + substrates anaerobic digestion
2) Bacteria run-off to surface water.

No cost extension request for NRCS CIG project entitled: Pathogen Reduction in a Community Based Anaerobic Digester
2 3 2010
Washington State University
Contract No. NRCS 68-3A75-6-129

a. The length of additional time required to complete the project and a justification for the extension.

We are requesting a no-cost extension of this project from the term date of May 6, 2010 to a term date of December 31, 2010.

The project is focused on the evaluation of pathogen reduction of a set of farms associated with a community anaerobic digester (AD), the AD itself, and multiple feedstocks used for methane production and electric generation. The project has included a period of monitoring for 1 year prior to and 2 years after (pre and post AD) the completion of the AD, a surface water quality monitoring component, microbial source tracking evaluation (are bugs coming out of the digester or from selected feedstocks different than bugs entering the digester), and a pasteurization component. We have completed one year of pre- AD monitoring in the late spring of 2008. Post AD monitoring and monitoring of manure and feedstocks has been in place since December of 2008 and sampling is scheduled to cease in Feb 2010 for general characterization of organism fate (die-off or kill during anaerobic digestion). We plan to complete all sampling for "microbial source tracking" via methods that will provide a comparative evaluation of the bacterial diversity of manure and substrates before and digested manure + substrates anaerobic digestion by September of 2010 and have all assays complete and summarized by December 2010.

To accomplish our original intent, we would like to extend the project completion date to December 31, 2010 so that we can accomplish the following project components:

2) Microbial source tracking effort (comparative evaluation of the bacterial diversity of manure and substrates before and digested manure + substrates anaerobic digestion) – sampling through September 2010 and complete assays by December 2010.
3) Bacteria run-off to surface water – complete phase 2 of the run-off work during the fall rains of 2010. (Phase 1 being conducted in February 2010)
4) Pasteurization – complete by end summer 2010.

D. Data Summary

The project was divided into 7 specific components:

1) surface water quality monitoring,
2) on-farm monitoring of pathogens,

3) pre- post AD monitoring of pathogens

4) Johnes surveillance,

5) pasteurization

6) bacteria die-off on soil after manure application

7) microbial source tracking.

8) fate of bacteria upon lagoon storage

The data summary section is organized as follows for each of the seven components:

a) description of activities
b) supporting data
c) Interpretive Statement

The Interpretive Statement is in blue lettering for ease of location.

1) Surface Water Quality Monitoring

The initial phase of the surface monitoring component was completed by the Snohomish Conservation District.

Author: William Bowe
Water Quality Specialist
Water Quality Summary Report
Washington State University Community Anaerobic Digester Subcontract G002133
March 14, 2011

ABSTRACT:

Personnel from Snohomish Conservation District performed twice-monthly sampling of three waterways in the vicinity of a community anaerobic digester near Monroe, WA. The three waterways were a slough whose historic upper-channel connection to riverine source waters was severed; an in-field drainage channel with intermittent pumping discharge; and a creek with active flow throughout the term of the sampling campaign.

Sampling occurred during the period March, 2007 through February, 2009. Sample sites bracketed reaches of channels adjacent to forage-crop fields expected to receive applications of pre-digestion or post-digestion effluent. On-site analytes were: Conductivity, Dissolved Oxygen, pH, Temperature and Turbidity. Laboratory analytes were: Ammonia, Fecal coliform, Orthophosphate, Total Nitrite/Nitrate and Total Kjeldahl Nitrogen. Data results are compared to extant State of Washington fresh-water quality standards.

Paired-*t* analyses for sample sites demarking a reach in respective channels showed significant mean differences for many of the analytes.

Correlation of precipitation events occurring during the sampling day, within 24 hours, 48 hours, and one week prior to sampling to data results showed differing precipitation responses for the three channels. Significant correlations for samples taken within 0, 24 and 48 hours after a precipitation event were typically found at those sites in the three channels characterized by active stream flow. Sites within the group of channels with sluggish or intermittent flow sometimes showed correlation to precipitation occurring earlier, i.e. 48 to 168 hours, and prior to sampling.

INTRODUCTION:

Three channels adjacent to the Qualco Energy Corporation (Qualco) Community Anaerobic Digester were selected for water quality analysis in anticipation of facility construction. The digester, Figure 16 and Inset 1, is located at the former Monroe Correctional Facility Honor Farm south of Monroe, WA. The project site and channels are located within the Snohomish Basin, Water Resource Inventory Area Seven (7).

Figure 16: Monitoring Sites and Location of the Qualco Digester at the Former Monroe Correctional Facility Honor Farm.

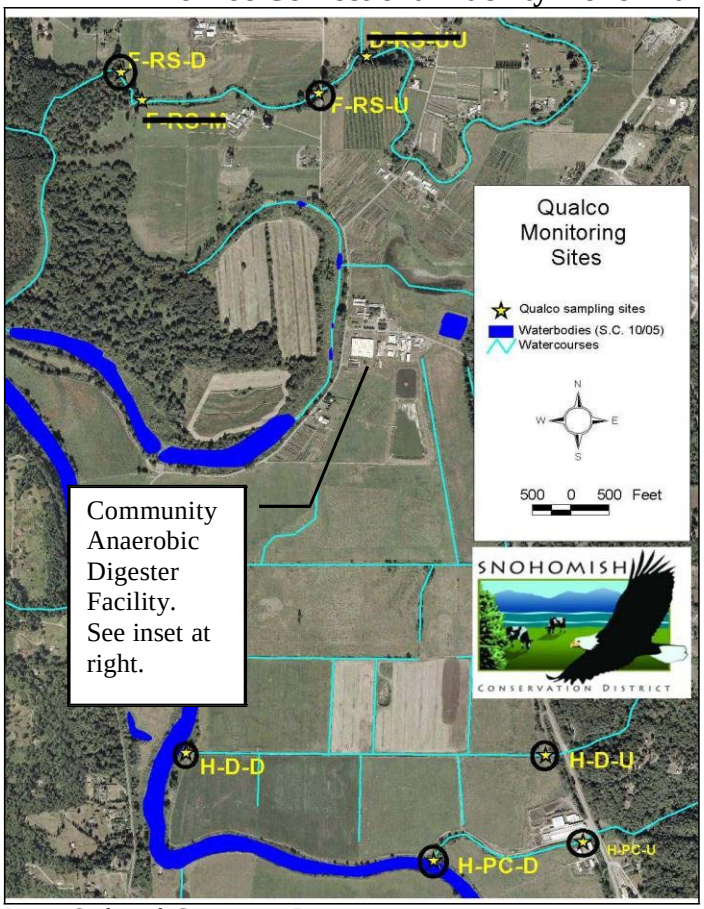
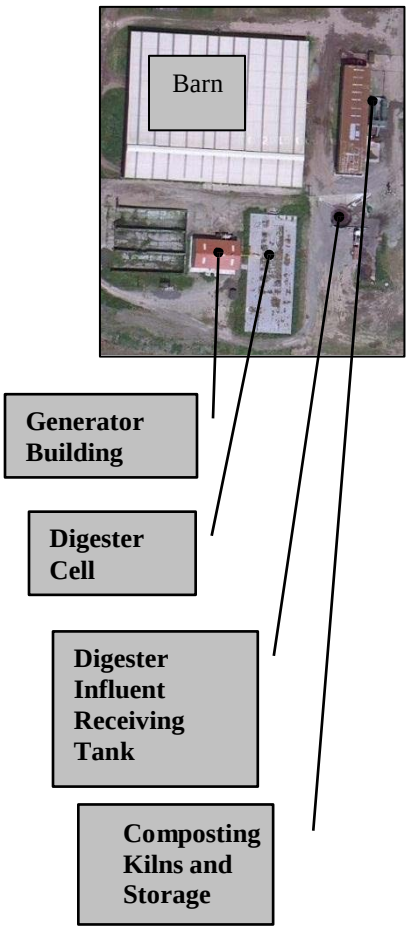


Image: Snohomish Conservation District

Inset 1: Community Anaerobic Digester

Image Source: 2010 Google – Digital Globe, GeoEye, USGS, Map Data 2010 Google.



The selected channels are adjacent to farms partnering in the development of the anaerobic digester system. Expectations are that farms will pipe dairy manure or related wastes to the digester and receive processed effluent from the digester for field application through return pipelines (Clark Group, 2005). Connection of the pipeline network to certain farms has not been completed as of this writing, hence this report for the period March, 2007 through February, 2009 does not provide the "before" and "after" comparisons originally envisioned.

CHANNEL DESCRIPTIONS:
Selected channel reaches are located in Riley Slough, Peoples Creek and an on-farm drainage channel, Table 3 and Figures 17, 19 and 21. Riley Slough is north of the digester facility, Peoples Creek and the drainage channel are located south of the digester facility. Transit Distance given in Table 3 is tortuous channel length between sample points measured via a GIS ruler utility.

Table 3: Monitoring sites adjacent to the Qualco digester project.

Water Body	Designator	Location in Channel	Latitude, Longitude (degrees)	Transit Distance (ft)
Riley Slough	F-RS-U	Upstream	47.822037, -	3400
Riley Slough	F-RS-D	Downstream	47.823161, -	
Drain Channel	H-D-U	Upstream	47.797907, -	4650
Drain Channel	H-D-D	Downstream	47.797775, -	
Peoples Creek	H-PC-U	Upstream	47.794955, -	2400
Peoples Creek	H-PC-D	Downstream	47.793964, -	

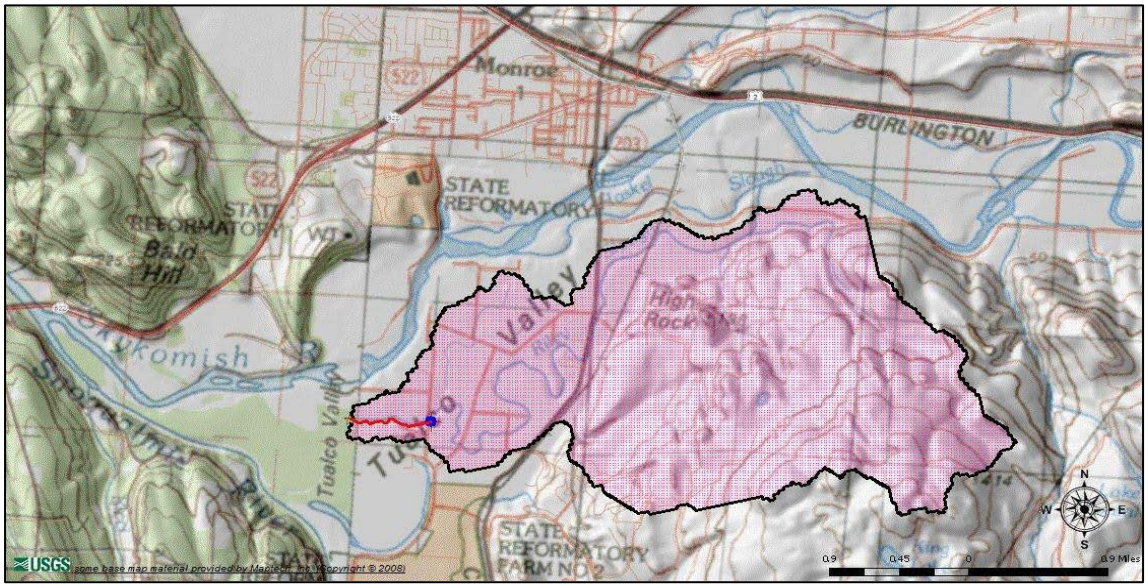
Riley Slough:

Riley Slough parallels the Skykomish River for four miles. Owing to channel tortuosity, its channel length approximates six miles as it transits southwesterly through the Tualco Valley and joins the Skykomish River a short distance above the Skykomish/Snoqualmie confluence. Riley Slough has an historical upstream connection to Haskell Slough at the northeast end of Tualco Valley which has been largely severed; agriculture has been the primary land use along the slough for many decades, and slough habitat has been influenced by flooding and grazing (SCD, 2003). The sampled reach is west of Tualco Loop Road near the confluence of the slough with the Skykomish River. See Figures 16a and 16b.

Figure 16a. Riley Slough upstream site. SCD photo March 2007.

Figure 16b. Riley Slough site. downstream SCD photo March 2007

Figure 17. Riley Slough Watershed. Sampled reach shown by red line at west end of the watershed.



Source: United State Department of the Interior, United State Geological Survey. StreamStats Water Resources Web Applicatio n.
StreamStats Washington; *http://water.usgs.gov/osw/streamstats/Washington.html*

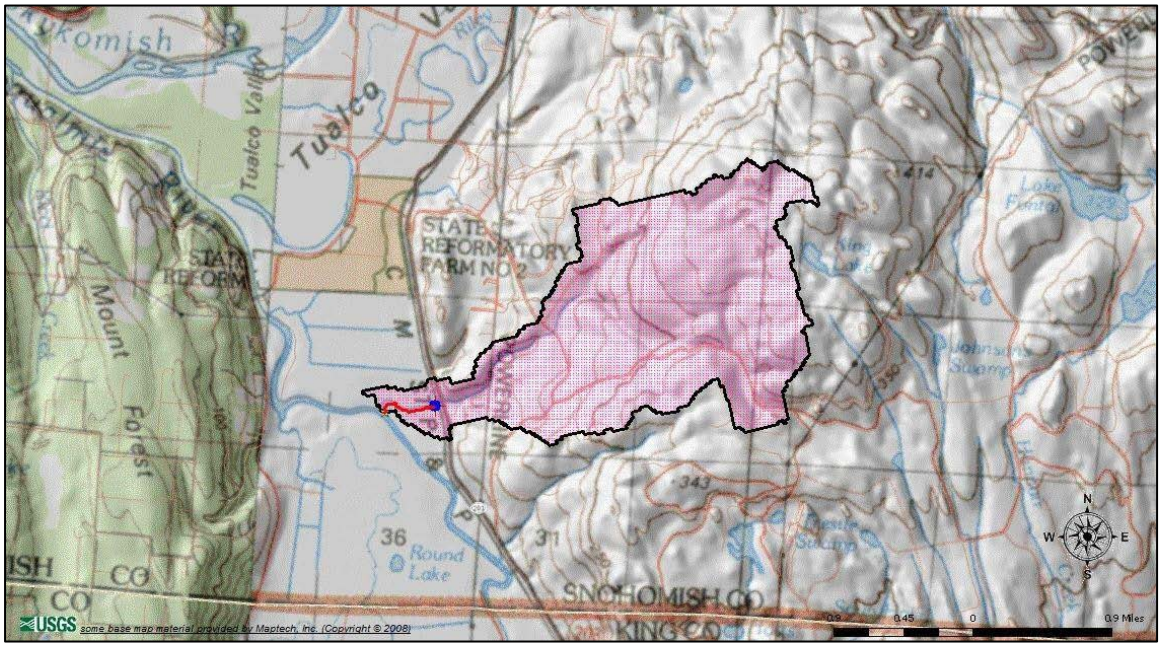
Peoples Creek:

The Peoples Creek watershed is located roughly four miles southeast of Monroe with origins on western aspect slopes east of the Monroe-Duvall Road (SR203). The watershed discharges westerly to the Snoqualmie River. Main stem Peoples Creek confluences with several small tributaries east of SR203 and crosses the highway one and one-half miles north of the Snohomish County/King County border. Uplands to the east of SR203 are composed of a mix of forest, rural residence and small agriculture parcels. Peoples Creek traverses the Snoqualmie River plain from its SR203 crossing to a confluence with the Snoqualmie River and the sampled reach for Peoples Creek is that part west of SR203, Figures 18a and 18b.

Figure 18a. Peoples Creek upstream site. SCD photo March 2007

Figure 18b. Peoples Creek downstream site. SCD Photo March 2007

Figure 19. Peoples Creek Watershed. Sampled reach shown by red line at west end of the watershed.



Source: United State Department of the Interior, United State Geological Survey. StreamStats Water Resources Web Application.
StreamStats Washington; *http://water.usgs.gov/osw/streamstats/Washington.html*

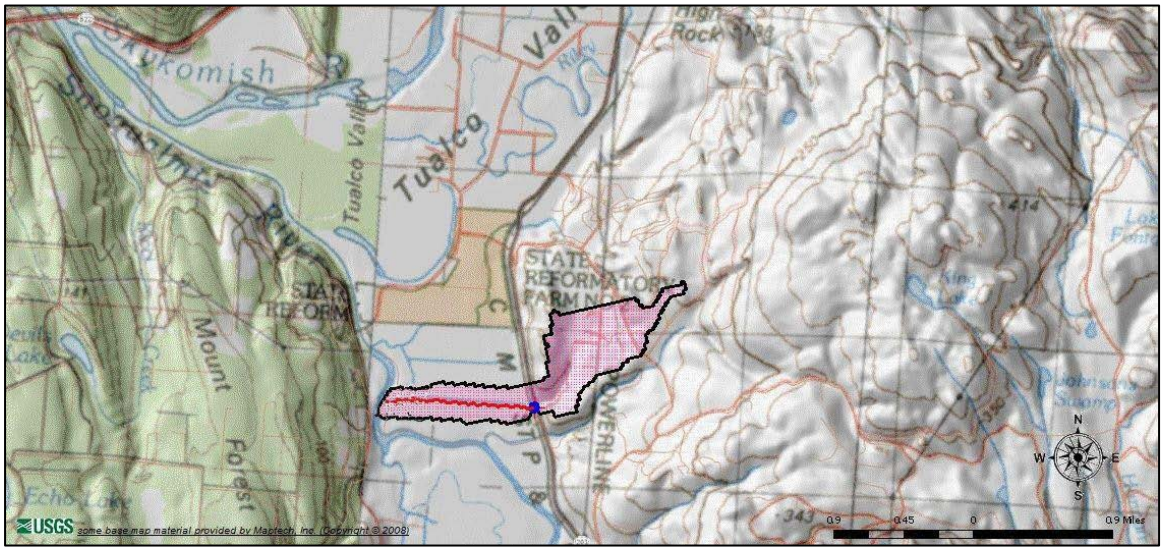
Drainage Channel:

The drainage channel has origins to the east of SR203 similar to Peoples Creek, albeit a much smaller contributing area on a lower flank of the slope. It crosses SR203 and proceeds west to the Snoqualmie River. Discharge from the drain channel to the river is driven by a pump system at the western terminus of the channel. The sampled reach for the drainage channel is west of SR203, consisting of that part crossing the Snoqualmie River plain. Figures 20a and 20b. The downstream sample site demarked in Figure 16 and pictured in Figure 20b, H-D-D, is located in the stilling pool of the drainage pump slightly upstream of the pump intake.

Figure 20a. Drainage channel upstream site at SR203 crossing. SCD photoMarch 2007.

Figure 20b. Drainage ditch downstream site. SCD photo April, 2007

Figure 21. Drainage Channel Watershed. Sampled reach shown by red line at west end of the watershed.



Source: United State Department of the Interior, United State Geological Survey. StreamStats Water Resources Web Applicatio n.
StreamStats Washington; *http://water.usgs.gov/osw/streamstats/Washington.html*

SAMPLE CHRONOLOGY and RAINFALL HISTORY:

Sample acquisition date and rainfall history for each sampling are given in Table 4. The nominal interval between sampling events was set at two weeks and was adhered to for the first fourteen months of the sampling sequence. A storm following a dry period in August, 2007 triggered a storm event sample on August 21, one week after the nominal August 14, 2007 sampling.

The interval was increased to two months in the summer and fall of 2008 to accommodate delays in construction of the digester and associated hookups to partnering farms yet maintain a base line of sample data. Plans were to resume the nominal two-week interval in the early spring of 2009, but sampling was stopped with the last accession on February 25, 2009. This
particular sampling was a storm-driven event one week after a relatively dry, nominal sampling taken on February 18.

Cumulative rainfall within the week prior to each sampling is parsed to examine the impact of rainfall timing and amount on each of the analytes. Precipitation is parsed according to the following schedule: precipitation on the day of sampling (day 0), twenty four hours prior to sampling (day 1) , forty eight hours prior to sampling (day 2) and 168 hours (day 7, one week) prior to sampling.

There was no dedicated rain gauge system at the channel sites. A series of cooperator and publicly-owned systems in the immediate area were gleaned for available data and staff were able to develop a contiguous record free of equipment and/or data malfunction errors. This data is summarized in Table 2.

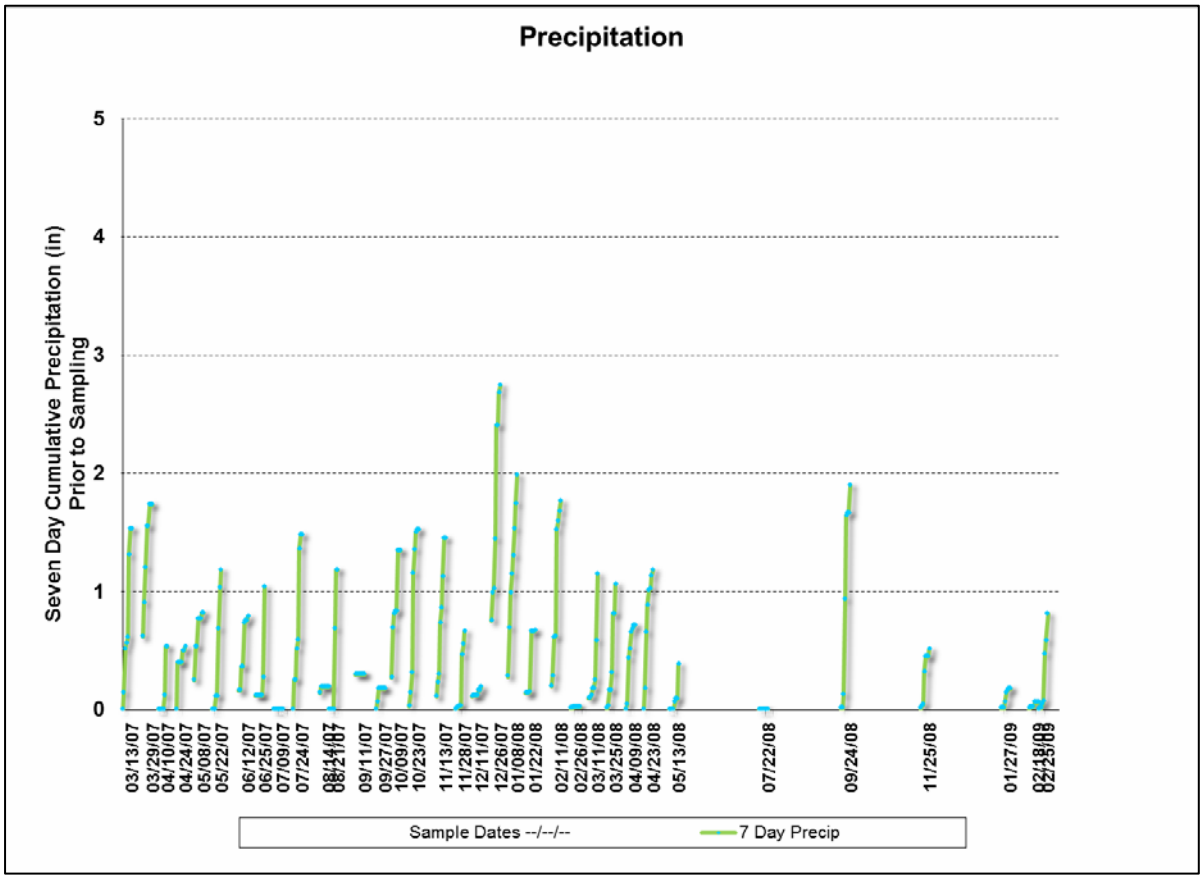
Table 4: Sample chronology and cumulative rainfall history. Precipitation amounts are given in inches.

Sampling Date mm/dd/yy	Rainfall: Day of Sampling	Cumulative Rainfall: One Day Prior	Cumulative Rainfall: Two Days Prior	Cumulative Rainfall: Seven Days Prior	Sampling Date mm/dd/yy	Rainfall: Day of Sampling	Cumulative Rainfall: One Day Prior	Cumulative Rainfall: Two Days Prior	Cumulative Rainfall: Seven Days Prior
03/13/07	**0**	**.22**	**.92**	**1.53**	**11/28/07**	**.11**	**.20**	**.63**	**.66**
03/29/07	**0**	**0**	**.18**	**1.73**	**12/11/07**	**.03**	**.03**	**.07**	**.19**
04/10/07	**0**	**.41**	**.53**	**.53**	**12/26/07**	**.06**	**.34**	**.34**	**2.74**
04/24/07	**.03**	**.03**	**.13**	**.53**	**01/08/08**	**.24**	**.45**	**.68**	**1.98**
05/08/07	**0**	**.05**	**.05**	**.82**	**01/22/08**	**.01**	**.01**	**.01**	**.67**
05/22/07	**.15**	**.50**	**1.07**	**1.18**	**02/11/08**	**.08**	**.17**	**.24**	**1.76**
06/12/07	**.04**	**.04**	**.06**	**.79**	**02/26/08**	**0**	**0**	**0**	**.02**
06/25/07	**.77**	**.92**	**.92**	**1.04**	**03/11/08**	**.56**	**.89**	**.96**	**1.14**
07/09/07	**0**	**0**	**0**	**0**	**03/25/08**	**.25**	**.25**	**.75**	**1.06**
07/24/07	**0**	**.12**	**.89**	**1.48**	**04/09/08**	**0**	**.03**	**.06**	**.71**
08/14/07	**0**	**0**	**0**	**.19**	**04/23/08**	**.05**	**.16**	**.17**	**1.18**
08/21/07	**0**	**.50**	**1.18**	**1.18**	**05/13/08**	**.29**	**.29**	**.32**	**.38**
09/11/07	**0**	**0**	**0**	**.30**	**07/22/08**	**0**	**0**	**0**	**0**
09/27/07	**0**	**0**	**0**	**.18**	**09/24/08**	**.24**	**.24**	**.26**	**1.90**
10/09/07	**0**	**0**	**.51**	**1.34**	**11/25/08**	**.06**	**.06**	**.06**	**.51**
10/23/07	**0**	**.02**	**.17**	**1.52**	**01/27/09**	**0**	**.02**	**.04**	**.18**
11/13/07	**0**	**.33**	**.59**	**1.45**	**02/18/09**	**0**	**0**	**0**	**.06**
					02/25/09	**.23**	**.34**	**.74**	**.81**

DATA GRAPHICS

Data for each analytical species and stream channel location are graphed in Appendix A Cumulative precipitation for seven days prior to the date of sampling is referenced to the left *y* axis whose full scale range is fixed for all charts. See Figure 22. Cumulative precipitation data points are indicated by a lozenge “diamond” marker and a connecting line from point to point within a given week prior to the date of sampling. This graphic of cumulative precipitation forms the background for all data plots.

Figure 22. Graphic showing seven-day precipitation antecedent to sampling.



Displacement of lines vertically from the *x* axis indicates precipitation received on the seventh day prior to sampling. If no precipitation was received, the cumulative depth line initiates from the *y* coordinate of zero on the *x* axis.

The graphs are arranged by: a) species within each of the channels and b) geographic order from north to south with Riley Slough, drainage channel and Peoples Creek in succession. The analytic result of interest and its numeric value are referenced to the right *y* axis. Due to concentration variations of the data, full scale ranges of these axes vary. A concerted effort is made to maintain a common full-scale range among a given species that provides good visual resolution for the majority of data points. Some data values are allowed to graph over the top
of the plot area, hence connecting lines for the data value appear to exit and reenter the plot area. Data values exceeding the scale range are placed in a text field at the top of the plot area. Channel location (upstream, downstream) for individual analytes are given by separate line plots within the chart. For a short time, a “middle” location within Riley Slough was accessed and eventually discontinued due to lack of flow during most of the sampling campaign. It is not shown in the Riley Slough graphics nor included in the statistical analyses and may be found in the data tables in Appendix E.
Water quality data for upstream and downstream sample locations on a given channel are superimposed on the rainfall graphic and presented in the following order:

Laboratory:	Units
Ammonia	mgN/L
Fecal Coliform	cfu/100mL
Orthophosphate	mgP/L
Total Kjeldahl Nitrogen	mgN/L
Total Nitrate and Nitrite	mgN/L

In Field:	
Conductivity	*u*S/cm
Dissolved Oxygen	mg/L
pH	units
Temperature	^{o}C
Turbidity	NTU

Where:

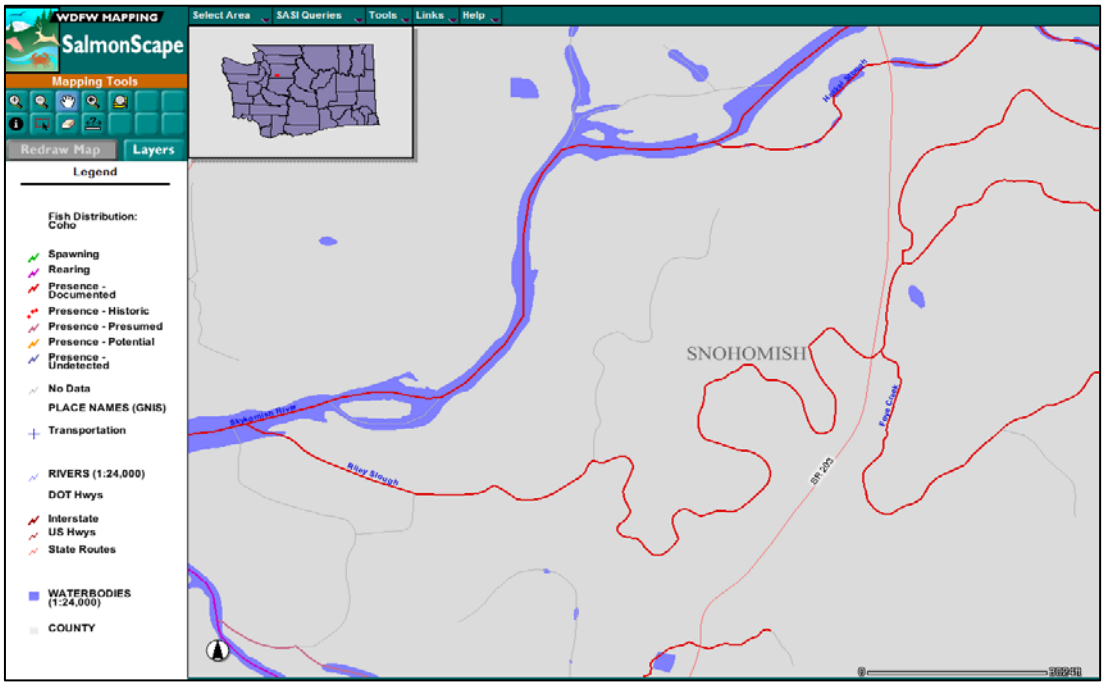
mg	=	milligram
N=	Nitrogen	
L =	Liter	
cfu	=	coliform forming unit
P =	Phosphorus	
*u*S	= microSiemens	
cm	=	centimeter
^{o}C	=	degrees Celsius
NTU	=	Nephelometric Turbidity Unit

Several of the graphics have breaks in data due to no flow conditions. This is most evident for the periods July 09 through November 13, 2007 and July 22, 2008 to September 24, 2008 in the Riley Slough data. Other intermittent gaps in data may be due to high water conditions in receiving river bodies creating temporary flooding of sample sites or may have been caused by equipment malfunctions. Data qualifiers are given by explanatory text entries in the raw data files in Appendices E, F and G for Riley Slough, drainage channel and Peoples Creek, respectively.

AQUATIC LIFE CRITERIA: Mapping of salmonid presence

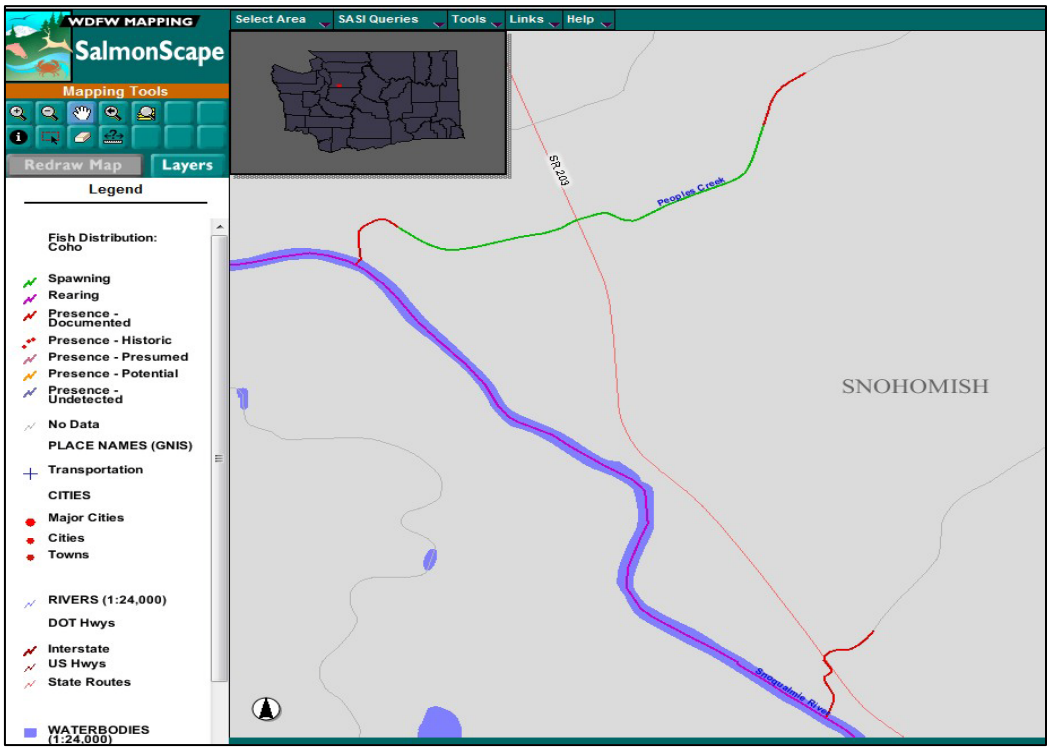
Salmonid presence (Coho salmon, *Oncorhynchus kisutch*) has been observed and mapped in Riley Slough and Peoples Creek. This mapping, taken from the Washington State Department of Fish and Wildlife SalmonScape web application (WDFW, 2011) is shown by the red line overlay of Riley Slough and Peoples Creek in Figures 23 and 24. A portion of Peoples Creek is mapped as spawning area for Coho and is shown as a green line overlay of the channel in Figure 10. The drainage channel is not mapped for Salmonids or other fish species.

Figure 23. Salmonid mapping in Riley Slough.



Source: Washington State Department of Fish and Wildlife. SalmonScape Web Application.
http://fortress.wa.gov/dfw/gispublic/apps/salmonscape/default.htm

Figure 24. Salmonid presence and spawning area mapping in Peoples Creek.



Source: Washington State Department of Fish and Wildlife. SalmonScape Web Application.
http://fortress.wa.gov/dfw/gispublic/apps/salmonscape/default.htm

Water Quality Standards

Washington Administrative Code (WAC) addresses aquatic life criteria for fresh waters of the state in Chapter 173-201A-200. Numeric values for the criteria vary according to fish species category and incidence of human contact. The criteria are summarized in publications issued by the Washington State Department of Ecology (Ecology, 2006 and Ecology, 2011) and can be found in an online format developed by the Washington State Legislature Office of the Code Reviser. Publications from Ecology refer final disposition of these criteria to the WACs and state, "…conflicts between the language contained in this document and the language contained in the official version of the regulation maintained by the Office of the Code Reviser, the language contained in the official version shall govern." (Ecology, 2006)

Criteria for Aquatic Life Category, *Salmonid Spawning, Rearing and Migration* are presented here to provide a comparison point for data collected. This criteria set is used for all channels although the drainage channel is not mapped for salmonids or other species. Criteria are defined for fecal coliform, dissolved oxygen, pH, temperature and turbidity in WAC 173-201A (Washington State Legislature, 2011a). Nitrogen compounds are not generally defined for surface waters other than acute and chronic toxicities for ammonia found in WAC 173-201A-240 (Washington State Legislature,
2011b) for toxic substances. Similarly, standards for phosphorus compounds are not generally defined at the present time for streams but are defined by WAC 173-201A-230 as lake nutrient criteria (Washington State Legislature. 2011c)

Ammonia

Acute toxicity exposure for ammonia is defined in the WACs as, "An 1-hour average concentration not to be exceeded more than once every three years on the average". Chronic toxicity exposure for ammonia is defined as,"A thirty-day average concentration of total ammonia nitrogen (in mg N/L) not to be exceeded more than once every three years on average. The highest four-day average within the thirty-day period should not exceed 2.5 times the chronic criterion".

Criteria levels for ammonia in fresh water vary with the temperature and pH. The United States Environmental Protection Agency (EPA, 2009) has a succinct description of the interelation of ammonia compound and ammonium ion presence to pH and temperature:

The chemical form of ammonia in water consists of two species, the more abundant of which is the ammonium ion (NH4+) and the less abundant of which is the non-dissociated or un-ionized ammonia (NH3) molecule; the ratio of these species in a given aqueous solution is dependent upon both pH and temperature. In general, the ratio of un-ionized ammonia to ammonium ion in fresh water increases by 10-fold for each rise of a single pH unit, and by approximately 2-fold for each 10OC rise in temperature from 0-30OC.

Threshold levels for problematic ammonia concentrations in fresh water are calculated by equations given in WAC 173-201A-240. These equations have differing constants according to 1) presence, absence or designated use of a water body by salmonids, 2) presence or absence of other fish early life stages, 3) pH range, 4) temperature range.
Not given here, the equations are available in the WACs and are contained in an interactive web-based spreadsheet series published by Greg Pelletier of the Washington Department of Ecology (Pelletier, 2010) as an aid to those preparing National Pollutant Discharge Elimination System (NPDES) permit application materials. An image of the user interface for the *NH3fresh3* tabbed worksheet within the *pwspread_v20101108* workbook is shown in Figure 24.

It may seem problematic to link the fresh-water bodies summarized in this report to NPDES permitting. The intent is to compare data results to current fresh water standards taken from the WACs. The WACs contain a fairly complex calculation protocol for defining ammonia criteria which happens to be summarized by Pelletier and placed in materials used by NPDES permit applicants.

The spreadsheet allows the user to enter conditions of existing or designated use by salmonids, presence or absence of non-salmonid early life stages, water temperature and water pH. The output field of the spreadsheet displays acute and chronic criteria for un ionized ammonia (milligrams Ammonia/Liter) and total ammonia nitrogen (milligrams Nitrogen/Liter). The maximum values for temperature and pH and their concurrent pH and temperatures, respectively, from each channel were processed by the web-based spreadsheet with the results shown in Tables 5 through 7. The processed points are not date-matched nor do they represent a particular month or season; data were selected on their maximal values which tends to bring forth an illustration of a worst-case situation.

Figure 24. Fresh water ammonia criteria calculator spreadsheet by Pelletier, 2010.

Freshwater un-ionized ammonia criteria based on Chapter 173-201A WAC
Amended November 20, 2006
VBA functions revised 08-Nov-2010

INPUT

1. Temperature (deg C):	11.4
2. pH:	7.11
3. Is salmonid habitat an existing or designated use?	Yes
4. Are non-salmonid early life stages present or absent?	Present

OUTPUT

1. Unionized ammonia NH3 criteria (mgNH3/L)	
Acute:	0.070
Chronic:	0.007
2. Total ammonia nitrogen criteria (mgN/L):	
Acute:	21.725
Chronic:	2.167

Data adjustments were made to convert reported laboratory values from a Nitrogen basis to an Ammonia basis for un-ionized ammonia criteria. A Standard Methods 4500–NH3- G automated phenate method which reports ammonia as milligrams nitrogen/Liter was employed by the reporting laboratory. These results are used unchanged for comparison to total ammonia nitrogen acute and chronic criteria. In the case of un-ionized ammonia, i.e. ammonia gas, the reported milligrams nitrogen/Liter are divided by the ratio of elemental nitrogen and gaseous ammonia molecular weights, N : NH3 = 14.01/17.03 =
0.82 to obtain ammonia concentration. In this context a reported value of 1.0 mgN/L becomes 1.0/0.82 = 1.22 mgNH3/L.

Table 5. Reported data results maxima for temperature and pH with concurrent reported ammonia data values and ammonia criteria for Riley Slough.

Location	Reported Temperature and pH Data Result. Date of observation given below maxima.		Reported Data Result NH3 as N (mg/L) at given Temperature and pH	Ammonia Criterion Calculated via *Pelletier, 2010*			Un-ionized Ammonia Criterion Calculated via *Pelletier, 2010*	
				Acute Total Ammonia Nitrogen (mgN/L)	Chronic Total Ammonia Nitrogen (mgN/L)	Data Result as un-ionized (mg NH3/L)	Acute Unionized NH3 (mgNH3/L)	Chronic Unionized NH3 (mgNH3/L)
Riley Slough Upstream	Tmax oC 06/25/07	pH at Tmax						
	16.5	6.54	0.08	32.101	1.897	0.10	.041	0.002
	pHmax 2/18/09	ToC at pHmax						
	6.87	5.3	0.09	26.736	2.303	0.11	0.031	0.003
Riley Slough Down- stream	Tmax oC 05/08/07	pH at Tmax						
	14.7	5.91	0.06	37.127	Calculates<0	0.07	0.010	Calculates<0
	pHmax 03/25/08	ToC at pHmax						
	6.92	6.8	0.10	25.752	2.264	0.12	0.037	0.003

Criteria values used in Table 6 are calculated using the drop menu selections of "no" and "absent" for input items 3 and 4, respectively. This is a departure from the analytic examination of data detailed above in that the channel is defined as non-salmonid/other fish species. A "spike" value for ammonia, 7.52 mg/L, was observed at the Drain Channel downstream site on December 26, 2007 and is included in Table 6.

Table 6. Reported data results maxima for Temperature and pH with concurrent reported ammonia data values and ammonia criteria for Drain Channel.

Location	Reported Temperature and pH Data Result Date of observation given below maxima.		Reported Data Result NH3 as N (mg/L) at given Temperature and pH	Ammonia Criterion Calculated via *Pelletier, 2010*			Data Result as un-ionized (mg NH3/L)	Un-ionized Ammonia Criterion Calculated via *Pelletier, 2010*	
				Acute Total Ammonia Nitrogen (mgN/L)	Chronic Total Ammonia Nitrogen (mgN/L)			Acute Unionized NH3 (mgNH3/L)	Chronic Unionized NH3 (mgNH3/L)
Drain Channel Upstream	Tmax oC 07/24/07	pH at Tmax							
	15.5	7.60	0.0015	17.032	3.730		0.002	0.231	0.051
	pHmax 02/18/09	ToC at pHmax							
	7.90	5.70	0.06	10.131	4.537		0.07	0.128	0.057
Drain Channel Down- stream	Tmax oC 07/09/07	pH at Tmax							
	18.1	6.65	0.43	30.547	1.685		0.52	0.057	0.003
	pHmax 09/27/07	ToC at pHmax							
	7.11	11.4	0.07	32.531	6.896		0.09	0.105	0.022
	Ammonia Spike: 12/26/07								
	pH	ToC							
	6.72	5.5	7.52	44.074	10.416		9.17	0.036	0.009

Table 7. Reported data results maxima for Temperature and pH with concurrent reported ammonia data values and ammonia criteria for Peoples Creek.

Location	Reported Temperature and pH Data Result Date of observation given below maxima.		Reported Data Result NH3 as N (mg/L) at given Temperature and pH	Ammonia Criterion Calculated via *Pelletier, 2010*			Un-ionized Ammonia Criterion Calculated via *Pelletier, 2010*	
				Acute Total Ammonia Nitrogen (mgN/L)	Chronic Total Ammonia Nitrogen (mgN/L)	Data Result as un-ionized (mg NH_3/L)	Acute Unionized NH_3 ($mgNH_3$/L)	Chronic Unionized NH3 (mgNH3/L)
Peoples Creek Upstream	Tmax oC 08/21/07	pH at Tmax						
	15.4	7.52	0.02	12.888	2.078	0.02	0.145	0.023
	pHmax 02/18/09	ToC at pHmax						
	7.91	4.8	0.05	6.643	1.652	0.06	0.080	0.020
Peoples Creek Down-stream	Tmax oC 07/09/07	pH at Tmax						
	16.2	7.30	0.0015	17.506	1.950	.002	0.126	0.014
	pHmax 07/22/08	ToC at pHmax						
	7.74	14.8	0.15	9.006	2.006	0.18	0.159	0.035

The sampling schema employed in this project was not of the rigor typically used in NPDES situations, hence it did not provide the number of daily samples needed for thirty-day chronic averaging nor was the sampling term of sufficient length to provide a three-year average for acute exposure. It did, however, provide temperature and pH
values for calculation of criterion levels to determine if instantaneous thresholds had been reached at any point during the sampling term. This information can be useful in gauging the dynamics of these water channels through the term of the sampling campaign.

The data presented here suggests that most sampling locales may have concerns with chronic values for un-ionized ammonia. Since the „upstream‟ locales represent a point at which the studied channel reach receives discharge from an upstream source, it is readily apparent that the general watershed above the studied reach contributes to this chronic exceedance.

Graphs for ammonia content are presented as described earlier with the resultant data superimposed over the precipitation graph.

Fecal coliform

WAC 173-201A-200 (*ibid*) contains a reference table known as Table 200 that lists aquatic life criteria and recreational contact criteria for fresh waters within the state.

Table 200 (2)(b) provides definition of fecal coliform standards based on expected human contact with a water body. There are three levels of recreational contact defined in the statute: Extraordinary Primary Contact, Primary Contact and Secondary Contact.

1) Extraordinary primary contact means waters providing extraordinary protection against waterborne disease or that serve as tributaries to extraordinary quality shellfish harvesting areas.

2) Primary contact recreation means activities where a person would have direct contact with water to the point of complete submergence including, but not limited to, skin diving, swimming, and water skiing.

3) Secondary contact recreation means activities where a person's water contact would be limited (e.g., wading or fishing) to the extent that bacterial infections of eyes, ears, respiratory or digestive systems, or urogenital areas would normally be avoided.

Secondary contact recreation context is chosen for this reporting; the standard for fecal coliform within this context is:

Fecal coliform organism levels must not exceed a geometric mean value of 200 colonies/100 mL, with not more than 10 percent of all samples (or any single sample when less than ten sample points exist) obtained for calculating the geometric mean value exceeding 400 colonies /100 mL.

There is a caveat within Table 200 regarding presentation of geometric mean data:

When averaging bacteria sample data for comparison to the geometric mean criteria, it is preferable to average by season and include five or more data collection events within each period. Averaging of data collected beyond a thirty-day period, or beyond a specific discharge event under investigation, is not permitted when such averaging would skew the data set so as to mask noncompliance periods. The period of averaging should not exceed twelve months, and should have sample collection dates well distributed throughout the reporting period.

The sample sequence used in this project does not handily avail itself to this protocol due to lack of data in certain channels caused by no-flow conditions and elongation of sampling interval at the end of the sequence as described previously. Table 6 contains fecal coliform results, seasonal geometric means and composite geometric means for the sample sites. The data is aligned on seasonal breaks bracketed around the no-flow/no data conditions experienced in Riley Slough.

Neither of the two sites on Riley Slough exceeded fecal coliform standards through the sampling sequence although there were two periods in the summers of 2007 and 2008 wherein no sampling occurred due to lack of flow in the channel. The

downstream drainage channel site had two instances of exceeding standards; once during winter, 2008 and again during summer, 2008. Both Peoples Creek sites exceeded standards during summer, 2007 and the downstream site exceeded standards during summer, 2008. This latter exceedance, based on a limited sample size of two, was the only instance of crossing the 400 cfu/100mL threshold among all sample sites.

Fecal coliform sample holding time limits (24 hours) were exceeded on May 13, 2008; July 22, 2008; and January 27, 2009. This situation arose from difficulties experienced with transshipment of the samples by courier to the laboratory. Qualifiers for these occurrences are noted in Appendices E, F and G. Examination of the fecal coliform graphics in Appendix A and data in Table 8 shows small differences in coliform counts for Riley Slough and Peoples Creek for the May 13, 2008 sampling; no sample was drawn for the drainage channel that day. There is a large difference in coliform counts for the drainage channel and Peoples Creek on July 22, 2008; no sample was drawn for Riley Slough that day. The sampling on January 27, 2009 shows a large difference in coliform counts for the drainage channel but similar counts for Riley Slough and Peoples Creek. It is unknown if this holding time exceedance had a material effect on the quality of samples submitted.

Table 8. Fecal coliform results, seasonal and composite geometric means.

		Riley Slough		Drainage Channel		Peoples Creek	
		Upstream	Downstream	Upstream	Downstream	Upstream	Downstream
		cfu/100mL	cfu/100mL	cfu/100mL	cfu/100mL	cfu/100mL	cfu/100mL
Sample Date							
3/13/2007		72	200	56	88	4	
3/29/2007		1	7	7	13	13	17
4/10/2007		16	36	20	40	140	68
4/24/2007		40	8	24	155	34	39
5/8/2007		888	34	26	12	35	14
5/22/2007		265	48	282	1000	171	163
6/12/2007		44	96	62	117	171	48
6/25/2007		576	520	8000	9200	900	1500
Spring 2007	**Geometric Mean**	**63.81**	**48.93**	**71.52**	**131.90**	**62.34**	**68.95**
7/9/2007				91	56	403	234
7/24/2007				76	324	242	222
8/14/2007				21	82	244	248
8/21/2007				33	146	340	548
9/11/2007				10	132	256	398
9/27/2007				5	72	132	110
Summer 2007	**Geometric Mean**	**No data**	**No data**	**24.92**	**112.84**	**254.76**	**260.02**
10/9/2007						66	18
10/23/2007				3	102	30	16
11/13/2007				156	610	40	52
11/28/2007		3	15	104	7	58	150
12/11/2007		21	7	156	1400	10	20
12/26/2007		15	18	64	34	25	48
Fall 2007	**Geometric Mean**	**9.81**	**12.36**	**54.62**	**115.70**	**32.36**	**35.94**
1/8/2008		10	5	100	90	48	74
1/22/2008		1	16	86	4400	33	26
2/11/2008		23	42			34	44
2/26/2008		14	15	19	106	7	12
3/11/2008		92	201	68	380	26	76
3/25/2008		13	20			128	10
Winter 2008	**Geometric Mean**	**12.52**	**24.23**	**57.74**	**355.38**	**32.84**	**30.29**
4/9/2008		36	56	64	20	14	35
4/23/2008		15	14	136	9	27	52
05/13/08		35*	100*			102*	172*
Spring 2008	**Geometric Mean**	**26.64**	**42.80**	**93.30**	**13.42**	**33.78**	**67.90**
07/22/08				72*	480*	84*	550*
09/24/08				55	100	78	400
Summer 2008	**Geometric Mean**	**No data**	**No data**	**62.93**	**219.09**	**80.94**	**469.04**
11/25/08		8	45	52	2	28	75
01/27/09		28*	12*	56*	880*	12*	27*
02/18/09		8	44	18	78	6	32
02/25/09		8	30	110	120	13	70
Fall 2008 - Winter 2009	**Geometric Mean**	**10.94**	**29.06**	**49.00**	**63.71**	**12.72**	**46.15**
*24 hr. holding time exceeded	**Geometric Mean (all)**	**22.46**	**31.16**	**52.19**	**115.58**	**51.03**	**71.64**

Conductivity

Conductivity, better termed specific conductance, is a measure of the ability of a substance (water, in this case) to conduct electric current. It provides an indication of dissolved products in the water although gives no assay for type or kind of product.

There are no fresh water standards extant, although there are references in the WACs for public water supplies (Chapters 246-290 and 246-291) for a limit of 700 *u*S/cm. Observed values for this sampling campaign were much lower than the limit for drinking water with a composite average of 131.6 *u*S/cm for all sites. The highest recorded value was 463.0 *u*S/cm for a sample taken from the drainage channel downstream location on December 26, 2007.

Dissolved Oxygen

Table 200, Section (1)(d), specifies a Lowest One-Day Minimum dissolved oxygen concentration of 8.0 mg/L for Aquatic Life Category, *Salmonid spawning, rearing, and migration.* This criterion is shown on the dissolved oxygen plots for the three channels as a horizontal line (although the drainage channel is not shown to be salmonid habitat). In general, the two sites on Peoples Creek and the upstream site of the drainage channel remained well oxygenated throughout the term of the sampling schedule. This is due to well-developed riffle flow upstream of each of the sample sites that provides adequate mixing.

Riley Slough's sites and the downstream sample site on the drainage channel were often challenged to maintain satisfactory oxygenation. These channels are moderately deep and tend to have a laminar, sluggish flow characteristic. Flow in Riley Slough was often sluggish or nonexistent; flow past the downstream sample point in the drainage channel was driven by a pumping plant that operated intermittently and was out of service for a part of summer, 2008.

pH

Section (1)(g) of Table 200 specifies pH as given below:

pH shall be within the range of 6.5 to 8.5 with a human-caused variation within the above range of less than 0.5 units.

As with dissolved oxygen, those channel locations with sluggish flow; Riley Slough upstream and downstream, and drainage channel downstream, were challenged to maintain pH within the standards-defined range. Staff did not delve into the "human- caused" aspect of the standard and its application to these channels.

Temperature

Instantaneous readings of water temperature were taken during on-site sampling activities and are reported here. The seven-day average of the daily maximum temperature (7- DADMax) establishes temperature criteria in a fresh water system and is given in Section (1)(c) of Table 200. It is defined in WAC 173-201A-200 as follows:

7-DADMax is the arithmetic average of seven consecutive measures of daily maximum temperatures. The 7-DADMax for any individual day is calculated by averaging that day's daily maximum temperature with the daily maximum temperatures of the three days prior and the three days after that date.

7-DADMax for Category *Salmonid spawning, rearing and migration* is 17.5 oC (63.5 oF). Temperature recording devices were not deployed at the observation sites during the term of the project, hence 7-DADMax data is not available. Data plots in Appendix A have an horizontal limit line at 17.5oC as reference and the instantaneous readings taken during site visits are plotted within the graphic field.

Turbidity

The Table 200 (1)(e) turbidity standard for Category *Salmonid spawning, rearing and migration* is:

Turbidity shall not exceed:
• 5 NTU over background when the background is 50 NTU or less;
or
• A 10 percent increase in turbidity when the background turbidity is more than 50 NTU.

Taken from the definitions within the WAC, background means

…the biological, chemical, and physical conditions of a water body, outside the area of influence of the discharge under consideration. Background sampling locations in an enforcement action would be up- gradient or outside the area of influence of the discharge. If several discharges to any water body exist, and enforcement action is being taken for possible violations to the standards, background sampling would be undertaken immediately up-gradient from each discharge.

Since the purpose of this field work was not pursuant to enforcement action, we simply take the upstream site in each channel as background to the downstream site in each channel. None of the upstream, i. e. background, sites had turbidity values greater than
50 NTU; downstream sites for Riley Slough and Peoples Creek typically maintained turbidity within standards and rarely gained five NTU over background.

The drainage channel downstream site, however, routinely exceeded the five NTU gain limit. Notations on the field sheets sometimes indicate ongoing flooding. For example, notations on the March 13, 2007 field sheet show that the adjacent Snoqualmie River water elevation is higher than sampling site water elevation and river water is entering the sampling site as backflow through the drainage pump discharge outlet. Other field sheet entries such as those on the January 22, 2008 note cessation of recent flooding and observe that trapped floodwaters from the river are likely continuing to drain from the

sampling site. Many of the field sheets have no entries that might explain the marked increase in turbidity at this location in comparison to the companion upstream site.

STATISTICAL ANALYSES: Paired *t* test:

A Paired Two Sample for Means *t* Test was conducted for each analyte within a channel reach. The null hypotheses for the tests are that the differences between means are zero; alternate hypotheses are that differences between means are not equal to zero and the null hypothesis is rejected if the absolute value of the calculated test statistic, |*t*|, is greater than the two-tail critical *t* value. Data and results from these analyses are shown in Appendix C. The results from the means tests are summarized in Table 9.

Table 9. Paired means testing significances for data results on channel reach sampling points.

Upstream *vs.* **D**ownstream	**Fecal Coliform**	**Ammonia**	**Ortho phosphate**	**Total Kjeldahl**	**Nitrate and Nitrite**	**Conductivity**	**Dissolved Oxygen**	**pH**	**Turbidity**	**Temperature**
Riley Slough Mean trend:	N U<D	N U>D	Y U>D	N U>D	Y U>D	N U>D	N U<D	N U<D	N U<D	N U<D
Drain Channel Mean trend:	Y U<D	Y U<D	Y U<D	Y U<D	Y U<D	Y U<D	Y U>D	Y U>D	Y U<D	Y U<D
Peoples Creek Mean trend:	N U<D	N U<D	N U<D	N U<D	N U<D	Y U<D	Y U>D	Y U>D	Y U<D	N U<D

This table shows that mean values for slightly more than half of analytes at upstream vs. downstream locations within the three channels are significantly different from one another, with the drain channel having significantly differing means for all analytes. A notation under each significance indicator shows the trend of the data, i.e., whether the means for an analyte are higher at the upstream or downstream collection point. For those cells showing no significant differences, the trend indication is statistically meaningless but presented here as an item of interest.

Samples representing a reach within respective channels were collected within a few minutes of one another and analyzed in like manner, inferring that differences between the two collection points are due to reach dynamics. As mentioned in the Introduction, this data was collected prior to the advent of digester effluent field applications. The ability to detect water quality changes in these channel reaches due to effluent

applications appears to be problematic since significant differences for many of the parameters of concern throughout the channel reaches are already extant.

Correlation

Each analytic result was examined by correlation analysis to determine a possible linear relationship between rainfall timing and results. Statistical analysis information is given in Appendices B, C and D for Riley Slough, drainage channel and Peoples Creek, respectively. Tables 10 through 12 present summaries of significance grouped by water channel and site location within the channel.

Table 10. Riley Slough Correlation Analysis for Rainfall Timing and Sample Acquisition.

		Significantly Correlated at *a*=0.05?									
	Rainfall Timing (Days)	**Fecal Coliform**	**Ammonia**	**Ortho phosphate**	**Total Kjeldahl N**	**Nitrate and Nitrite**	**Conductivity**	**Dissolved Oxygen**	**pH**	**Turbidity**	**Temperature**
Riley Slough Upstream	0	N	N	N	N	N	N	N	N	N	N
	1	N	Y	N	N	N	N	N	N	N	N
	2	N	N	N	N	N	N	N	N	N	N
	7	N	N	N	N	N	Y	Y	N	N	N
Riley Slough Downstream	0	Y	N	N	N	N	N	N	N	N	N
	1	N	N	N	N	N	N	N	N	N	N
	2	N	N	N	N	N	Y	N	N	N	N
	7	N	N	N	Y	N	Y	N	N	N	N

Table 11. Drainage Channel Correlation Analysis for Rainfall Timing and Sample Acquisition.

		Significantly Correlated at *a*=0.05?									
	Rainfall Timing (Days)	**Fecal**	**Ammonia**	**Ortho phosphate**	**Total**	**Nitrate and**	**Conductivity**	**Dissolved**	**pH**	**Turbidity**	**Temperature**
Drain Channel Upstream	0	Y	Y	N	Y	N	Y	N	N	Y	N
	1	N	N	Y	N	N	N	N	N	Y	N
	2	N	N	N	N	N	N	N	N	N	N
	7	N	N	N	N	N	N	N	N	N	N
Drain Channel Downstream	0	Y	N	N	N	Y	N	N	N	N	N
	1	N	N	Y	N	Y	Y	N	Y	N	N
	2	N	Y	Y	Y	N	Y	N	N	Y	N
	7	N	Y	Y	Y	N	N	N	N	N	N

Table 12. Peoples Creek Correlation Analysis for Rainfall Timing and Sample Acquisition.

		Significantly Correlated at *a*=0.05?									
	Rainfall Timing (Days)	**Fecal**	**Ammonia**	**Ortho phosphate**	**Total**	**Nitrate and**	**Conductivity**	**Dissolved**	**pH**	**Turbidity**	**Temperature**
Peoples Creek Upstream	0	Y	N	N	Y	Y	Y	N	N	Y	N
	1	N	N	Y	N	N	N	N	N	N	Y
	2	N	N	N	N	Y	N	N	N	N	N
	7	N	N	N	N	N	N	N	N	N	N
Peoples Creek Downstream	0	Y	N	N	Y	N	Y	N	N	Y	N
	1	N	N	Y	N	N	N	N	N	N	Y
	2	N	N	N	N	Y	N	N	N	N	N
	7	N	N	N	N	Y	N	N	N	N	N

Interpretive Statement (CONCLUSIONS)

Stagnant or slow moving waters at sample sites were found to depart from extant fresh water aquatic life criteria.

The paired-sample two means *t* test comparing data means for upstream and downstream locations within individual channels often indicated significant differences for parametric data. One particular channel, an in-field drainage channel, showed significant differences for all measured parameters. These differences are identified prior to application of pre- digestion and post-digestion effluent from a community anaerobic digester and indicate difficulties which might arise from using channel reach sites to discern water quality differences imparted by effluent species.

Examination of precipitation timing during or prior to a sampling foray for an effect on data results did not show clearly defined effects. Significant correlations for samples taken within 0, 24 and 48 hours after a precipitation event were typically found at those sites in the three channels characterized by active stream flow. Sites within the group of channels with sluggish or intermittent flow sometimes showed correlation to precipitation occurring earlier, i.e. 48 to 168 hours, prior to sampling.

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Appendices

Appendix A Data Plots

Appendix B
Riley Slough site data, correlation statistics, means differences *t* test

Appendix C
Drainage channel site data, correlation statistics, means differences *t* test

Appendix D
Peoples Creek site data, correlation statistics, means differences *t* test

Appendix E
Riley Slough data arranged Ecology EIM format

Appendix F
Drainage channel data arranged Ecology EIM format

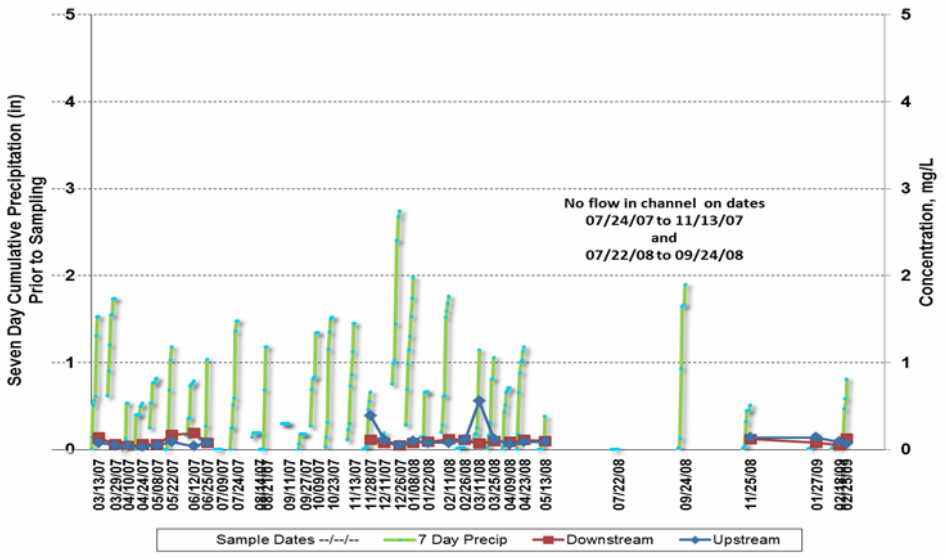
Appendix G
Peoples Creek data arranged Ecology EIM format

Appendix A

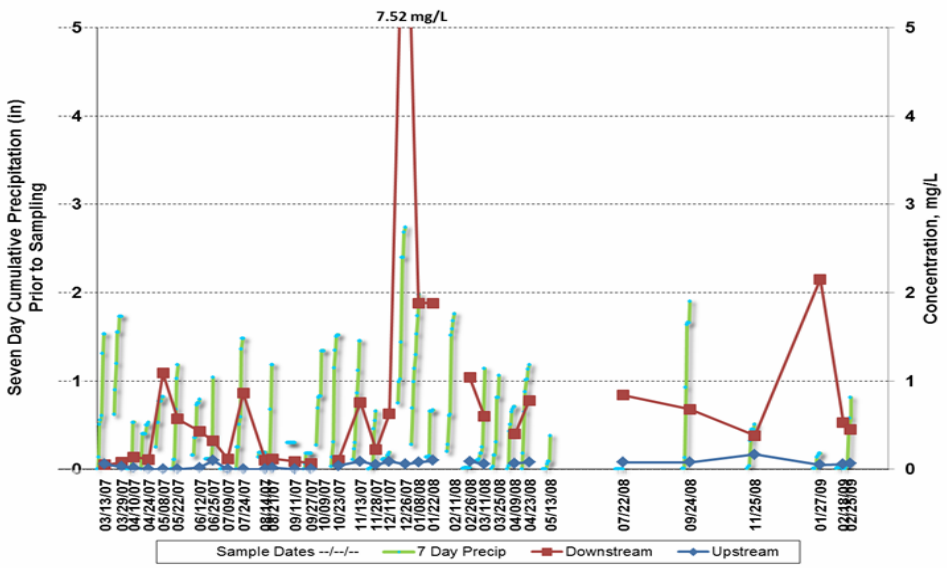
Data Plots
for

Ammonia
Fecal coliform
Orthophosphate
Total Kjeldahl Nitrogen
Total Nitrate and Nitrite
Nitrogen
Conductivity
Dissolved Oxygen
pH
Temperature
Turbidity

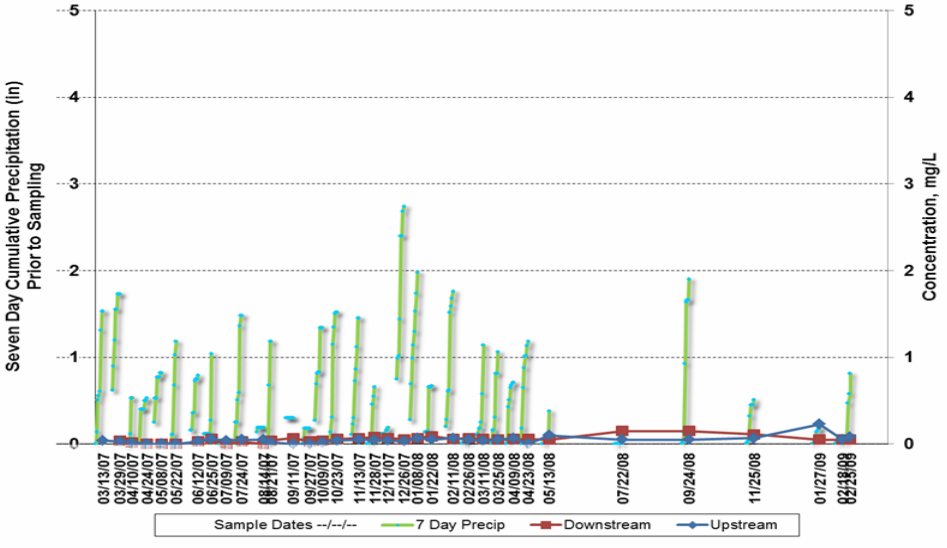
Ammonia in Riley Slough

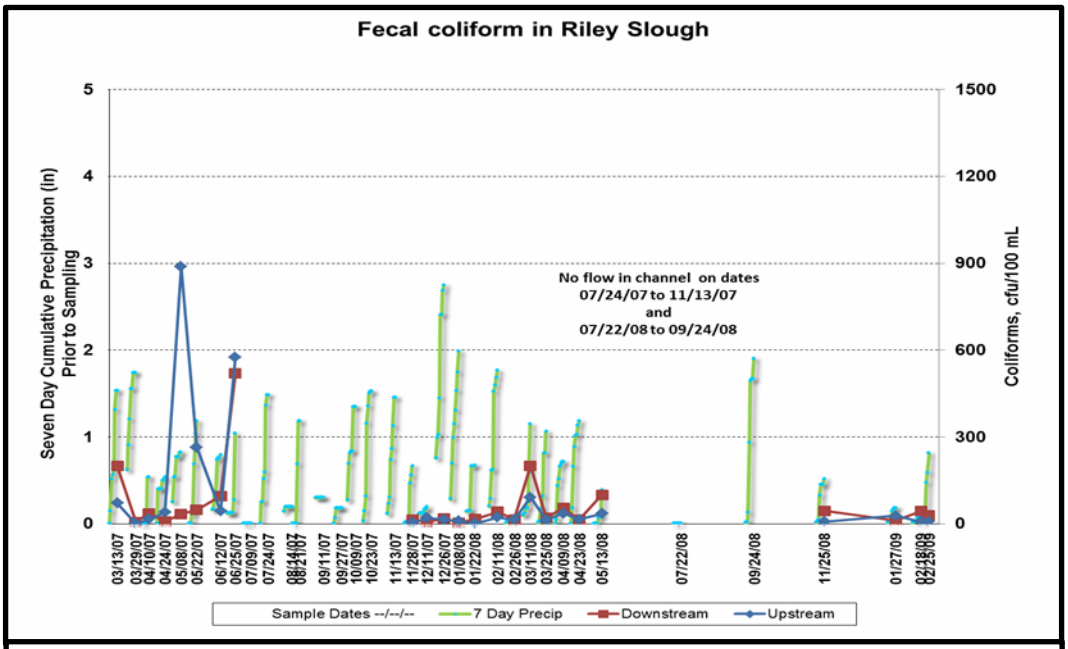


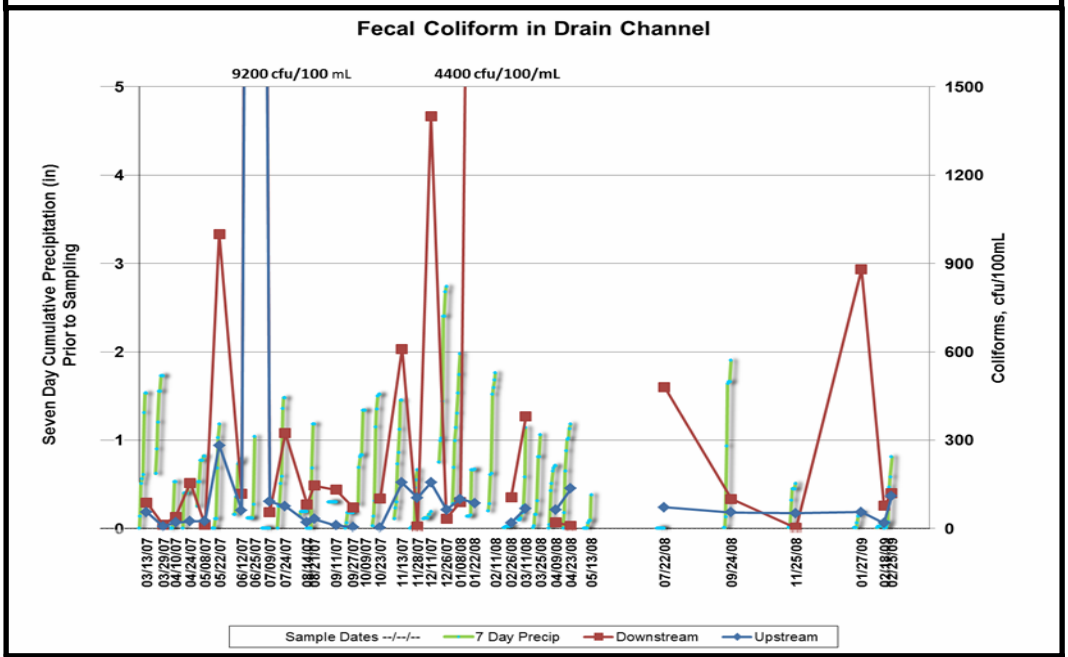
Ammonia in Drain Channel

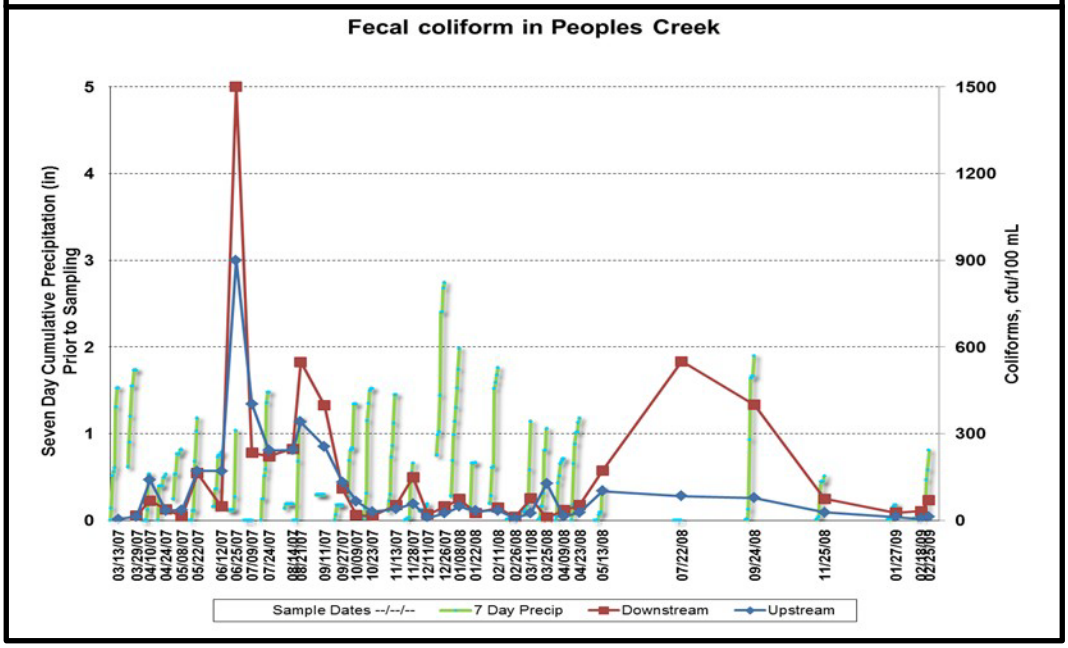


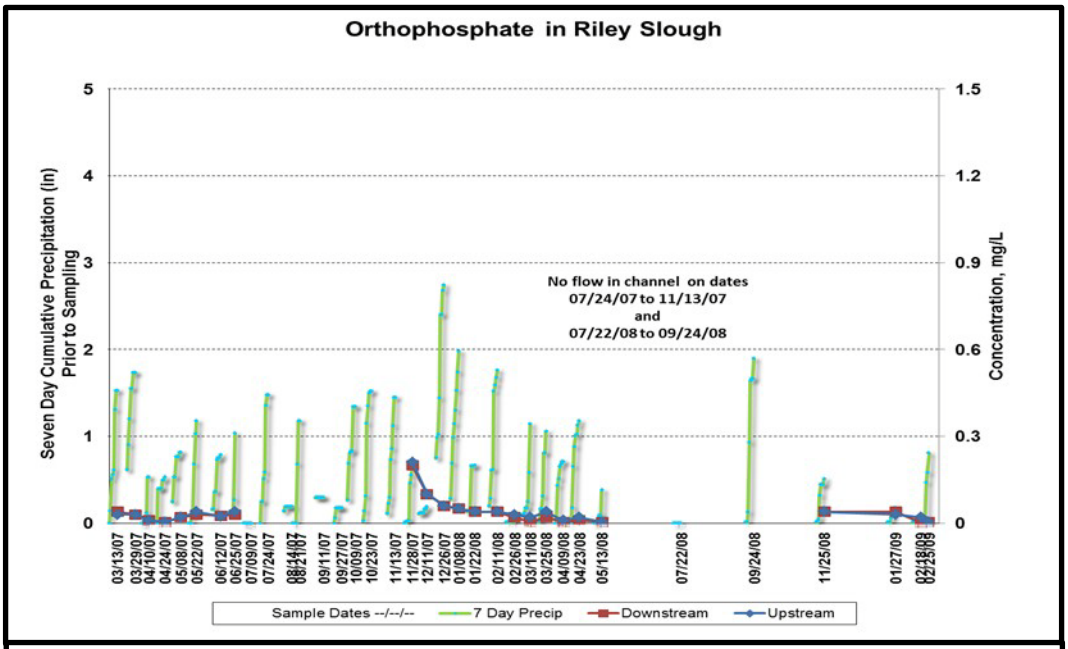
Ammonia in Peoples Creek

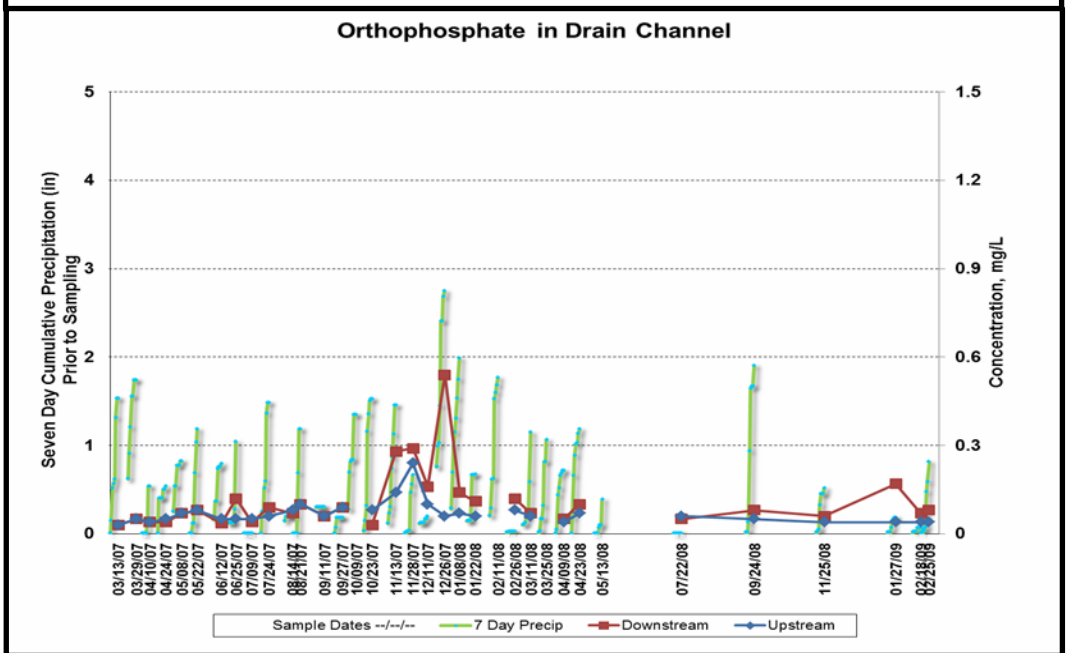


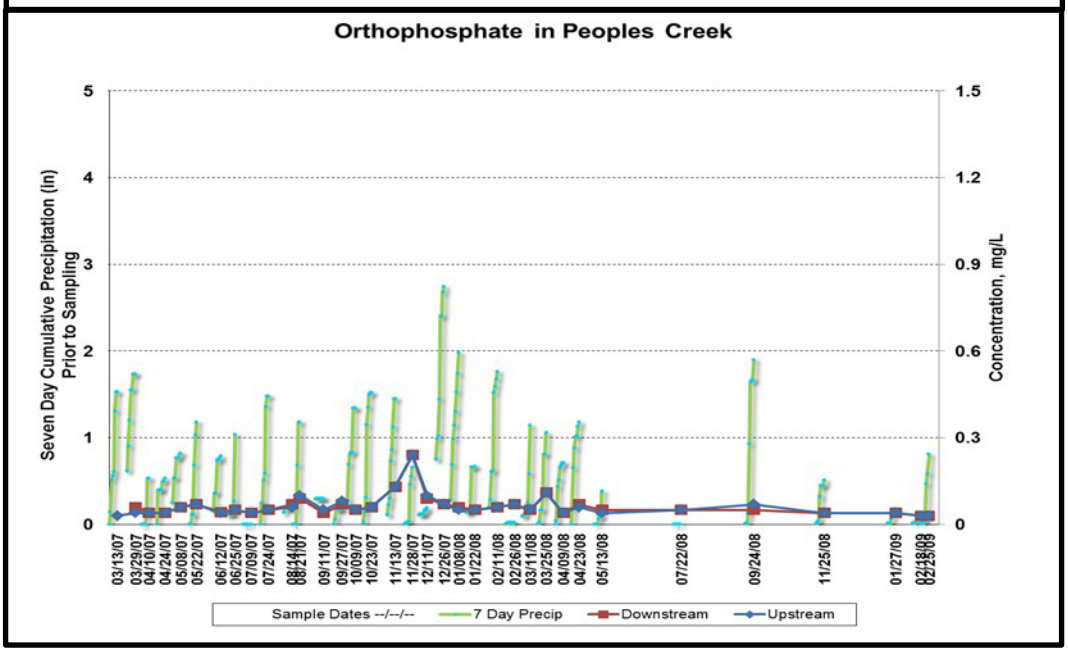

Fecal coliform in Riley Slough
Seven Day Cumulative Precipitation (in) Prior to Sampling
Coliforms, cfu/100 mL
No flow in channel on dates
07/24/07 to 11/13/07
and
07/22/08 to 09/24/08
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream

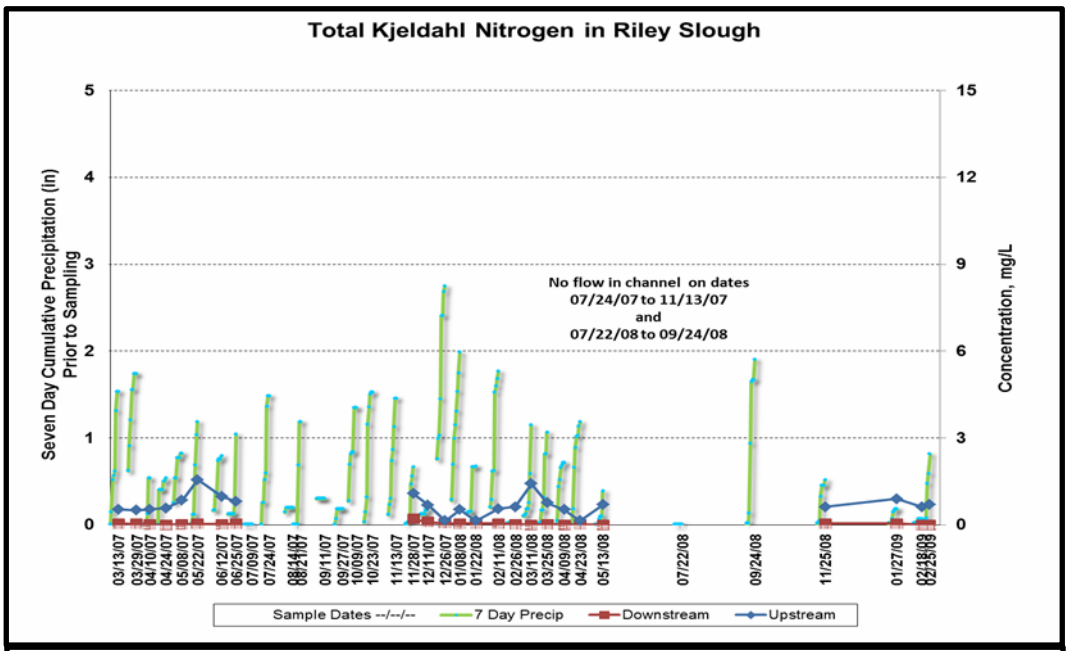

Fecal Coliform in Drain Channel
9200 cfu/100 mL
4400 cfu/100/mL
Seven Day Cumulative Precipitation (in) Prior to Sampling
Coliforms, cfu/100mL
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream

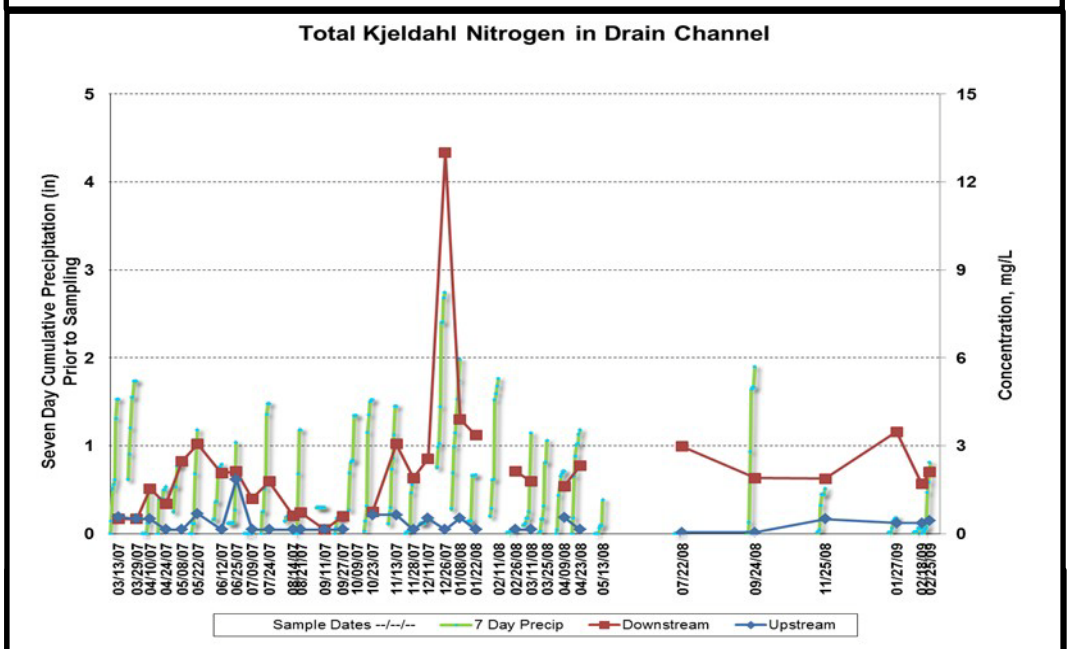

Fecal coliform in Peoples Creek
Seven Day Cumulative Precipitation (in) Prior to Sampling
Coliforms, cfu/100 mL
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream

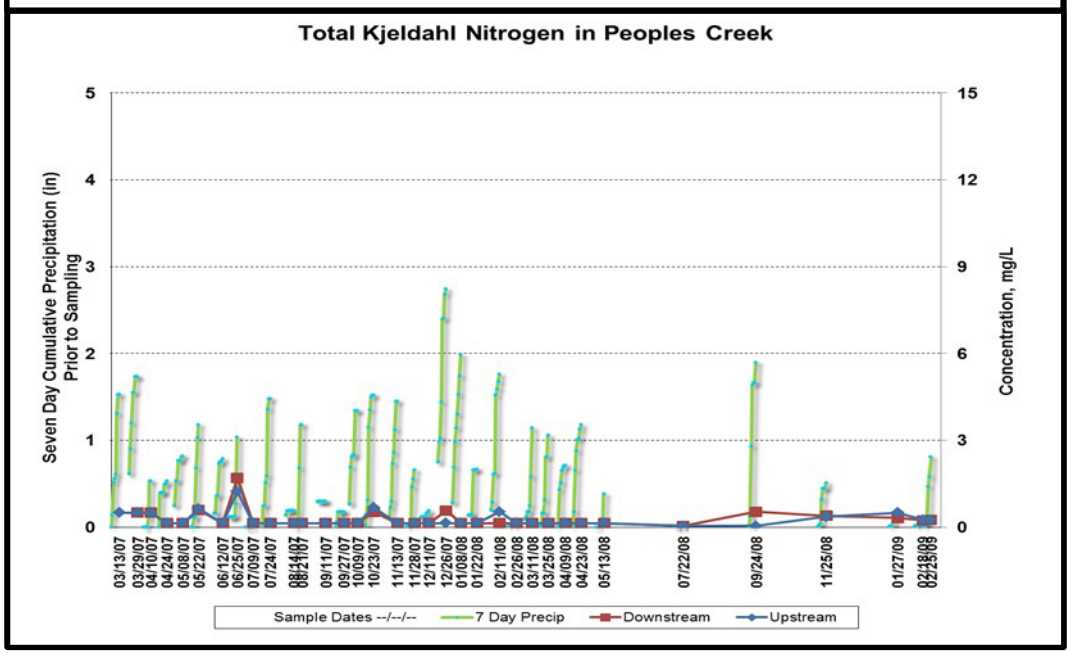

Orthophosphate in Riley Slough
Seven Day Cumulative Precipitation (in) Prior to Sampling
Concentration, mg/L
No flow in channel on dates
07/24/07 to 11/13/07
and
07/22/08 to 09/24/08
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream

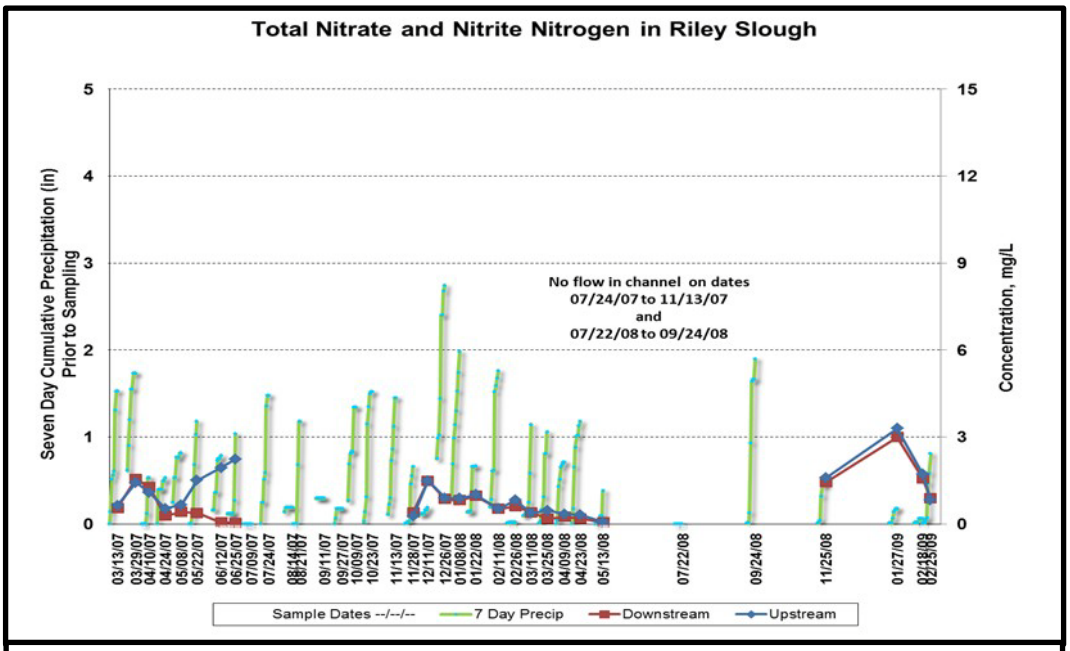

Orthophosphate in Drain Channel
Seven Day Cumulative Precipitation (in) Prior to Sampling
Concentration, mg/L
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream

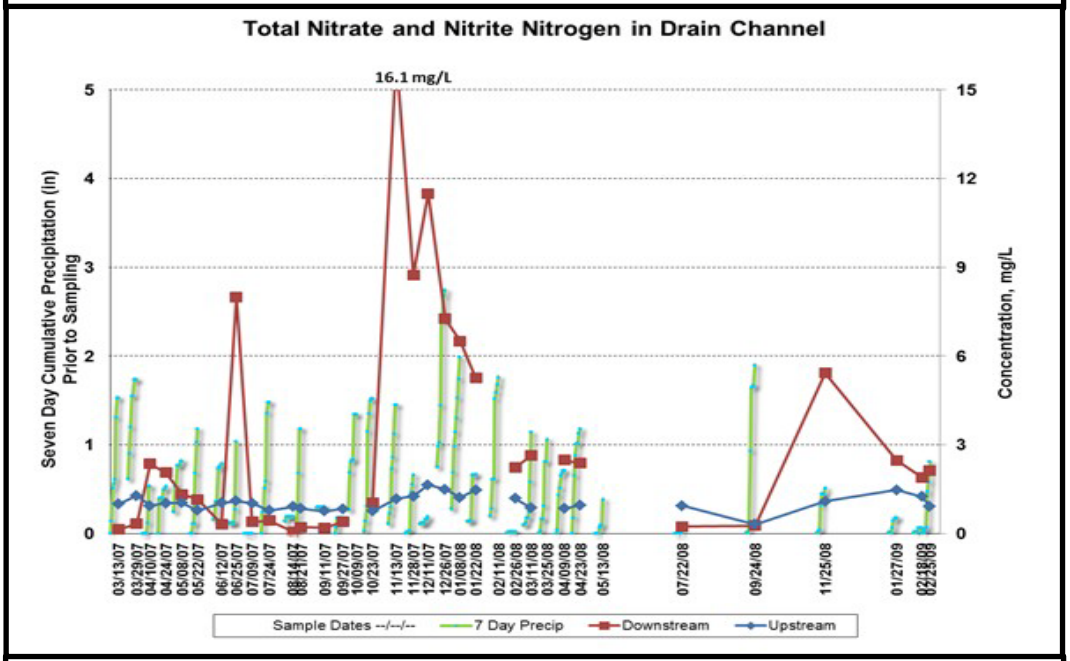

Orthophosphate in Peoples Creek
Seven Day Cumulative Precipitation (in) Prior to Sampling
Concentration, mg/L
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream

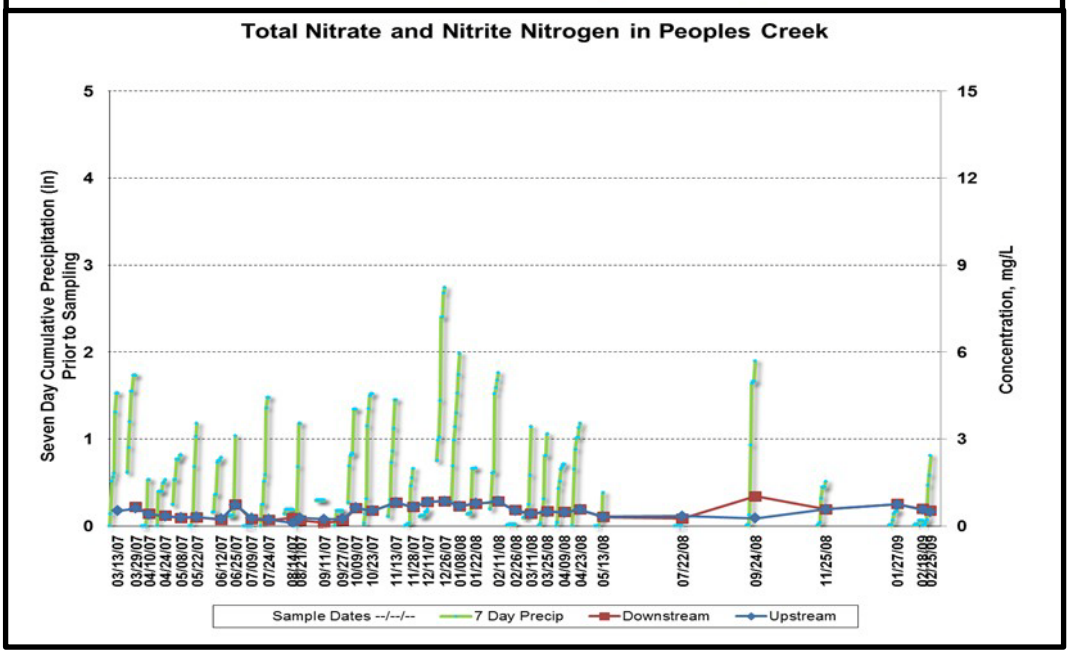

Total Kjeldahl Nitrogen in Riley Slough
Seven Day Cumulative Precipitation (in) Prior to Sampling
Concentration, mg/L
No flow in channel on dates
07/24/07 to 11/13/07
and
07/22/08 to 09/24/08
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream

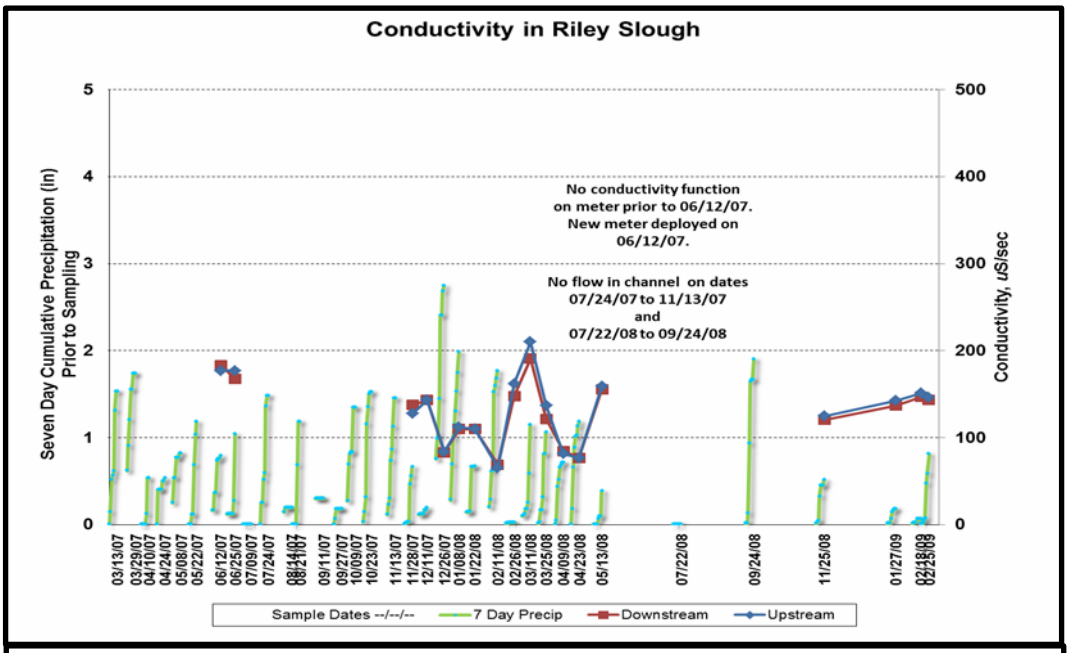

Total Kjeldahl Nitrogen in Drain Channel
Seven Day Cumulative Precipitation (in) Prior to Sampling
Concentration, mg/L
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream

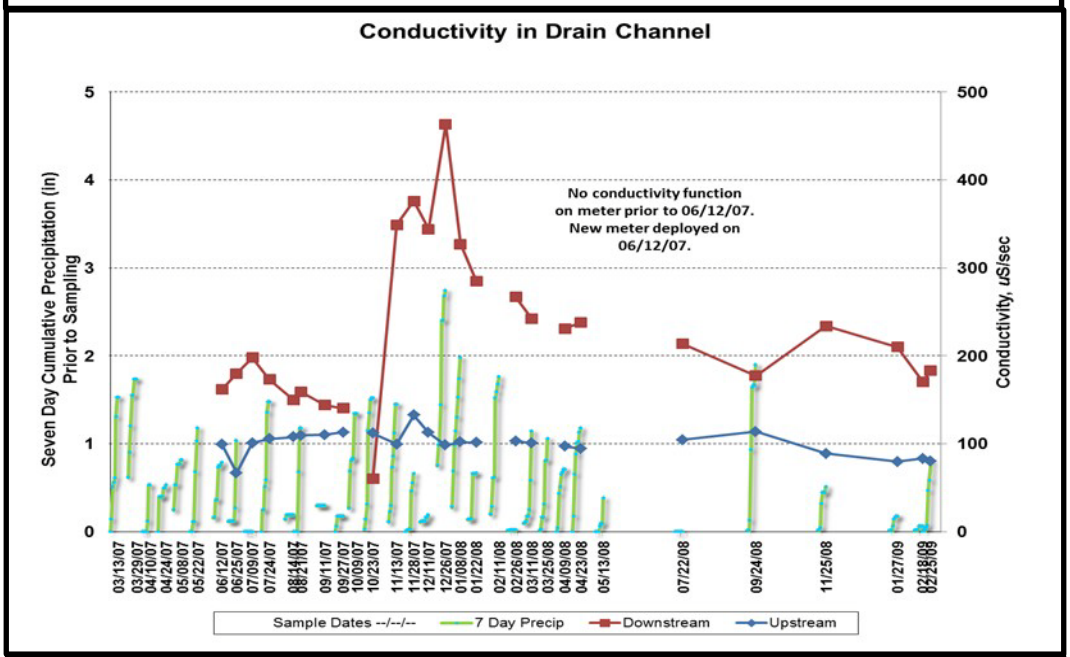

Total Kjeldahl Nitrogen in Peoples Creek
Seven Day Cumulative Precipitation (in) Prior to Sampling
Concentration, mg/L
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream

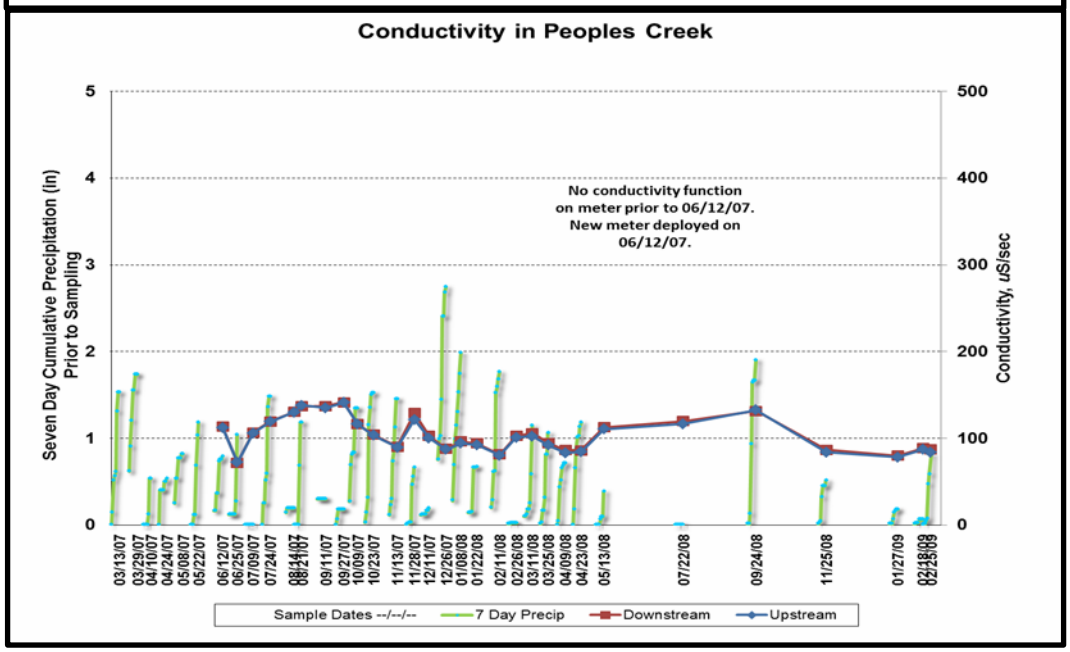

Total Nitrate and Nitrite Nitrogen in Riley Slough
Seven Day Cumulative Precipitation (in) Prior to Sampling
Concentration, mg/L
No flow in channel on dates
07/24/07 to 11/13/07
and
07/22/08 to 09/24/08
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream

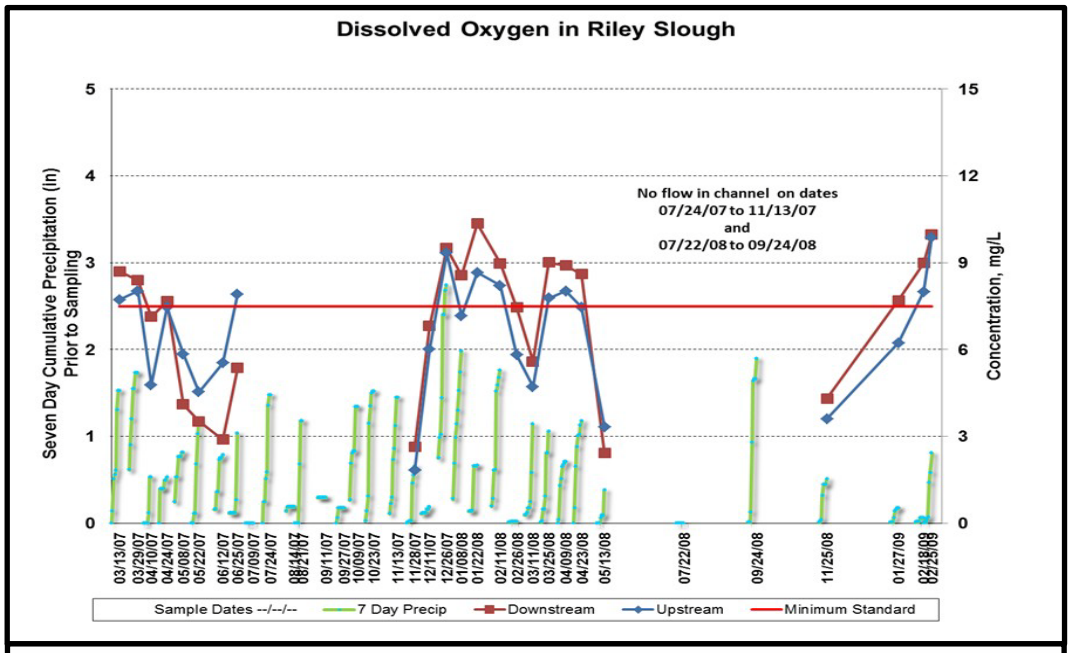

Total Nitrate and Nitrite Nitrogen in Drain Channel
16.1 mg/L
Seven Day Cumulative Precipitation (in) Prior to Sampling
Concentration, mg/L
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream

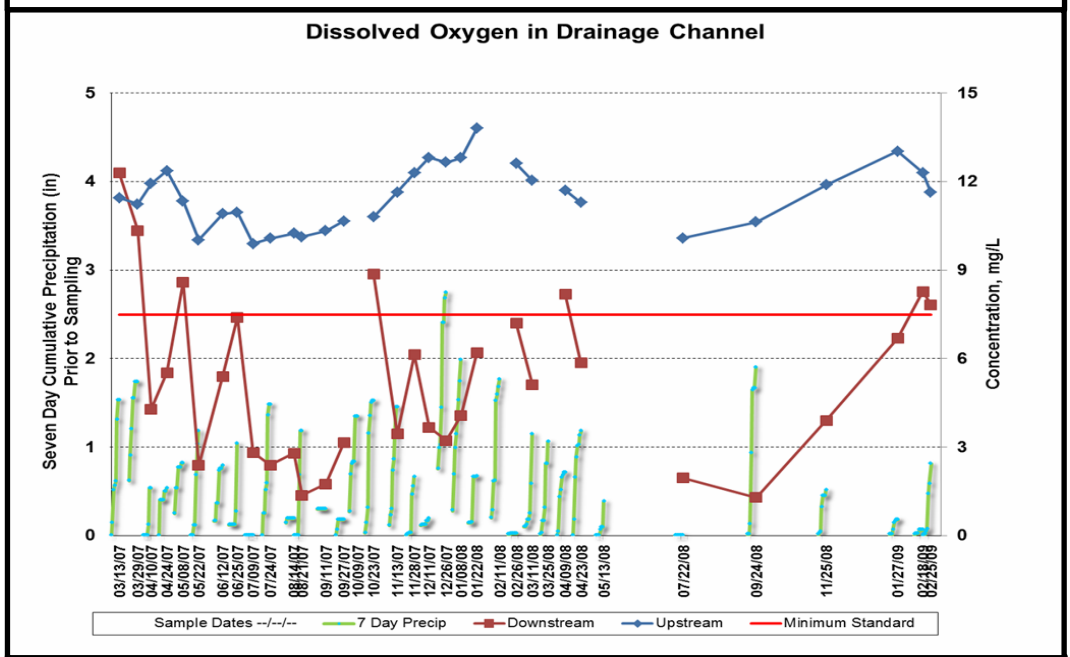

Total Nitrate and Nitrite Nitrogen in Peoples Creek
Seven Day Cumulative Precipitation (in) Prior to Sampling
Concentration, mg/L
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream

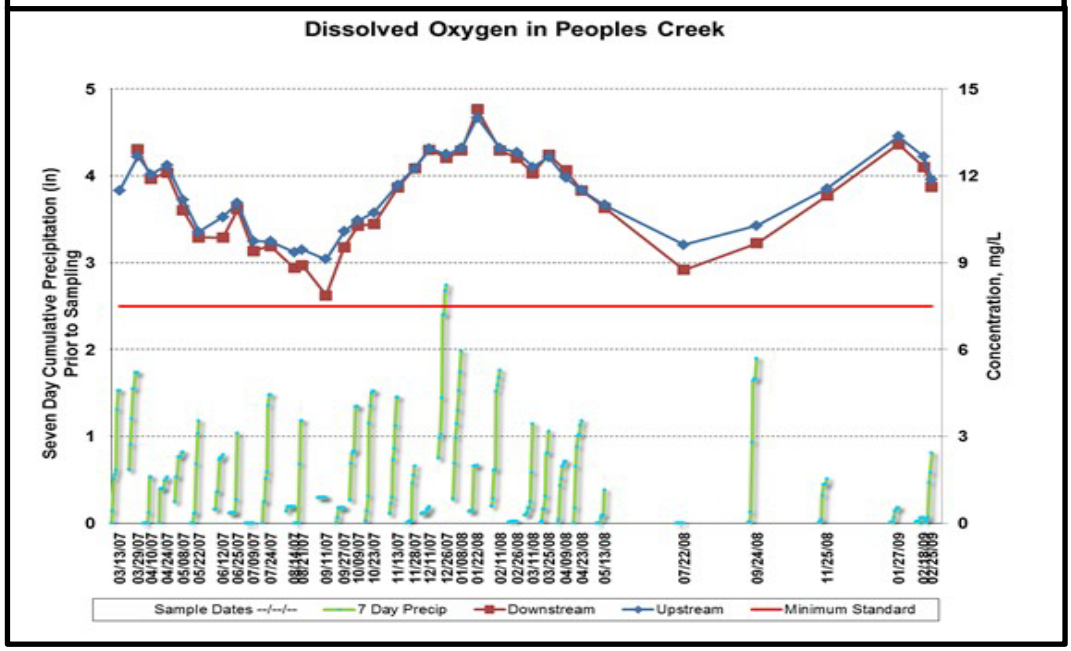

Conductivity in Riley Slough
Seven Day Cumulative Precipitation (in) Prior to Sampling
Conductivity, uS/sec
No conductivity function on meter prior to 06/12/07. New meter deployed on 06/12/07.
No flow in channel on dates 07/24/07 to 11/13/07 and 07/22/08 to 09/24/08
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream

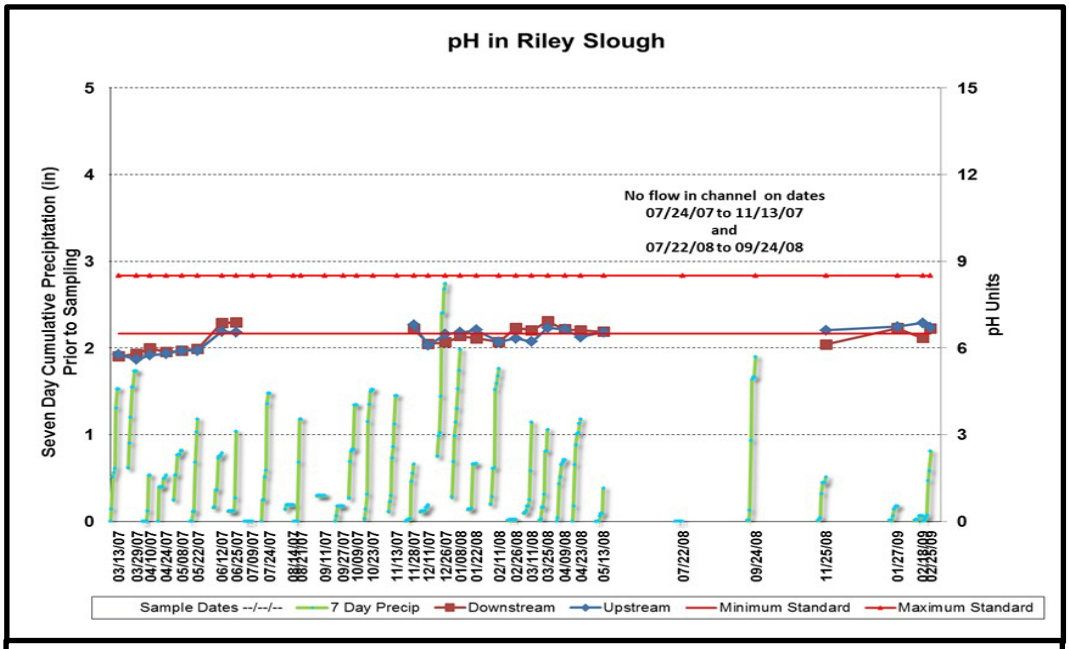

Conductivity in Drain Channel
Seven Day Cumulative Precipitation (in) Prior to Sampling
Conductivity, uS/sec
No conductivity function on meter prior to 06/12/07. New meter deployed on 06/12/07.
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream

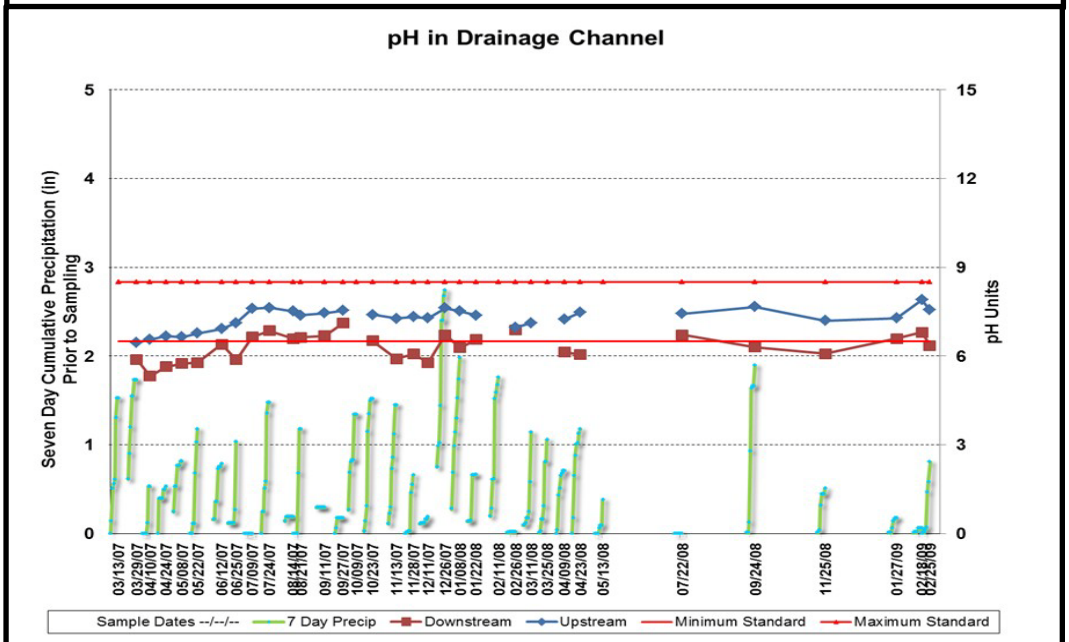

Conductivity in Peoples Creek
Seven Day Cumulative Precipitation (in) Prior to Sampling
Conductivity, uS/sec
No conductivity function on meter prior to 06/12/07. New meter deployed on 06/12/07.
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream

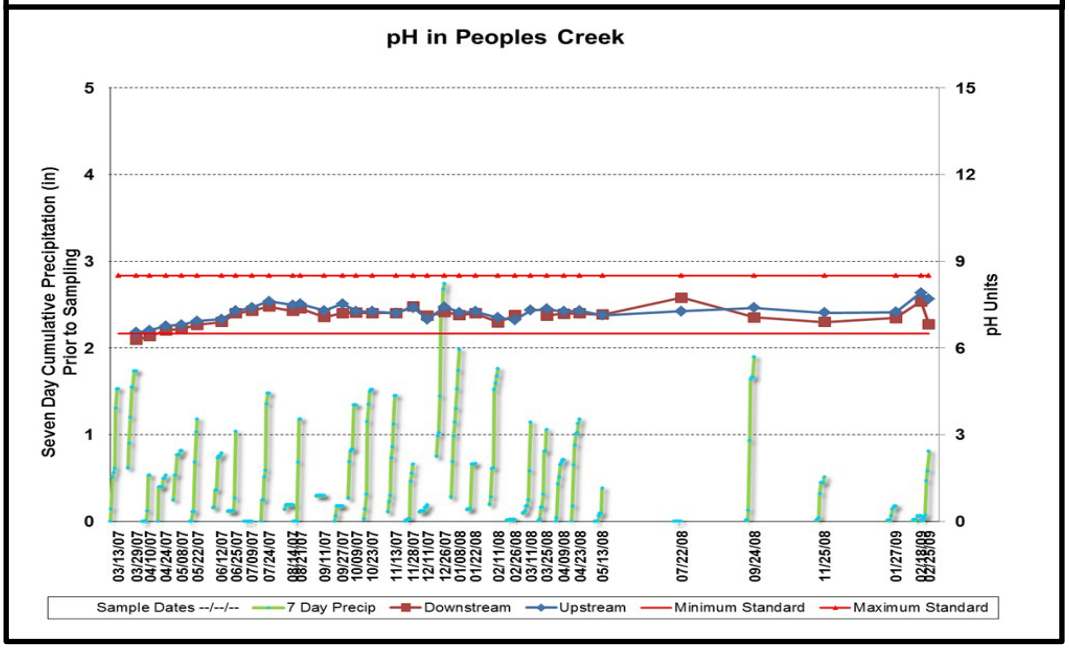

Dissolved Oxygen in Riley Slough
No flow in channel on dates
07/24/07 to 11/13/07
and
07/22/08 to 09/24/08
Seven Day Cumulative Precipitation (in) Prior to Sampling
Concentration, mg/L
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream
Minimum Standard

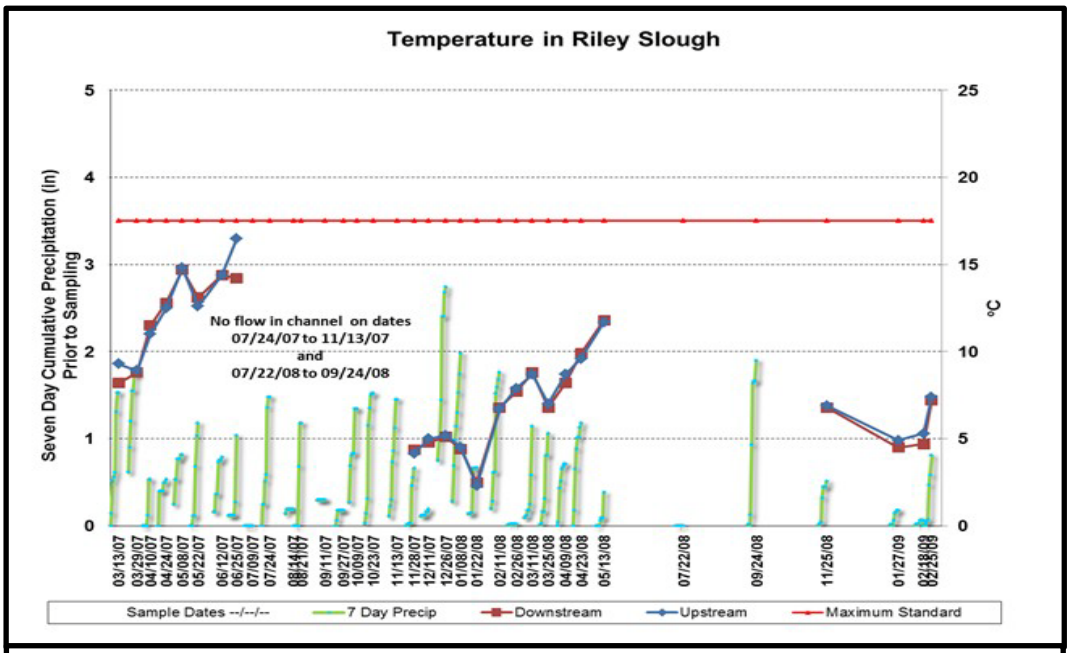

Dissolved Oxygen in Drainage Channel
Seven Day Cumulative Precipitation (in) Prior to Sampling
Concentration, mg/L
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream
Minimum Standard

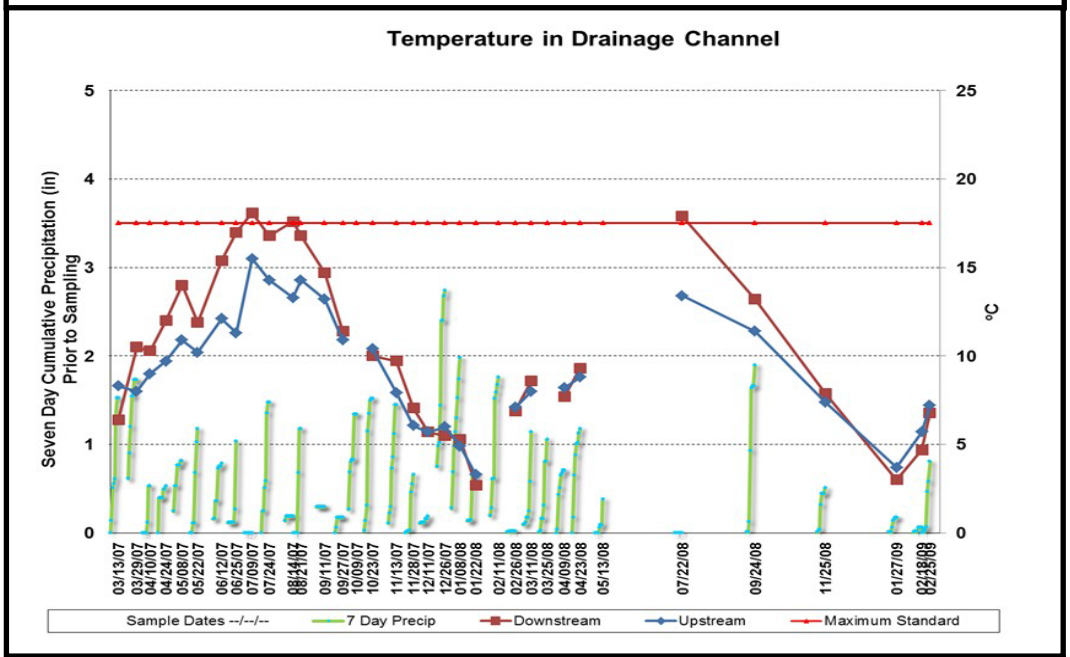

Dissolved Oxygen in Peoples Creek
Seven Day Cumulative Precipitation (in) Prior to Sampling
Concentration, mg/L
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream
Minimum Standard

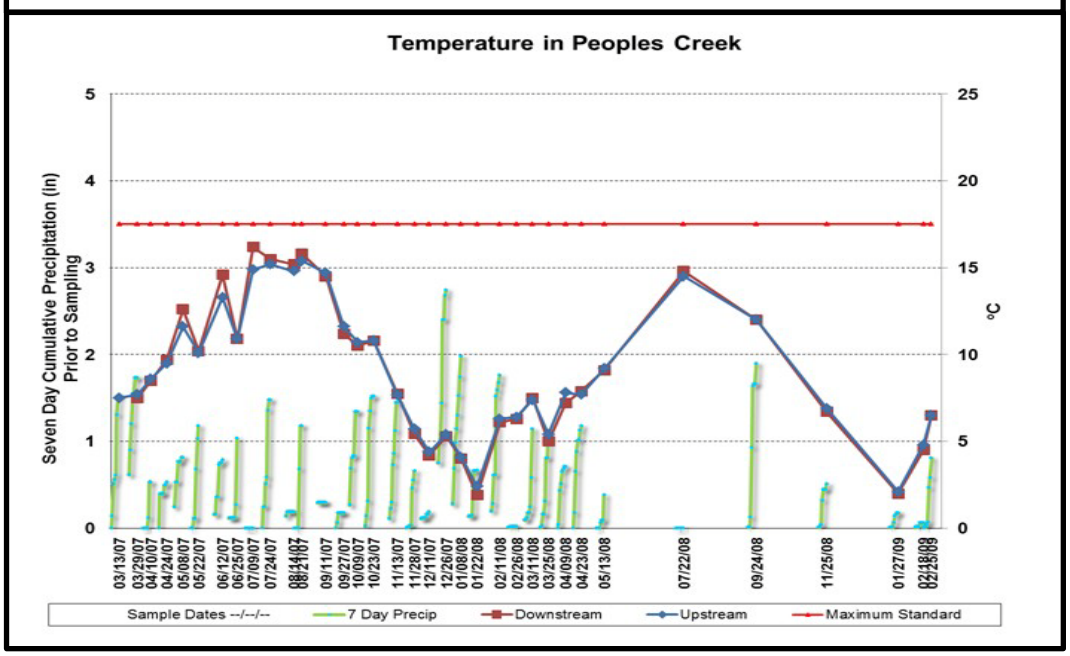

pH in Riley Slough
No flow in channel on dates
07/24/07 to 11/13/07
and
07/22/08 to 09/24/08
Seven Day Cumulative Precipitation (in)
Prior to Sampling
pH Units
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream
Minimum Standard
Maximum Standard


pH in Drainage Channel
Seven Day Cumulative Precipitation (in)
Prior to Sampling
pH Units
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream
Minimum Standard
Maximum Standard

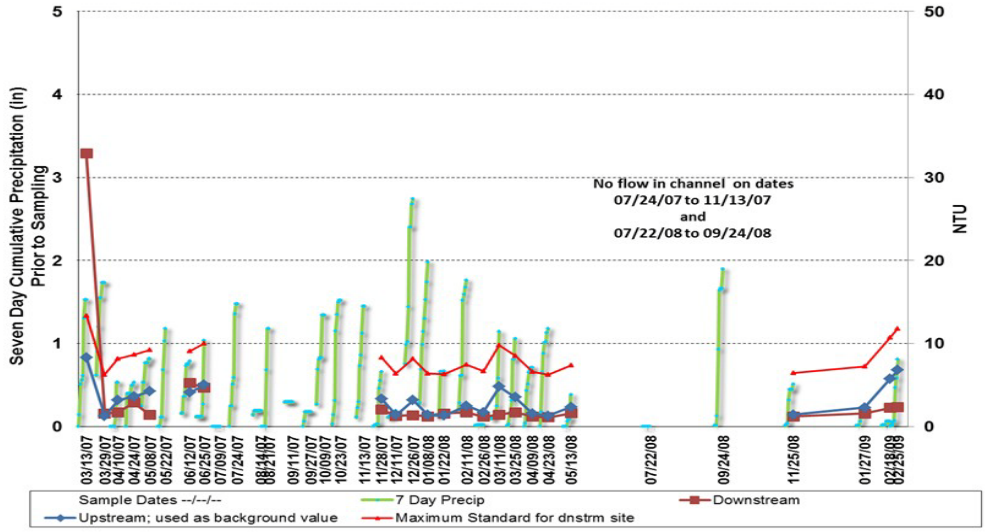

pH in Peoples Creek
Seven Day Cumulative Precipitation (in)
Prior to Sampling
pH Units
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream
Minimum Standard
Maximum Standard


Temperature in Riley Slough
Seven Day Cumulative Precipitation (in) Prior to Sampling
°C
No flow in channel on dates
07/24/07 to 11/13/07
and
07/22/08 to 09/24/08
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream
Maximum Standard

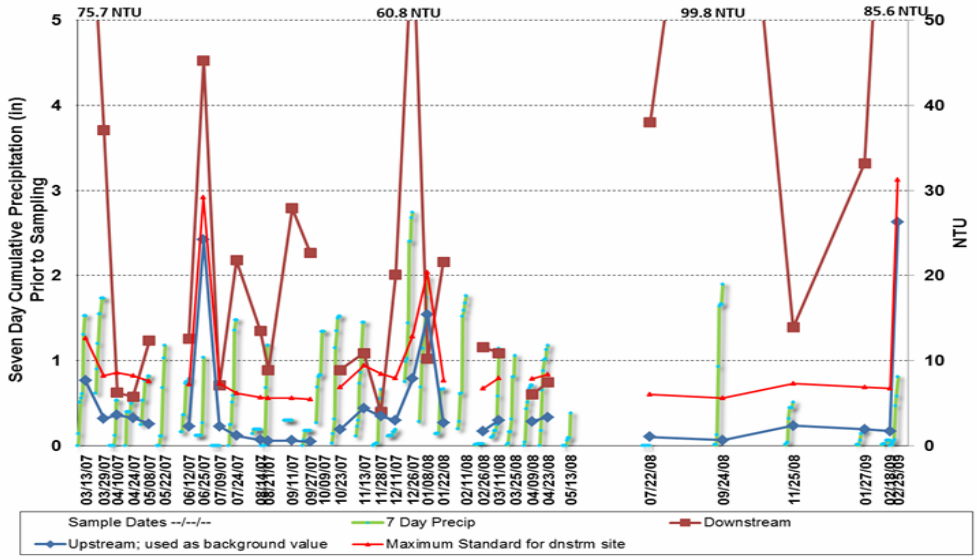

Temperature in Drainage Channel
Seven Day Cumulative Precipitation (in) Prior to Sampling
°C
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream
Maximum Standard


Temperature in Peoples Creek
Seven Day Cumulative Precipitation (in) Prior to Sampling
°C
Sample Dates --/--/--
7 Day Precip
Downstream
Upstream
Maximum Standard

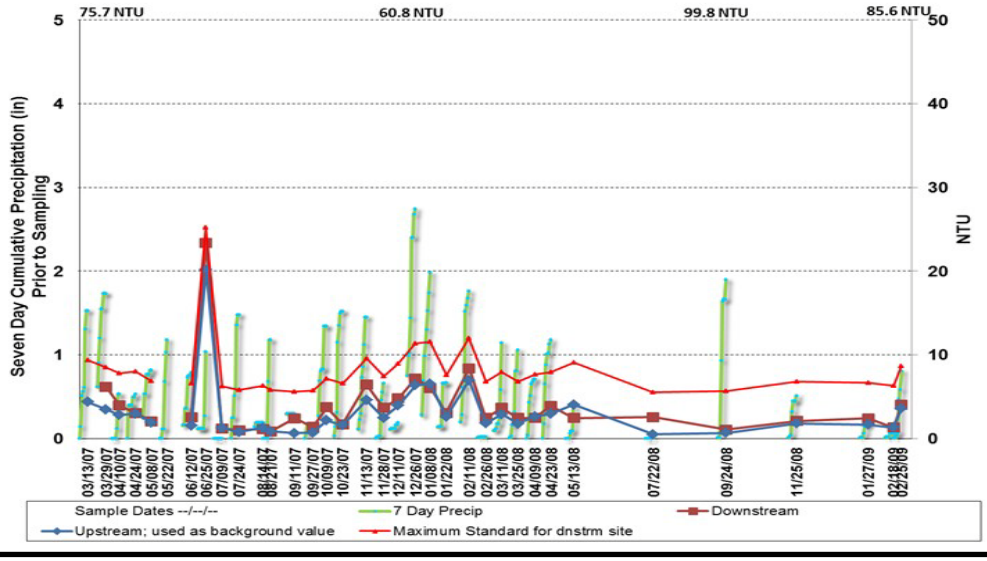
Turbidity in Riley Slough



Turbidity in Drainage Channel



Turbidity in Peoples Creek



Water Quality Monitoring – Surface Water Run-off plot evaluations –In 2010 and early 2011 we conducted a series of surface water quality runoff plot evaluations. AD and non-AD manure were applied to grass plots with a slope, and plots were surrounded with edging-border to contain and direct runoff to a central collection point. Manures were applied just prior to anticipated heavy rainfall events. Plot dimensions were 5 ft wide and 15 ft in length (top to bottom). Manures were applied on the top 10 ft of each plot , allowing the bottom 5 to serve as a natural buffer strip., except in the 3 2011 evaluation where manures were applied to the top 14 feet and 1 foot served a natural buffer. Runoff water samples were assayed for total coliforms, fecal coliforms, and e-coli.

Manure application dates – June 1, 2010; October 25, 2010; November 19, 2010; and March 1, 2011.

Note: this part of the project proved to be the most difficult. Significant challenges were: 1) acquiring sloped land which could be divided in plots that drained or produced over-land runoff flow consistently at each rain event, 2) subsurface flow as a result of voles or moles (subsurface trails that carried storm event water), and 3) variation in subsurface soil that resulted in ground water upwelling through plots ("spring effect").

Interpretive statement - The findings on bacterial movement to surface water are summarized in table 13 with supporting chi-square statistics. The data suggests that the number of surface water run-off samples observed positive for total coliform bacteria are statistically significantly reduced when AD manure was applied compared to nonAD manure. The associations between number positive for fecal coliforms and for *E. coli* were not statistically significant.

Table 13. Summary of presence of bacteria in surface run-off water from manure amended grass plots.

Treatment	**Number Positive Samples (%)**				**Number Samples**
AD Manure		Total Coliforms	Fecal Coliforms	*E. coli*	
	Summer 2010	-	4 (31%)	2 (15%)	13
	Fall10-Spring11	27 (47%)	4 (7%)	25 (43%)	58
	March 2011	7 (41%)	3 (18%)	16 (94%)	17
	Combined	**34 (45%)**	**11 (12%)**	**43 (49%)**	**88**
NonAD Manure					
	Summer 2010	-	5 (50%)	3 (30%)	10
	Fall10-Spring11	35 (74%)	2 (4%)	17 (36%)	47
	March 2011	13 (87%)	0	12 (80%)	15
	Combined	**48 (77%)**	**7 (10%)**	**32 (44%)**	**72**
Control					
	Fall10-Spring11	19 (73%)	0	18 (69%)	26

Chi-Square Test for Heterogeneity or Independence
for Count = TotalColiforms ManureType

Collapsed on Series

TestColiforms	**Control**	**NonAD**	**AD Manure**	
Negative Obs	7	14	41	62
Expected	9.89	23.58	28.53	
Cell Chi-Sq	0.84	3.89	5.45	
Positive Obs	19	48	34	101
Expected	16.11	38.42	46.47	
Cell Chi-Sq	0.52	2.39	3.35	
	26	62	75	163

Overall Chi-Square 16.45
P-value 0.0025
Degrees of Freedom 4 (from non-collapsed table)

The associations between source type and Fecal coliforms or E.coli in the collapsed tables are not significant.

Chi-Square Test for Heterogeneity or Independence for Count = Fecal Coliforms ManureType

Test		Collapsed on Series Control	NonAD	AD Manure	
Negative	Obs	26	65	77	168
	Expected	23.48	65.03	79.48	
	Cell Chi-Sq	0.27	0.00	0.08	
Positive	Obs	0	7	11	18
	Expected	2.52	6.97	8.52	
	Cell Chi-Sq	2.52	0.00	0.72	
		26	72	88	186

Overall Chi-Square	3.59
P-value	0.7320
Degrees of Freedom	6 (from non-collapsed table)

Chi-Square Test for Heterogeneity or Independence for Count = E.coli ManureType

Test		Collapsed on Series Control	NonAD	AD Manure	
Negative	Obs	8	40	45	93
	Expected	13.00	36.00	44.00	
	Cell Chi-Sq	1.92	0.44	0.02	
Positive	Obs	18	32	43	93
	Expected	13.00	36.00	44.00	
	Cell Chi-Sq	1.92	0.44	0.02	
		26	72	88	186

Overall Chi-Square	4.78
P-value	0.5723
Degrees of Freedom	6 (from non-collapsed table)

2) On-Farm Monitoring of Pathogens

Samples were collected on a monthly basis from 6 farms for 18 months prior to the start-up of the community anaerobic digester. Results are summarized in tables 14 – 18 and graphs 25 – 32.

Interpretive statement – Even though Campylobacter is a pathogen for humans, its prevalence (> 65 % of samples) in bovine feces is consistent with it being bovine commensal enteric flora on dairy farms. The prevalence of Listeria on the farms was low with < 15% of samples being positive. The prevalence of *Mycobacterium avium* subspecies *paratuberculosis* (*M. paratuberculosis* or MAP) ranges from 23.5 to 100% across farms and is consistent with data collected via blood sampling in cooperation with the Washington State Department of Agriculture Johnes surveillance testing (see later in report). The prevalence of Salmonella ranged from 0 to 72% across farms with it being essentially an "endemic" organism on farm 3. The concentration of generic *E. coli* (GEC) and fecal Enterococcus observed on the farms over time was in the range of expected values and was statistically different between farms for both organisms. Farms 3 and 6 were evaluated for GEC and Enterococcus before and after the AD started up (before and after December 2008). Farm 3 contributed manure to the AD and farm 6 was intending to pump manure to the AD but needed to make additional enhancements to their manure handling system to provide a high quality source of manure that was not sand laden. When concentrations of GEC and Enterococcus in on-farm fecal samples were compared pre- and post AD start-up between these two farms, no differences were observed.

Table 14 Presence-absence of bacteria in manure from collaborator farms.

	Number of on-farm samples with one or more test positive replicates				
Farm	Campylobacter	*E. coli* O157:H7	Listeria	*Mycobacterium paratuberculosis*	Salmonella
1	11/17 (65%)	0/17 (0%)	0/16(0%)	4/17 (24%)	2/17 (12%)
2	13/17 (76%)	1/17 (6%)	1/16(6%)	4/17 (24%)	1/17 (6%)
3	22/29 (76%)	0/28 (0%)	4/28(14%)	19/29 (66%)	21/29 (72%)
4	4/4 (100%)	1/ 4 (25%)	0/4 (0%)	4/4 (100%)	0/4 (0%)
5	17/17 (100%)	1/17 (6%)	1/16(6%)	7/17 (41%)	6/17 (35%)
6	22/26 (85%)	0/25 (0%)	2/25(8%)	17/26 (65%)	2/26 (8%)
Overal1	89/110 (81%)	3/108(3%)	8/105 (8%)	55/110 (50%)	32/110(29%)

Kruskal-Wallis One-Way Nonparametric AOV for LogGEC by Farm

Farm	Mean Rank	Sample Size
1	70.3	16
2	61.7	16
3	28.1	28
4	53.7	3
5	83.0	16
6	42.9	25
Total	52.5	104

Kruskal-Wallis Statistic 44.3921
P-Value, Using Chi-Squared Approximation 0.0000

Parametric AOV Applied to Ranks

Source	DF	SS	MS	F	P
Between	5	40186.0	8037.21	14.85	0.0000
Within	98	53055.0	541.38		
Total	103	93241.0			

Total number of values that were tied 31
Max. diff. allowed between ties 0.00001

Kruskal-Wallis All-Pairwise Comparisons Test of LogGEC by Farm

Farm	Mean	Homogeneous Groups
5	83.000	A
1	70.250	AB
2	61.656	AB
4	53.667	ABC
6	42.900	BC
3	28.143	C

Alpha 0.05
Critical Z Value 2.935 Critical Value for Comparison 24.364 TO 55.708
There are 3 groups (A, B, etc.) in which the means
are not significantly different from one another.

Kruskal-Wallis One-Way Nonparametric AOV for LogEnt by Farm

Farm	Mean Rank	Sample Size
1	48.9	14
2	45.2	13
3	44.5	23
4	14.0	1
5	58.2	13
6	28.0	21
Total	43.0	85

Kruskal-Wallis Statistic 14.9999
P-Value, Using Chi-Squared Approximation 0.0104

Parametric AOV Applied to Ranks

Source	DF	SS	MS	F	P
Between	5	9136.1	1827.22	3.43	0.0074
Within	79	42026.4	531.98		
Total	84	51162.5			

Total number of values that were tied 25
Max. diff. allowed between ties 0.00001

Kruskal-Wallis All-Pairwise Comparisons Test of LogEnt by Farm

Farm	Mean	1	2	3	4	5
1	48.857					
2	45.231	3.626				
3	44.543	4.314	0.687			
4	14.000	34.857	31.231	30.543		
5	58.154	9.297	12.923	13.610	44.154	
6	28.024	20.833	17.207	16.520	14.024	30.130*

Alpha 0.05
Critical Z Value 2.935 Critical Value for Comparison 21.865 TO 75.179
The homogeneous group format can't be used
because of the pattern of significant differences.

Table 15. Summary of Descriptive Statistics for on farm Log Enterococcus

Farm	Median Log Enterococcus	1st quartile	3rd quartile	N
1	3.7934	3.2357	4.3942	14
2	3.5683	3.0936	4.1561	13
3	3.6813	3.1143	3.9732	23
4	2.8457	NA	NA	1
5	4.0253	3.5793	4.4137	13
6	3.1495	2.8413	3.5426	21

Table 16. Summary of Descriptive Statistics for on farm Log GEC

Farm	Median Log GEC	1st quartile	3rd quartile	N
1	5.0000	4.8281	5.4038	16
2	4.9287	4.6882	5.2021	16
3	4.3323	3.8675	4.5309	28
4	5.0000	4.2381	5.0000	3
5	5.0000	5.1762	5.6916	16
6	4.4742	4.2041	4.9773	25

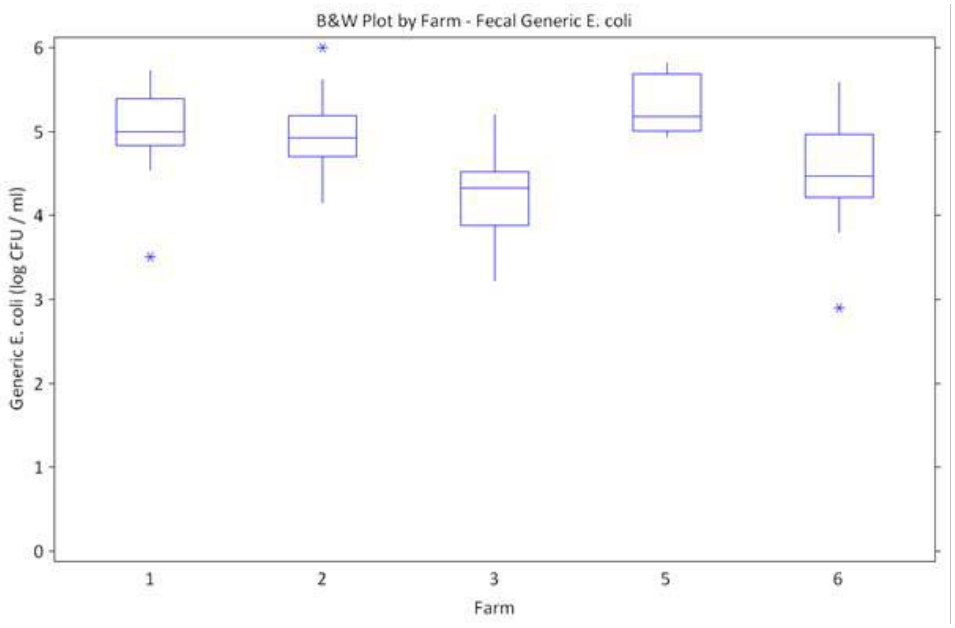


Figure 25. Box and Whisker plots of Log GEC for on farm fecal samples

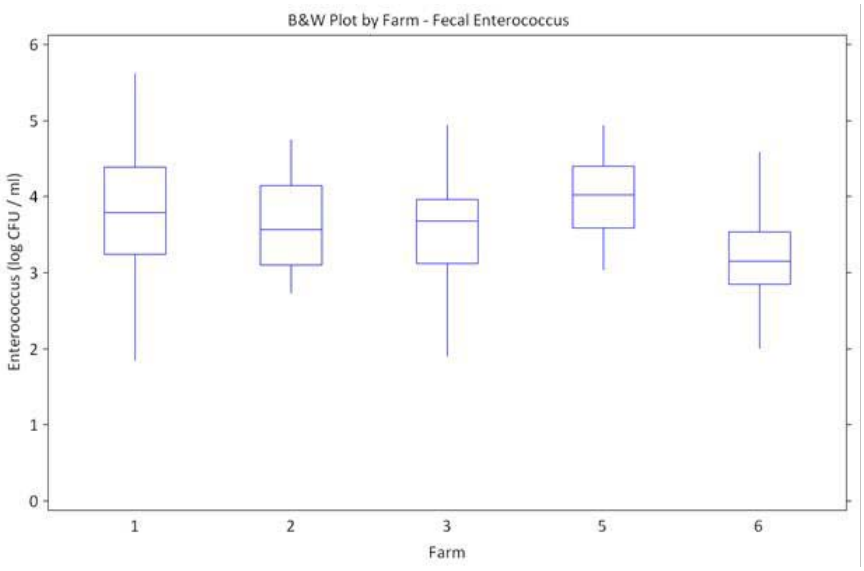


Figure 26. Box and Whisker plots of Log ENT for on farm fecal samples

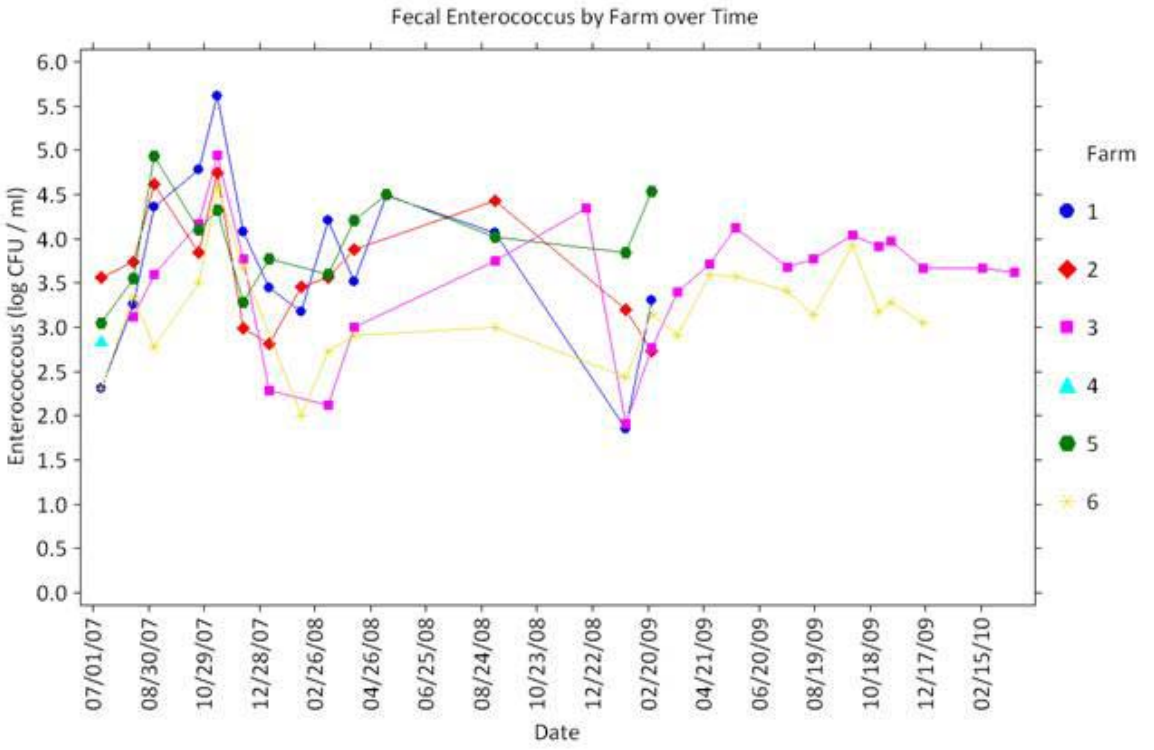


Figure 27. Temporal plot of Log ENT for on farm fecal samples

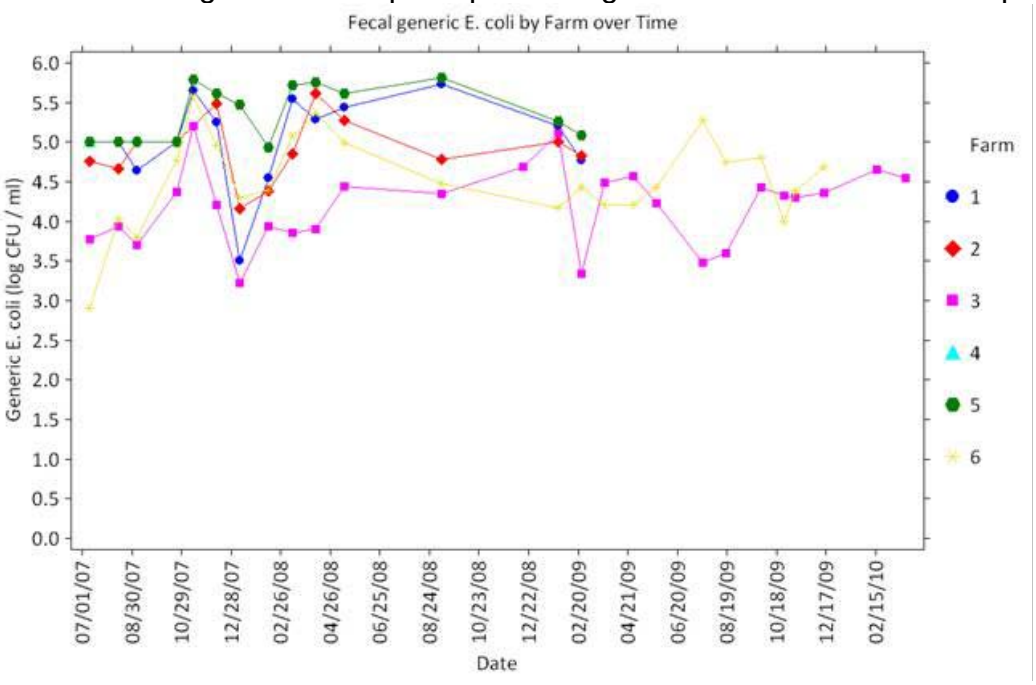


Figure 28. Temporal plot of Log GEC for on farm fecal samples

Subsequent analyses to further evaluate on-farm data and

Comparison between Farm 3 (Werkhoven) and Farm 6 across digester startup 12/15/08:

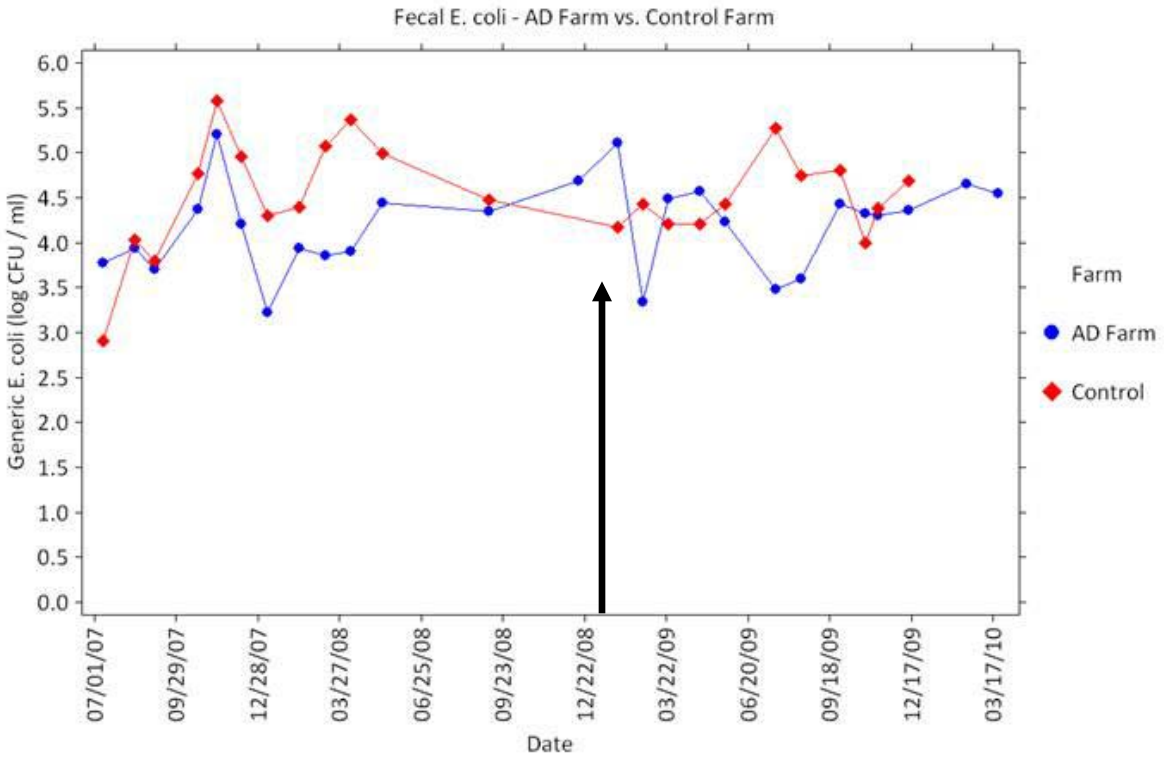


Figure 29 – Generic e-coli during the period of pre- and post- AD startup. Digester went on-line December 2008.

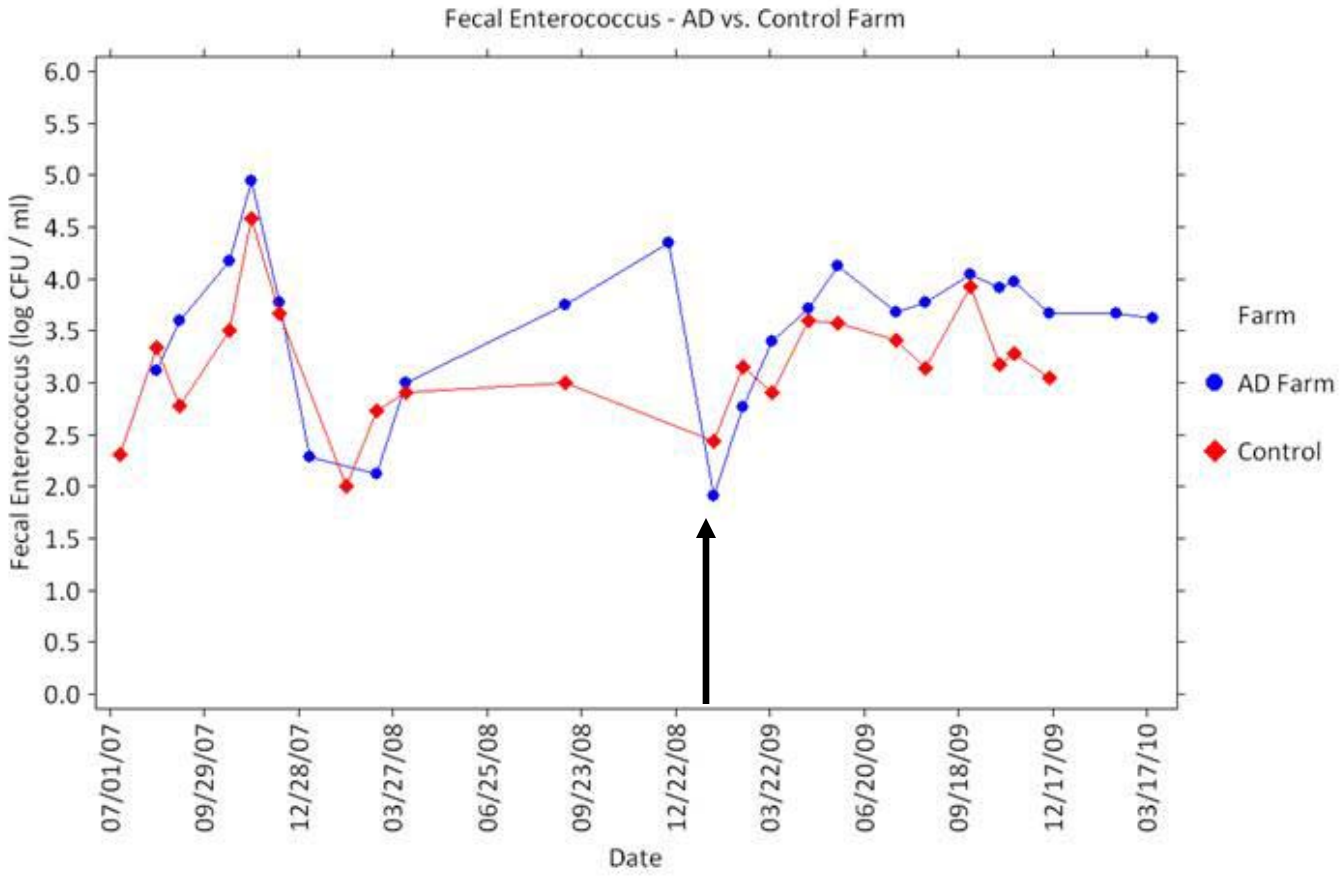


Figure 30 – Enterococcus during the period of pre- and post- AD startup. Digester went on-line December 2008.

Table 17 Generic *E. coli* across the time period of when the AD began operation.

On-farm Fecal Generic *E. coli* (log CFU/ml) across AD Startup					
Pre-Start	**Farm**	**N**	**1st Quartile**	**Median**	**2nd Quartile**
	AD Farm	15	3.8574	4.2069	4.4503
	Control Farm	14	4.2315	4.7553	5.0189
Post-start					
	AD Farm	13	3.9163	4.3617	4.5561
	Control Farm	11	4.2041	4.4314	4.7482

Table 18. Enterococcus across the time period of when the AD began operation.

On-farm Fecal Enterococcus (log CFU/ml) across AD Startup					
Pre-Start	**Farm**	**N**	**1st Quartile**	**Median**	**2nd Quartile**
	AD Farm	10	2.8206	3.6752	4.2177
	Control Farm	10	2.6257	2.9520	3.5470
Post-start					
	AD Farm	13	3.5107	3.6813	3.9409
	Control Farm	11	3.0418	3.1764	3.5799

Kruskal-Wallis One-Way Nonparametric AOV for GECLog by Cat

Cat	Mean Rank	Sample Size
30	21.2	15
31	24.5	13
60	34.0	14
61	29.0	11
Total	27.0	53

Kruskal-Wallis Statistic	5.5622
P-Value, Using Chi-Squared Approximation	0.1350

Parametric AOV Applied to Ranks

Source	DF	SS	MS	F	P
Between	3	1326.2	442.070	1.96	0.1328
Within	49	11072.3	225.965		
Total	52	12398.5			

Total number of values that were tied	9
Max. diff. allowed between ties	0.00001

Kruskal-Wallis One-Way Nonparametric AOV for ENTLog by Cat

Cat	Mean Rank	Sample Size
30	25.5	10
31	28.0	13
60	16.2	10
61	19.0	11
Total	22.5	44

Kruskal-Wallis Statistic	6.2244
P-Value, Using Chi-Squared Approximation	0.1012

Parametric AOV Applied to Ranks

Source	DF	SS	MS	F	P
Between	3	1026.74	342.248	2.26	0.0966
Within	40	6066.26	151.656		
Total	43	7093.00			

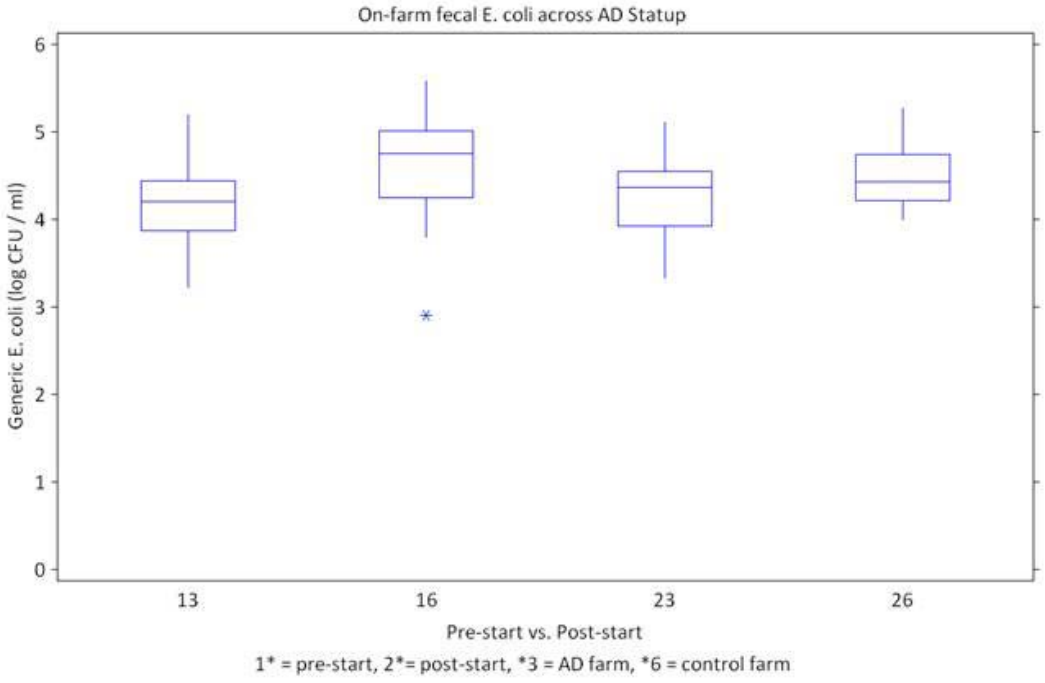


Figure 31 – Box – Whisker plots of *E.coli* across the time period that the AD began operation.

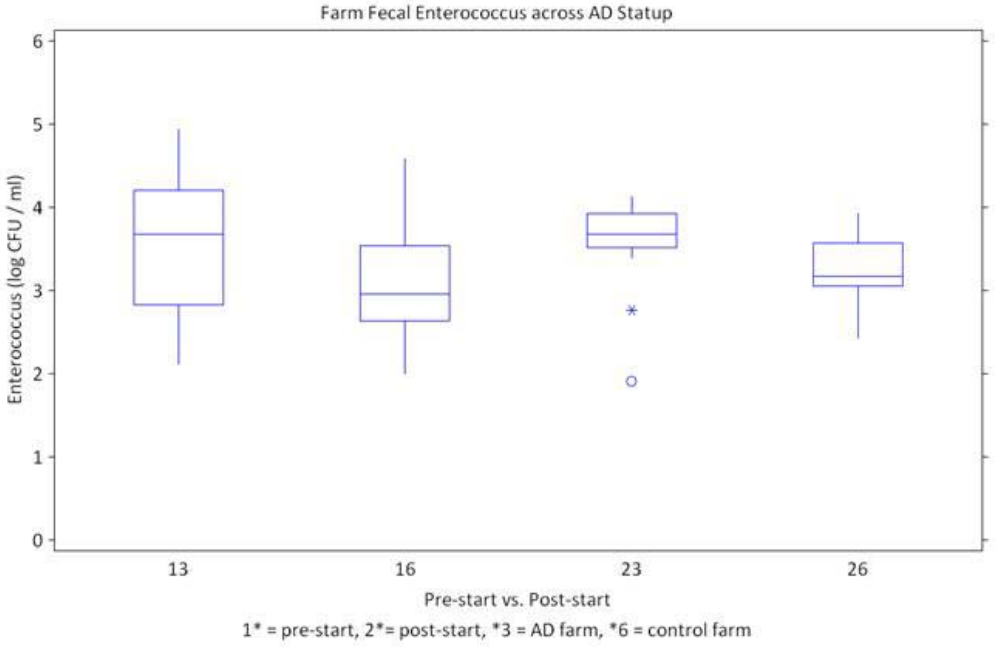


Figure 32– Box – Whisker plots of enterococcus across the time period that the AD began operation.

3) Pre- Post AD Monitoring of Pathogens

Samples of manure and various manure streams were collected and analyzed at monthly or 2x/month intervals from December 2008 through March 2010. Figure 33 and 34 summarize the Box and Whisker plots of GEC and enterococci bacteria at different points in the manure handling system. Table 19 and 20, and figures 33 and 34 summarize information related to the fate of bacteria when evaluated in fresh feces, fresh manure, bedding, feedstocks, the manure and feedstock mixture (receiver), post AD effluent, post AD solids, post AD liquids, and aerobic composted solids.

Interpretive Statement - The findings demonstrate that the AD treatment resulted in a 2 $\log_{10}$ reduction in Enterococci (LogEnt, receiver tank – median 3.93, to AD Effluent - median 2.78) and a 2.5 $\log_{10}$ reduction generic E-coli (LogGEC, receiver tank - median 4.51 to AD Effluent - median 2.02). Composting the manure solids after AD resulted in a further reduction to median Enterococcus count of 0 and median generic E. coli count of 0.

The presence-absence data for Campylobacter, Listeria, *Mycobacterium paratuberculosis*, and Salmonella are summarized in Table 2. Common patterns were: 1) Campylobacter – a reduction in presence after AD, and no detection in composted solids; 2) Listeria – little or no reduction due to AD, and no detection in composted solids; 3) *Mycobacterium paratuberculosis* – small reduction in presence after AD, and no detection in composted solids; and, 4) Salmonella – increased detection after AD, and no detection in composted solids. With the exception of one commodity sample (eggwaste) with a detection of Salmonella, the selected bacteria were not detected in these sources.

Table 19. Description of sampling points and number of samples for bacterial counts or presence-absence of bacteria (see figures 3 and 4, and table 2 for data).

Sampling Location	Description	Log Enterococci # samples	Log GEC # samples
Feces	Feces sampled at the farm	25	25
Farm Flow	Manure as received at the digester via 1 mile pipeline from the farm	26	27
Bedding	Mixed shavings and manure from dry-cow and heifer barn at digester site	22	23
Commodity	Addition feedstocks received at digester: includes whey, fish-stick processing waste, blood from slaughter plant, and egg waste,	6	6
Receiving Tank	Manure and feedstock mix	27	26
Effluent after anaerobic digestion	Effluent emerging from anaerobic digester	30	30
AD Solids	Solids after anaerobic digestion and liquid-solids separation	26	24
SepLiquid	Liquid after anaerobic digestion and liquid-solids separation	44	44
Compost	Composted AD solids	21	21
Calf Barn	Surface liquid run-off from calf barn at digester site (does not enter digester, but goes to the AD effluent storage lagoon)	10	9

Figure 33. Box-Whisker plot of generic e-coli bacteria in pre- and post AD materials. Wilcoxon Rank Sum Test indicated that the reduction in Log GEC due to anaerobic digestion, Receiver site compared to Effluent site, was a statistically significant.

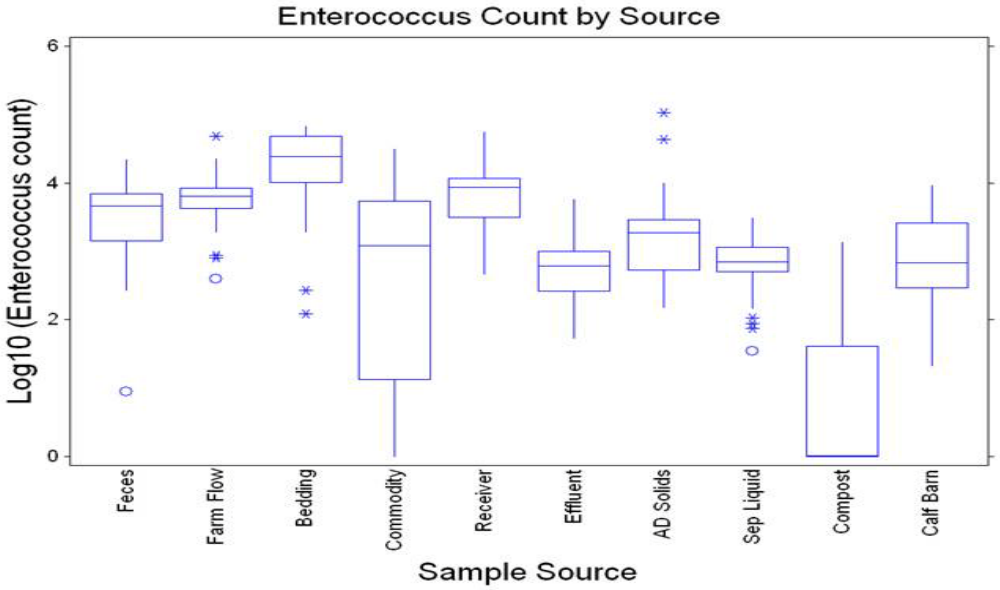


Figure 34. Box-Whisker plot of enterococci bacteria in pre- and post AD materials. Wilcoxon Rank Sum Test indicated that there was the reduction in Log enterococci due to anaerobic digestion, Receiver site compared to Effluent site, was statistically significant.

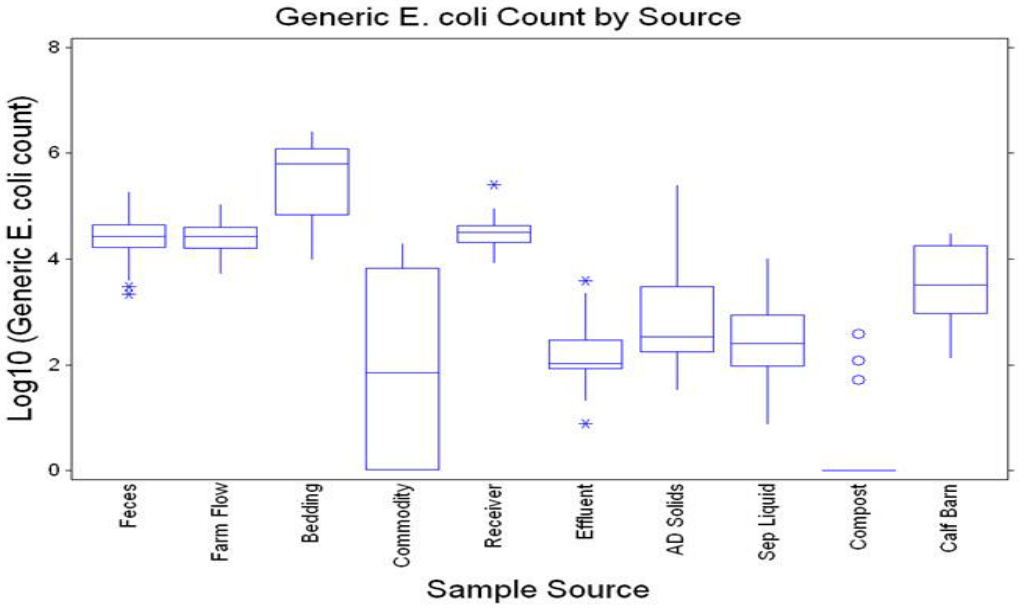


Table 20. Presence-absence of bacteria in pre- and post-AD materials

	Number of post-AD startup samples with one or more test positive replicates				
Sample Origin	Campylobacter	*E. coli* O157:H7	Listeria	*Mycobacterium paratuber culosis*	Salmonella
Farm 3	22/46 (48%)	0/44(0%)	5/47(11%)	48/69 (70%)	37/67 (55%)
Tipping	0/3 (0%)	0/3(0%)	0/3 (0%)	0/6 (0%)	1/6(17%)*
AD InFlow	7 14 (50%)	1/14(7%)	0/14 (0%)	22/26 (85%)	24/26 (92%)
Post AD	11/53 (21%)	0/52(0%)	7/56(12%)	72/101 (71%)	93/97 (96%)
Compost	0/11 (0%)	0/10(0%)	0/11 (0%)	0/21 (0%)	2/20 (10%)
Runoff	4/ 6 (67%)	0/5(0%)	1/6 (17%)	4/11 (36%)	4/10 (40%)
Overal1	44/133 (33%)	1/128(1%)	13/137(9%)	146/234(62%)	159/226 (70%)

* source was egg waste

Chi-Square Test for Heterogeneity or Independence
for 1 = Campylobacter Type

		Type			
Campy		**Farm**	**Inflow**	**Tipping**	**PostAD**
Negative	Obs	32	7	2	44
	Expected	42.07	9.50	1.36	35.29
	Cell Chi-Sq	2.41	0.66	0.30	2.15
Positive	Obs	30	7	0	8
	Expected	19.93	4.50	0.64	16.71
	Cell Chi-Sq	5.09	1.39	0.64	4.54
		62	14	2	52

		Type	
Campy		**Compost**	
Negative	Obs	10	95
	Expected	6.79	
	Cell Chi-Sq	1.52	
Positive	Obs	0	45
	Expected	3.21	
	Cell Chi-Sq	3.21	
		10	140

Overall Chi-Square	21.93
P-value	0.0002
Degrees of Freedom	4

4) Johnes Surveillance – individual cow evaluation via blood sample

Interpretive Statement - The prevalence of Johnes infected animals (table 21) was low with one herd having no test positive animals. The results from the monthly cultures from on-farm pooled manure samples correlated with the infection prevalence detected by the individual cow blood sampling completed by the WSDA. The blood test is relatively insensitive compared to the fecal culture test.

Table 21 summarizes the results of Johnes surveillance conducted by the Washington State Department of Agriculture.

Farm	# cows in herd	# cows sampled	# cows positive
A	550	220	0
B	150	50	1
C	350	181	2
D	650	226	2
E	750	324	2

5) Pasteurization

Two proprietary technologies were evaluated for their merit for bacterial reduction.

In 2008 we evaluated a proprietary technology (EDIN Technology, Tacoma, WA) that was designed to interrupt the life-cycle of bacteria via magnetic forces. The system worked via passing manure through a pipe that had been lined in its inner walls with strong magnets. The concept was that the magnetic force would disrupt the normal functioning of bacterial cell membranes and result in cell death. The unit was run at multiple flow rates of dairy manure: static for 15 minutes, and flows of ~ 200 ml.min, 400 ml/min, 2000 ml/min, and 5600 ml/min.

A second technology (G 2 Sorb Water Management Unit) developed by EDIN technologies (Tacoma, WA) for water filtration was also evaluated. This system relies on the attraction “foreign” substances to the adsorbing material.

Interpretive Statement - The magnetic device had no apparent effect of on viability of generic *E.coli* under any of the conditions evaluated (see table 23). The G-2 sorb technology had no apparent effect of on generic *E. coli* and Enterococcus in manure (see table 23).

Table 22
Anaerobic Digestion - Magnetic Field Device Test

Spiral Plated: 100ul per plate - Counts via WASP Spiral Plater

Sample #		Sample	Coliform cfu/ml
1A		Pre-treatment	7.7E+04
1B		Pre-treatment	5.7E+04
2A		Pre-treatment	5.5E+04
2B		Pre-treatment	4.7E+04
3A		Pre-treatment	5.7E+04
3B		Pre-treatment	5.2E+04
	Pre-treatment	**Average**	**5.8E+04**
4A		Static - 15min	5.4E+04
4B		Static - 15min	5.1E+04
5A		Static - 15min	4.9E+04
5B		Static - 15min	4.4E+04
6A		Static - 15min	5.2E+04
6B		Static - 15min	4.9E+04
	Static - 15min	**Average**	**5.0E+04**
7A		< 200 ml/min	5.6E+04
7B		< 200 ml/min	5.4E+04
8A		< 200 ml/min	4.8E+04
8B		< 200 ml/min	4.9E+04
	< 200 ml/min	**Average**	**5.2E+04**
9A		400 ml/min	5.0E+04
9B		400 ml/min	5.3E+04
10A		400 ml/min	5.1E+04
10B		400 ml/min	5.4E+04
	400 ml/min	**Average**	**5.2E+04**
11A		2000 ml/min	3.7E+04
11B		2000 ml/min	3.9E+04
12A		2000 ml/min	2.9E+04
12B		2000 ml/min	2.6E+04
	2000 ml/min	**Average**	**3.3E+04**
13A		5600 ml/min - Full Open	5.4E+04
13B		5600 ml/min - Full Open	5.5E+04
14A		5600 ml/min - Full Open	4.4E+04
14B		5600 ml/min - Full Open	5.1E+04
	5600 ml/min - Full Open	**Average**	**5.1E+04**

Table 23. Anaerobic Digestion - Filtration Unit

Spiral Plated: 100ul per plate - Counts via WASP Spiral Plater

Sample #		Sample	Coliform cfu/ml	Enterococcus cfu/ml
1		Pre-Filtration	1.1E+05	1.2E+05
2		Pre-Filtration	1.4E+05	1.2E+05
3		Pre-Filtration	1.1E+05	1.5E+05
4		Pre-Filtration	1.1E+05	1.5E+05
5		Pre-Filtration	1.0E+05	1.4E+05
	Pre-Filtration	**Average**	**1.1E+05**	**1.4E+05**
6		Post-Filtration	1.0E+05	1.5E+05
7		Post-Filtration	1.1E+05	1.4E+05
8		Post-Filtration	1.4E+05	1.3E+05
9		Post-Filtration	1.2E+05	1.4E+05
10		Post-Filtration	1.0E+05	1.4E+05
	Post-Filtration	**Average**	**1.1E+05**	**1.4E+05**

6) Bacteria Die-Off on Soil after Manure Application

In 2007 bacteria die-off data was collected on six occasions after undigested manure had been applied by the dairy producer during normal operations to grass to be harvested for silage. The results are summarized in figure 35 After an initial rapid rise in fecal coliform or e-coli on soil, there was a rapid rate of decline to pre-application levels within days to a few weeks.

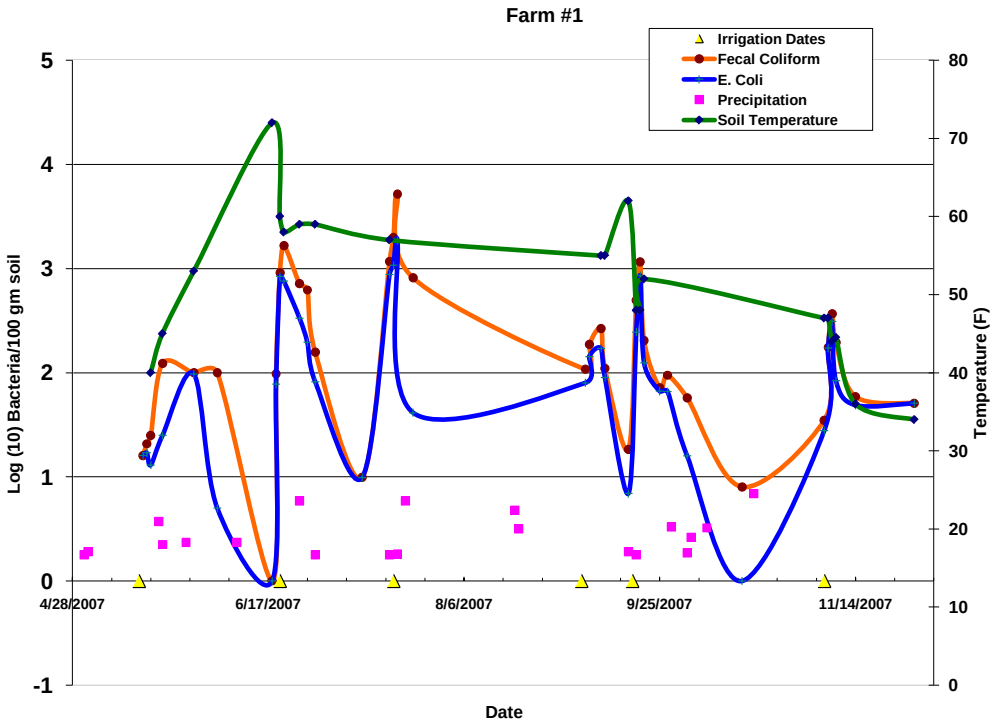


Figure 35. Log bacteria counts on soil after aerial manure application at farm 6.

In 2009 – 2010 the survival or die-off of fecal coliform and *Escherichia coli* on soil after manure application was characterized in replicated plots. Fresh and AD manure was applied to replicated plots of grass to be harvested for silage. Manure was applied via two methods, subsurface application and surface broadcast application. Subsurface deposition was accomplished with a 3.05 meter Aerway® Sub Surface Deposition (SSD) (Model AW1000-2B48-D) with a custom Banderator® attachment for application of manure through eight PVC pipes attached to the Banderator® tines. Tines were set to drop ~ 10 cm below the soil surface creating intermittent slices 12.5 cm in length at the surface. Surface broadcast of non AD manure [before digestion, or (BD)] and AD manure was applied using drop hoses connected with the Aerway® ^{TM} system in the up position. (see figure 36)

Figure 36. Manure application tank with Aerway® ^{TM} applicator.

Soil cores of 1 inch deep by 2 inch diameter were sampled from each plot with after manure application to determine the die-off of fecal coliform and *Escherichia coli*. These organisms are used as indicator pathogens because they are commonly present in the fecal material of warm blooded animals, and are affected by anaerobic digestion. The rate of indicator bacteria decline is presented in Table 24 for each trial. The slope of the line over time began at the peak day of bacterial concentration and continued until the final day of sampling, prior to the next manure application.

Interpretive Statement - Statistical significances are presented in Table 25 as an average of all trials over two seasons. Soil receiving the before digestion-broadcast applied (BD-B) manure saw the greatest reduction rate in fecal coliform (-0.254), followed by AD-SSD manure (-0.170). Terminal day sampling indicated AD-SSD had the fewest fecal coliforms (2.096 $\log_{10}$ CFU 100g soil^{-1}), while BD-B had significantly more (3.445 $\log_{10}$ CFU 100g soil^{-1}). The greatest rate of decline of bacteria numbers occurred when ambient temperatures were highest. This study found that over five different application trials, with varying environmental conditions, anaerobically digested manure had significantly fewer indicator bacteria, both initially and at the end of the sampling period in a field of forage grasses.

Table 24. Individual die-off rate of bacteria over each sampling period (rate = log CFU/100 gm soil/day).

			Treatment			
			AD-SSD	AD-B	BD-SSD	BD-B
			Rate of bacterial decline (day^{-1})			
2009		Fecal coliform				
	May		-0.0489	-0.0185	-0.0162	0.0096
		E. coli	-0.0345	-0.0143	-0.0357	0.0443
		Fecal coliform				
	Jun		-0.0834	-0.0794	-0.1129	-0.0621
		E. coli	-0.0645	-0.0722	-0.0939	-0.0580
		Fecal coliform				
	Aug		-0.2454	-0.3119	-0.2457	-0.7166
		E. coli	-0.3284	-0.1994	-0.2313	-0.3257
2010		Fecal coliform				
	June		-0.2496	-0.0995	-0.1451	-0.1102
		E. coli	-0.1700	-0.0900	-0.1268	-0.0924
		Fecal coliform				
	July		-0.2235	-0.1446	0.0712	-0.4307
		E. coli	-0.1691	-0.1154	0.0523	-0.3373

AD-SSD, Anaerobically digested – subsurface deposition; AD-B, Anaerobically digested – broadcast applied; BD-SSD, Before digestion – subsurface deposition;
BD-B, Before digestion – broadcast applied.

Table 25. Average rate of bacterial die-off from all sampling periods during 2009 and 2010 seasons. (rate = log CFU/100 gm soil/day)

	Fecal coliform		*E. coli*	
	Rate of bacterial decline (day^{-1})			
AD-SSD	-0.170	ab	-0.153	a
AD-B	-0.131	b	-0.098	ab
BD-SSD	-0.090	b	-0.087	b
BD-B	-0.254	a	-0.145	ab

AD-SSD, Anaerobically digested – subsurface deposition; AD-B, Anaerobically digested – broadcast applied; BD-SSD, Before digestion – subsurface deposition; BD-B, Before digestion – broadcast applied.

7) Microbial Source Tracking

The original intent of this component was to be able to look at surface water quality at the study site before and after manure from all farms had been anaerobically digested. As previously mentioned the opportunity to make the full pre- post comparison did not come to fruition.

However, during outreach events associated with this project we have been presented on numerous occasions with questions like, "will processing manure with anaerobic digestion technology create super bugs" and "does the AD process result in hardier bugs in the processed manure" .

In 2010 we designed a series of evaluations of pre- and post digested manure to try to determine if the bacteria in manure after AD were different than prior to AD. The evaluations included:

a) bacterial fermentation profiles – Generic *E. coli*:

The sugar fermentation profiles of 80 generic *E. coli* isolated from inputs prior to AD were compared to those of 83 generic E. coli isolated from post-AD liquid and solid materials or 163 total GEC isolates. The profile consisted of the sugars Adonitol, Dulcitol, Melibiose, Raffinose, Rhamnose, Salicin, Sorbose, Sucrose and the indicator medias MAC and MUG.

b) antibiotic resistance profiles (Kirby-Bauer disk diffusion method) - Generic *E. coli* and Salmonella:

The antibiotic profiles of 182 generic *E. coli* and 164 Salmonella isolated from inputs prior to AD were compared to those of 115 generic *E. coli* and 317 Salmonella isolated from post-AD liquid and solid materials or 297 total GEC isolates. The following antibiotic discs (12) were used: Amikacin (AN 30); Amoxicillin/clavulanic acid (AmC 30); Ampicillin (AM 10); Cephalothin (CF 30); Ceftiofur (XNL); Chloramphenicol (C 30); Gentamicin (GM 10); Nalidixic acid (NA 30); Streptomycin (S 10); Sulfisoxazole (G .25); Tetracycline (TE 30); and Sulfamethoxazole/trimethoprim (SXT).

c) Salmonella sergrouping:

Salmonella isolates were serogrouped to determine if serogroup profiles differed between pre-AD inputs and post AD liquid and solid materials. This was of interest to determine if Salmonella serogroups differed in their ability to survive AD.

d) Rep-PCR – Generic *E. coli*:

REP-PCR was used to determine if the generic *E. coli* isolated from inputs prior to AD differed genetically from those isolated from post-AD liquid and solid

materials. Repetitive extragenic palindromic chain reaction (REP-PCR) establishes the distribution of REP elements across the entire bacterial genome of each isolate. The PCR products form a pattern that acts as the common genomic fingerprint of closely related strains of the bacterial species. This method is known for its ease of application and interpretation, high discrimination power, good reproducibility and not requiring extensive DNA extraction.

a) bacterial fermentation profiles – Generic *E. coli*:

Using the fermentation results from the 8 sugars Adonitol, Dulcitol, Melibiose, Raffinose, Rhamnose, Salicin, Sorbose, and Sucrose as indicator variables (1 = fermented, 0 = not fermented), a cluster analysis based on Ward's method was performed. This grouped the isolates into 20 clusters based on their similarity/dissimilarity. A Chi Square analysis was then performed on these clusters to determine if isolate AD status (pre-AD or post-AD) was homogeneously distributed across these clusters, which it was.

Interpretive Statement – Because AD status (pre- vs. post-AD) was not statistically significantly associated with this set of fermentation cluster memberships (figure 37), the generic *E. coli* isolated pre- and post AD manure did not differ in their ability to ferment sugars.

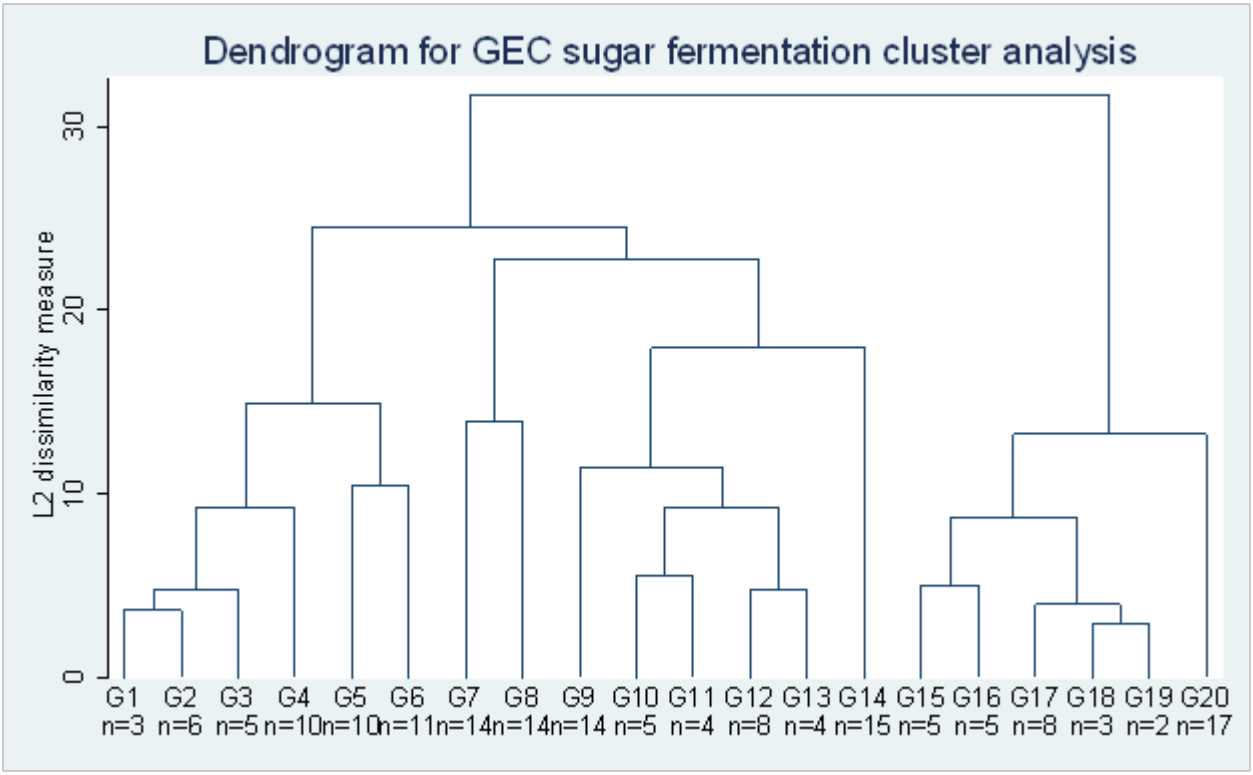


Figure 37. Dendogram of the sugar fermentation cluster analysis of generic *E. coli.*

Looking at how Pre / Post AD is distributed in these clusters:

Cluster Group No	AD Status Pre	Post
1	2	1
2	3	3
3	2	3
4	8	2
5	2	8
6	7	4
7	5	9
8	9	5
9	6	8
10	2	3
11	3	1
12	4	4
13	4	
14	10	5
15	3	2
16	1	4
17	3	5
18	1	2
19	1	1
20	4	13

Running a Chi Square on that: AD status (pre- vs. post AD) is not statistically significantly associated with this set of fermentation cluster memberships.

Pearson chi2(19) = 25.5411 Pr = 0.143

b) antibiotic resistance

The overall summary of antibiotic resistance of GEC isolates is shown in table 26. The initial statistical analysis looked at the number of antibiotics that each isolate was resistant. Subsequent statistical analyses follow.
The overall summary of antibiotic resistance of salmonella isolates is shown in table 27.

Interpretive Statement – The proportion generic *E. coli* isolated from post-AD liquids and solids resistant to one or more antibiotics was not statistically significantly different from those isolated from farm-origin effluent, which constituted at least 70% of the AD inflow, or from those isolated from tipping materials. The proportion of Salmonella isolates resistant to one or more antibiotics also was not statistically significantly different across the AD system inflows and outflows.

*Generic e-coli and antibio*tic resistance

Table 26 Summary of antibiotic resistance of GEC isolates

	Bovine blood	Whey	Egg Waste	Paper Pulp	Receiving tank	Sugar waste	Werk Farm	Werk Inflow tank	Bedding	Fish stick waste	AD effluent	Ad solids	Transfer tank	Before transfer tank	Compost
# sples	49	1	5	29	19	1	13	36	21	1	29	28	22	34	2
# resistant	6	1	0	3	8	0	2	11	5	0	7	3	5	8	0
AmcAmCF	2			1	1									2	
AmcCF	1														
Cf	1										1				
SGTe	1														
AmCf		1													
AmCfc				1											
AmCfXnlC				1											
AmCfSGTe					2										
AmSTe	1				1										
CfCSGTe					1										
GTe					2		2	4	1			1			
Te					1			1							
AmcAmCf SGTe								1			1		1		1
An								1							
CSGTe								2			2				
SGTe								1	3				3	4	
S								1							
AmS									1						
Am											3	1			
G												1			
AmCGTe													1		
AmTe														1	

Cross Tabulation of Source by generic E.coli Resistance Status

Source	Number of Antibiotics GEC isolates were resistant to 0	1	2	3	4	5	6	7	
Farm	53	7	5	7	1	1	1	0	75
Row %	70.7%	9.3	6.7	9.3	1.3	1.3	1.3	0.0	25.3
InFlow	11	1	0	4	0	1	1	1	19
	57.9%	5.3	0.0	21.1	0.0	5.3	5.3	5.3	6.4
Post	92	6	3	7	3	1	2	1	115
	80.0%	5.2	2.6	6.1	2.6	0.9	1.7	0.9	38.7
Tipping	76	4	1	5	2	0	0	0	88
	86.4%	4.5	1.1	5.7	2.3	0.0	0.0	0.0	29.6
	232	18	9	23	6	3	4	2	297

Chi-Square Test for Heterogeneity or Independence
for Source vs. GEC Resistance Status (collapsed to reduce 0 cells)

Source		Number of Antibiotics GEC isolates were resistant to 0	1	2	3	4	
Farm	Observed	53	7	5	7	3	75
	Expected	58.59	4.55	2.27	5.81	3.79	
	Cell Chi-Sq	0.53	1.33	3.27	0.24	0.16	
InFlow	Obs	11	1	0	4	3	19
	Expected	14.84	1.15	0.58	1.47	0.96	
	Cell Chi-Sq	0.99	0.02	0.58	4.35	4.34	
Post	Observed	92	6	3	7	7	115
	Expected	89.83	6.97	3.48	8.91	5.81	
	Cell Chi-Sq	0.05	0.13	0.07	0.41	0.24	
Tipping	Obs	76	4	1	5	2	88
	Expected	68.74	5.33	2.67	6.81	4.44	
	Cell Chi-Sq	0.77	0.33	1.04	0.48	1.34	
		232	18	9	23	15	297

Overall Chi-Square	20.69
P-value	0.0551
Degrees of Freedom	12

The resistance of Salmonella isolates is not statistically significantly different between across the AD system.

Table 27 Summary of antibiotic resistance of salmonella isolates.

	Bovine blood	Whey	Egg Waste	Paper Pulp	Receiving tank	Sugar waste	Werk Farm	Werk Inflow tank	Bedding	Fish stick waste	AD effluent	Ad solids	Transfer tank	Before transfer tank	Compost
# sples	49	1	5	29	19	1	13	36	21	1	29	28	22	34	2
# resistant	6	1	0	3	8	0	2	11	5	0	7	3	5	8	0
AmcAmCF	2			1	1									2	
AmcCF															
Cf	1										1				
SGTe															
AmCf	1	1													
AmCfc				1											
AmCfXnlC				1											
AmCfSGTe					2			1							
AmSTe	1				1										
CfCSGTe					1										
GTe					2		2	4	1			1			
Te					1			1							
AmcAmCf SGTe											1		1	1	
An								1							
CSGTe								2			2				
SGTe	1							1	3				3	4	
S								1							
AmS									1						
Am											3	1			
G												1			
AmCGTe													1		
AmTe														1	

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Chi-Square Test for Heterogeneity or Independence for 1 = Resist Source

Resist		Source: Farm	InFlow	PostAD	
R	Observed	15	15	38	68
	Expected	12.63	10.36	45.00	
	Cell Chi-Sq	0.44	2.07	1.09	
S	Observed	74	58	279	411
	Expected	76.37	62.64	272.00	
	Cell Chi-Sq	0.07	0.34	0.18	
		89	73	317	479

Overall Chi-Square	4.20
P-value	0.1222
Degrees of Freedom	2

Looking only at the Salmonella isolates from the receiving tank onward:

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Chi-Square Test for Heterogeneity or Independence for 1 = Resist Source

Resist		Source: InFlow	PostAD	
R	Observed	15	38	53
	Expected	9.92	43.08	
	Cell Chi-Sq	2.60	0.60	
S	Observed	58	279	337
	Expected	63.08	273.92	
	Cell Chi-Sq	0.41	0.09	
		73	317	390

Overall Chi-Square	3.70
P-value	0.0543
Degrees of Freedom	1

c) Sero-groups of salmonella

Salmonella Typing Salmonella isolates were classified by serogroup and serotype (see table 28). The salmonellae are a heterogeneous group of bacteria in the *Salmonella* genus of the family *Enterobacteriaceae.* The taxonomy and nomenclature of *Salmonella* have changed over the years and is still evolving. Currently, the CDC recognizes two species of *Salmonella* which are divided into seven subspecies: *S. enterica* (six subspecies) and *S. bongori* (one subspecies). These subspecies are divided into over 50 serogroups based on which somatic (O) antigens are present. The serogroups are further divided into over 2500 serotypes based on flagellar (H) antigens. *Salmonella* serotypes are recognized by antigenic formulas listed in the document called the White-Kauffmann-Le Minor Scheme. Updating this scheme is the responsibility of the WHO Collaborating Centre for Reference and Research on *Salmonella,* which is located at the Pasteur Institute, Paris, France. Most *Salmonella* serotypes isolated from humans and warm-blooded animals belong to *Salmonella enterica* subspecies 1 in Oserogroups A, B, C1, C2, D, E1, E2, E3, and E4.

Salmonella serogroup: Bacterial identification systems, such as the VitekTM, APITM, and MicroScanTM, are reliable in the biochemical identification of *Salmonella* to the genus level. These systems however, do not identify the salmonellae into serogroups or serotypes. To identify *Salmonella* serogroups, numerous O-grouping antisera along with control antigens, are necessary. The FDIU has antisera to perform agglutination testing and recognize the following O-serogroups: Groups A, B, C1, C2, D, E and numerous other less common serogroups. In addition, antiserum to detect the capsular or virulence (Vi) antigen is also available to screen for *Salmonella* serotype (Group D). At the FDIU, serogrouping is routinely performed on all biochemically recognized salmonellae for confirmation and reporting.

Analyzing only the three most numerous Salmonella serogroups, B (66 isolates), C1 (95 isolates), and E1 (268 isolates) and the sources being Farm 3, Inflow and Post AD, the simplest log linear model that fits the data is [Serogroup*Resistance Status][Serogroup*Source] (Goodness of fit summary statistics Likelihood Chi-square = 9.74, 6 df, p = 0.1359). Based on the predicted counts from this model, passing through the digester has no effect on the proportion of a serogroup that are antibiotic resistant. Serogroup is significantly associated with source and with antibiotic resistance.

On page 33 of the USAHA Committee on Salmonella report (report-sal-2009) is a table of the most common non-clinical cattle isolate serotypes for 2008.

1) Kentucky C3
2) Montevideo C1
3) Dublin D1
4) Cerro K
5) Newport C2

Table 28 Summary of salmonella serogroups and serotypes

Site	Calf barn runoff	Whey	Fish Stick waste	Egg Waste	Dry Cow pen	Werk feces	Werk In-flow	Receiving tank	Post Ad leffluent	Post Ad Solids	Effluent before transfer	Transfer Tank	Compost
# sples	21	1	6	1	62	28	78	77	92	72	63	63	58
# pos	8	0	0	1	15	16	50	58	71		58	60	2
B								3	10	6		4	2
B/C1									1				
B/C2								1			1		
B/E1							1	1	4	2	7	3	
B/K Mixed/II K:18:Z4,Z23:								1					
B Heidelberg									7	1	3	1	
B Heidelberg/Kemo									1		3		
C1	4				13		1	8	3	11	4	5	
C1 – Tennessee	1							1		1	1	1	
C1/B									1				
C1/E1											5		
C1/C2												1	
C1/E1								1	4				
C1/E1 Tennessee/Anatum								1	1	1			
C1/E1												5	
C1/E1 Infantis										1		1	
C1/K									1			1	
C1/K Tennessee/Cerro									1			5	
C2										2		1	
D1					1		1						
D1/E1											1		
E1	3				1	12	33		38	23	22	34	
E1/Anatum												1	
E1 Vejle							1				1		
E1/C1 Tennessee/Anatum											1	1	
E1/B									1	1	6		
E1/C1										1			
K						3	9						
K – Cerro						1	2		1	2		2	
K/C2											1		
Mixed/K II K:18:Z4,Z23:-							1						
K/E1 II K:18:Z4, Z23:-												1	
Poly A				1									
ACGKSAmcSulCaz, Dublin							1						

Salmonella serogroup and resistance status appear to be related to isolate source in sufficiently complex ways that the data cannot be represented by simpler models.

Cross Tabulation of Serogroup by Source
Controlling for Status = Resistant

Serogroup	Source: Farm	InFlow	PostAD	Tipping	
B	0	1	14	0	15 (23%)
C1	0	4	5	0	9 (9%)
C2	0	8	6	0	14 (93%)
D1	1	0	0	0	1 (33%)
E1	1	0	5	0	6 (2%)
K	13	2	7	0	22 (73%)
PA	0	0	0	0	0
	15(17%)	15(21%)	37(12%)	0	67 (14%)

Cross Tabulation of Serogrp by Source
Controlling for Status = Susceptible

Serogroup	Source: Farm	InFlow	PostAD	Tipping	
B	0	6	45	0	51
C1	22	12	52	0	86
C2	0	1	0	0	1
D1	1	0	1	0	2
E1	49	34	179	0	262
K	2	4	2	0	8
PA	0	1	0	1	2
	74	58	279	1	412

Cases Included 479 Missing Cases 2

Interpretive Statement – The proportion of the most numerous Salmonella serogroups (B, C1 and E1) that are resistant to one or more antibiotics is not affected by passage through the anaerobic digester but is associated with serogroup (resistant 23% B, 9% C1, and 2% for E1 – see table 29).

Table 29 – Summary of proportion of antibiotic resistant salmonella by source and serotype in fitted model

Proportion Resistant by Source			
	Source		
Serogroup	Farm	InFlow	PostAD
B	-	23%	23%
C1	9%	9%	9%
E1	2%	2%	2%

Chi-Square Test for Heterogeneity or Independence for Source vs. Serogroup

Source		Serogroup B	C1	E1	
Farm	Observed	0	22	50	72
	Expected	11.22	15.91	44.87	
	Cell Chi-Sq	11.22	2.33	0.59	
InFlow	Obs	7	16	34	57
	Expected	8.88	12.59	35.53	
	Cell Chi-Sq	0.40	0.92	0.07	
PostAD	Obs	60	57	184	301
	Expected	46.90	66.50	187.60	
	Cell Chi-Sq	3.66	1.36	0.07	
		67	95	268	430

Overall Chi-Square	20.61
P-value	0.0004
Degrees of Freedom	4

Chi-Square Test for Heterogeneity or Independence
for Resistance status vs. Serogroup

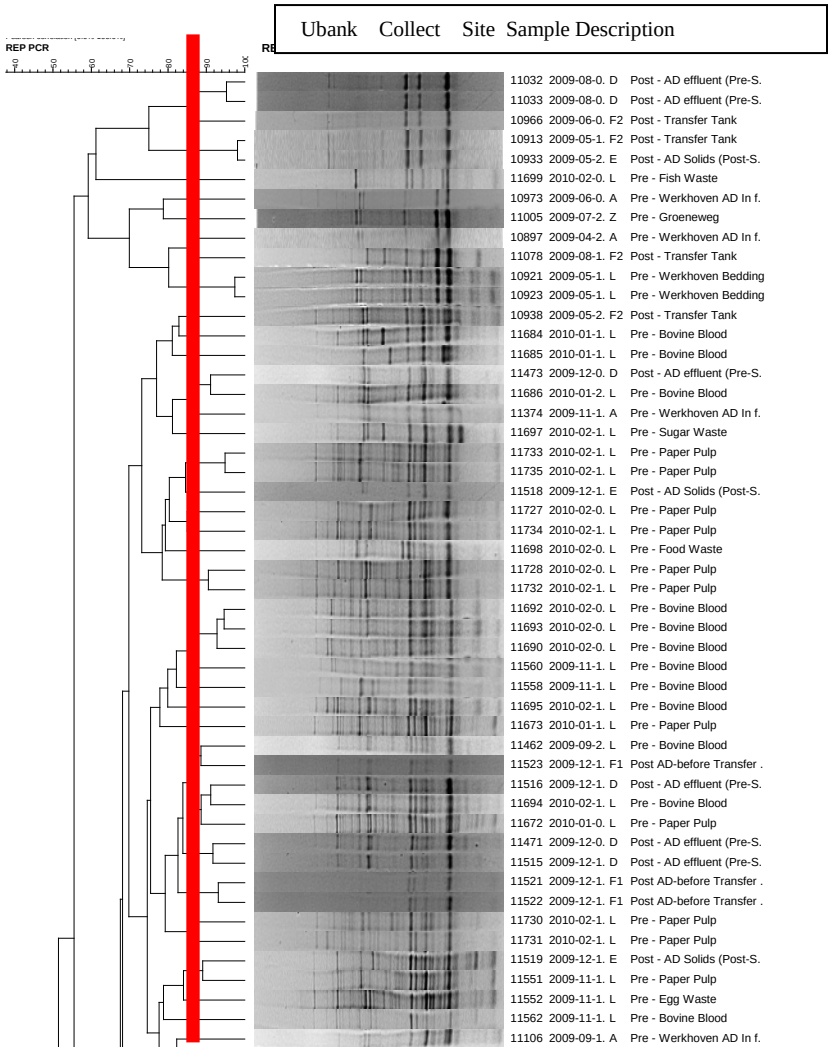
Resistance		Serogroup B	Serogroup C1	Serogroup E1	
R	Observed	15	9	6	30
	Expected	4.62	6.64	18.74	
	Cell Chi-Sq	23.37	0.84	8.66	
S	Observed	51	86	262	399
	Expected	61.38	88.36	249.26	
	Cell Chi-Sq	1.76	0.06	0.65	
		66	95	268	429

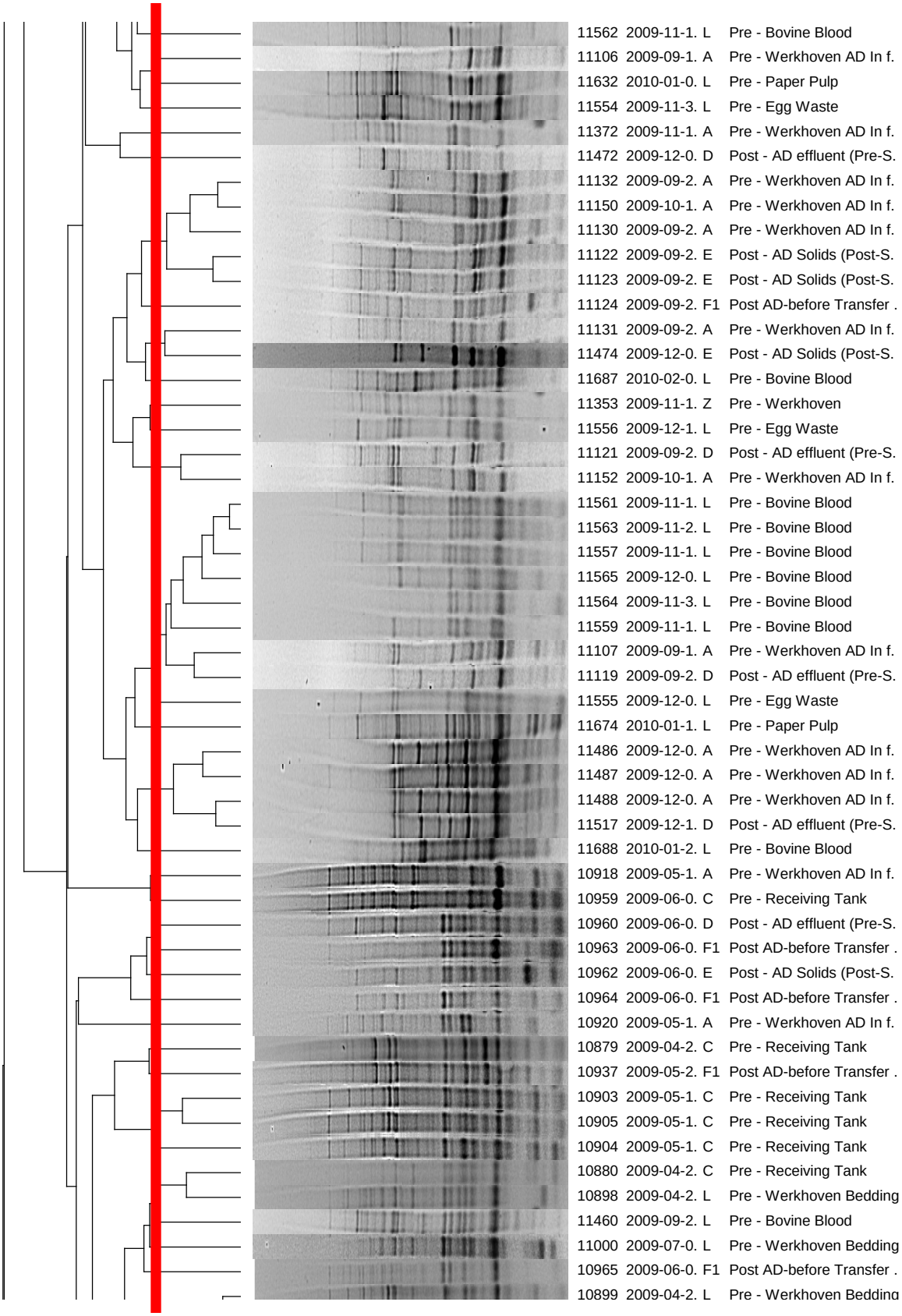
Overall Chi-Square	35.33
P-value	0.0000
Degrees of Freedom	2

d) Rep-PCR

AD GEC REP-PCR: 253 Isolates Tested

Interpretive Statement - If isolates are 85% similar they could be considered clonal. Since we are seeing pre and post AD GEC in the different clusters of relatedness it would be safe to say that the Digester is not selecting for one particular strain of GEC. If the Digester was selecting for different bacteria, we would see a large cluster of Post-AD GEC that are more than 85% similar, and that is not seen in this dendogram.

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11106 2009-09-1. A Pre - Werkhoven AD In f.
11632 2010-01-0. L Pre - Paper Pulp
11554 2009-11-3. L Pre - Egg Waste
11372 2009-11-1. A Pre - Werkhoven AD In f.
11472 2009-12-0. D Post - AD effluent (Pre-S.
11132 2009-09-2. A Pre - Werkhoven AD In f.
11150 2009-10-1. A Pre - Werkhoven AD In f.
11130 2009-09-2. A Pre - Werkhoven AD In f.
11122 2009-09-2. E Post - AD Solids (Post-S.
11123 2009-09-2. E Post - AD Solids (Post-S.
11124 2009-09-2. F1 Post AD-before Transfer .
11131 2009-09-2. A Pre - Werkhoven AD In f.
11474 2009-12-0. E Post - AD Solids (Post-S.
11687 2010-02-0. L Pre - Bovine Blood
11353 2009-11-1. Z Pre - Werkhoven
11556 2009-12-1. L Pre - Egg Waste
11121 2009-09-2. D Post - AD effluent (Pre-S.
11152 2009-10-1. A Pre - Werkhoven AD In f.
11561 2009-11-1. L Pre - Bovine Blood
11563 2009-11-2. L Pre - Bovine Blood
11557 2009-11-1. L Pre - Bovine Blood
11565 2009-12-0. L Pre - Bovine Blood
11564 2009-11-3. L Pre - Bovine Blood
11559 2009-11-1. L Pre - Bovine Blood
11107 2009-09-1. A Pre - Werkhoven AD In f.
11119 2009-09-2. D Post - AD effluent (Pre-S.
11555 2009-12-0. L Pre - Egg Waste
11674 2010-01-1. L Pre - Paper Pulp
11486 2009-12-0. A Pre - Werkhoven AD In f.
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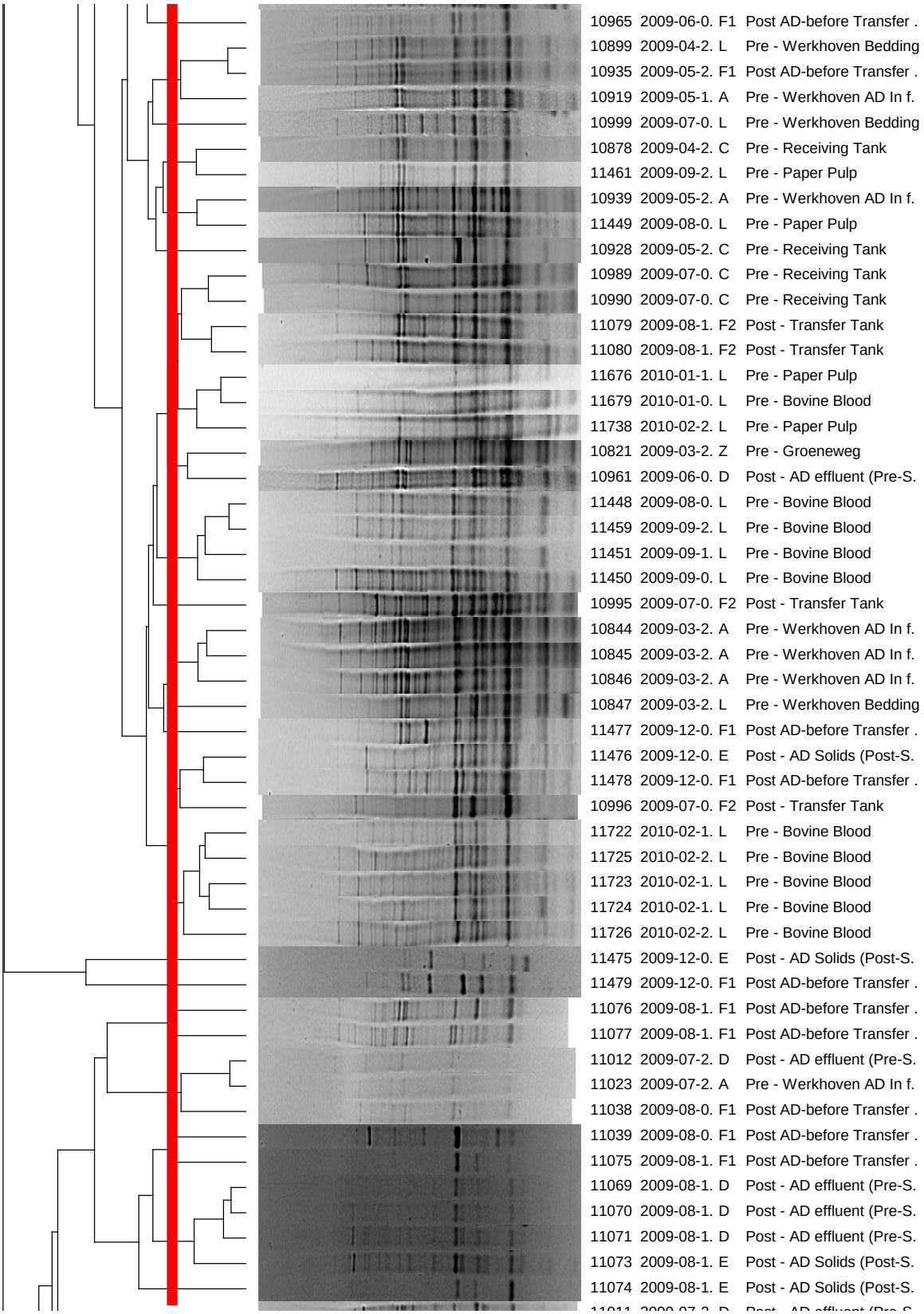
11488 2009-12-0. A Pre - Werkhoven AD In f.
11517 2009-12-1. D Post - AD effluent (Pre-S.
11688 2010-01-2. L Pre - Bovine Blood
10918 2009-05-1. A Pre - Werkhoven AD In f.
10959 2009-06-0. C Pre - Receiving Tank
10960 2009-06-0. D Post - AD effluent (Pre-S.
10963 2009-06-0. F1 Post AD-before Transfer .
10962 2009-06-0. E Post - AD Solids (Post-S.
10964 2009-06-0. F1 Post AD-before Transfer .
10920 2009-05-1. A Pre - Werkhoven AD In f.
10879 2009-04-2. C Pre - Receiving Tank
10937 2009-05-2. F1 Post AD-before Transfer .
10903 2009-05-1. C Pre - Receiving Tank
10905 2009-05-1. C Pre - Receiving Tank
10904 2009-05-1. C Pre - Receiving Tank
10880 2009-04-2. C Pre - Receiving Tank
10898 2009-04-2. L Pre - Werkhoven Bedding
11460 2009-09-2. L Pre - Bovine Blood
11000 2009-07-0. L Pre - Werkhoven Bedding
10965 2009-06-0. F1 Post AD-before Transfer .
10899 2009-04-2. L Pre - Werkhoven Bedding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11461 2009-09-2. L Pre - Paper Pulp
10939 2009-05-2. A Pre - Werkhoven AD In f.
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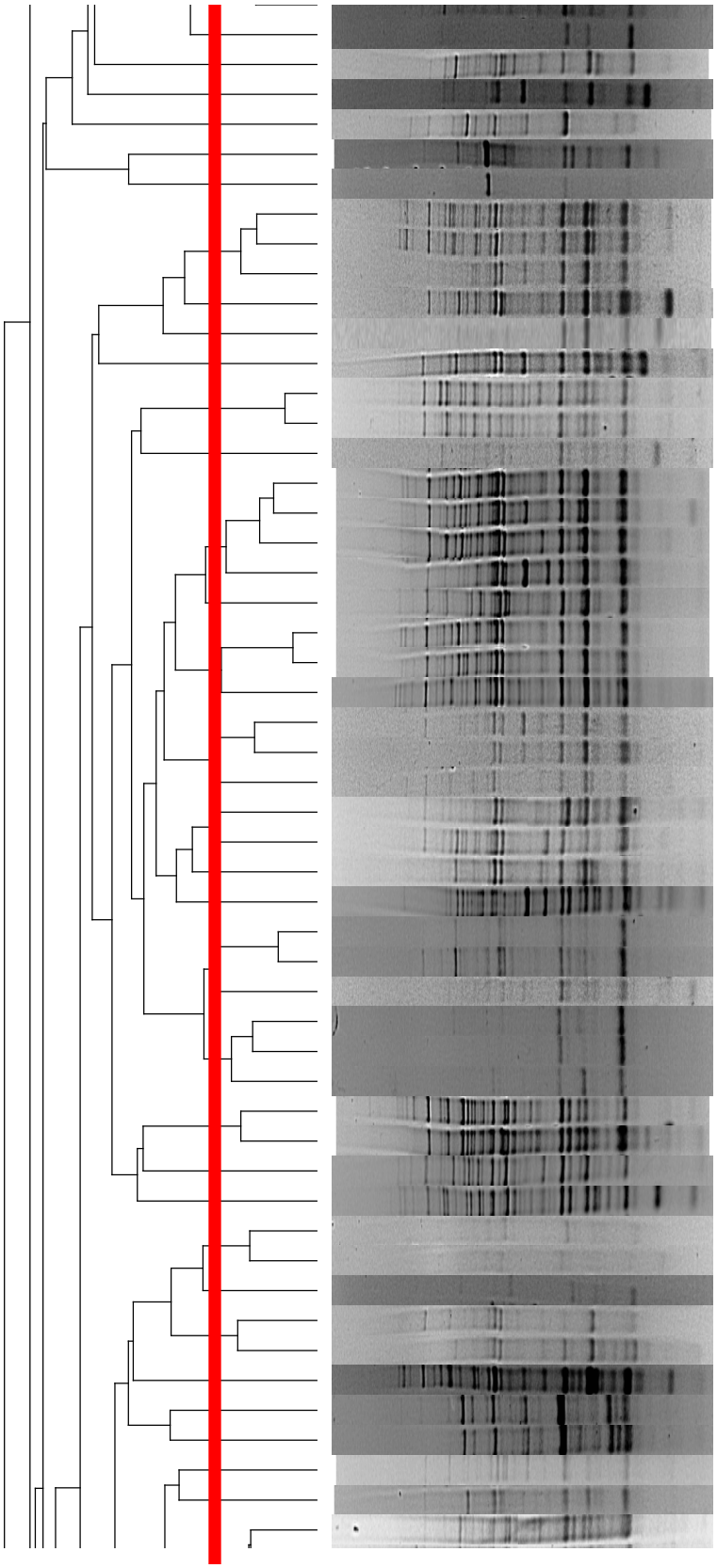
10928 2009-05-2. C Pre - Receiving Tank
10989 2009-07-0. C Pre - Receiving Tank
10990 2009-07-0. C Pre - Receiving Tank
11079 2009-08-1. F2 Post - Transfer Tank
11080 2009-08-1. F2 Post - Transfer Tank
11676 2010-01-1. L Pre - Paper Pulp
11679 2010-01-0. L Pre - Bovine Blood
11738 2010-02-2. L Pre - Paper Pulp
10821 2009-03-2. Z Pre - Groeneweg
10961 2009-06-0. D Post - AD effluent (Pre-S.
11448 2009-08-0. L Pre - Bovine Blood
11459 2009-09-2. L Pre - Bovine Blood
11451 2009-09-1. L Pre - Bovine Blood
11450 2009-09-0. L Pre - Bovine Blood
10995 2009-07-0. F2 Post - Transfer Tank
10844 2009-03-2. A Pre - Werkhoven AD In f.
10845 2009-03-2. A Pre - Werkhoven AD In f.
10846 2009-03-2. A Pre - Werkhoven AD In f.
10847 2009-03-2. L Pre - Werkhoven Bedding
11477 2009-12-0. F1 Post AD-before Transfer .
11476 2009-12-0. E Post - AD Solids (Post-S.
11478 2009-12-0. F1 Post AD-before Transfer .
10996 2009-07-0. F2 Post - Transfer Tank
11722 2010-02-1. L Pre - Bovine Blood
11725 2010-02-2. L Pre - Bovine Blood
11723 2010-02-1. L Pre - Bovine Blood
11724 2010-02-1. L Pre - Bovine Blood
11726 2010-02-2. L Pre - Bovine Blood
11475 2009-12-0. E Post - AD Solids (Post-S.
11479 2009-12-0. F1 Post AD-before Transfer .
11076 2009-08-1. F1 Post AD-before Transfer .
11077 2009-08-1. F1 Post AD-before Transfer .
11012 2009-07-2. D Post - AD effluent (Pre-S.
11023 2009-07-2. A Pre - Werkhoven AD In f.
11038 2009-08-0. F1 Post AD-before Transfer .
11039 2009-08-0. F1 Post AD-before Transfer .
11075 2009-08-1. F1 Post AD-before Transfer .
11069 2009-08-1. D Post - AD effluent (Pre-S.
11070 2009-08-1. D Post - AD effluent (Pre-S.
11071 2009-08-1. D Post - AD effluent (Pre-S.
11073 2009-08-1. E Post - AD Solids (Post-S.
11074 2009-08-1. E Post - AD Solids (Post-S.



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11003	2009-07-2.	Z	Pre - Werkhoven
11035	2009-08-0.	E	Post - AD Solids (Post-S.
11680	2010-01-0.	L	Pre - Bovine Blood
11017	2009-07-2.	F1	Post AD-before Transfer .
11019	2009-07-2.	F2	Post - Transfer Tank
11018	2009-07-2.	F1	Post AD-before Transfer .
11016	2009-07-2.	F1	Post AD-before Transfer .
10934	2009-05-2.	E	Post - AD Solids (Post-S.
10922	2009-05-1.	L	Pre - Werkhoven Bedding
11454	2009-09-1.	L	Pre - Bovine Blood
11455	2009-09-1.	L	Pre - Bovine Blood
11014	2009-07-2.	E	Post - AD Solids (Post-S.
10987	2009-06-2.	A	Pre - Werkhoven AD In f.
10988	2009-06-2.	L	Pre - Werkhoven Bedding
11440	2009-06-2.	L	Pre - Bovine Blood
10982	2009-06-2.	C	Pre - Receiving Tank
10994	2009-07-0.	F2	Post - Transfer Tank
10980	2009-06-2.	Z	Pre - Werkhoven
10981	2009-06-2.	Z	Pre - Groeneweg
10940	2009-05-2.	A	Pre - Werkhoven AD In f.
11001	2009-07-0.	L	Pre - Werkhoven Bedding
11013	2009-07-2.	E	Post - AD Solids (Post-S.
11010	2009-07-2.	D	Post - AD effluent (Pre-S.
11457	2009-09-1.	L	Pre - Paper Pulp
11458	2009-09-2.	L	Pre - Paper Pulp
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10972	2009-06-0.	A	Pre - Werkhoven AD In f.
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11015	2009-07-2.	E	Post - AD Solids (Post-S.
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10974	2009-06-0.	L	Pre - Werkhoven Bedding
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10975	2009-06-0.	L	Pre - Werkhoven Bedding
11671	2010-01-0.	L	Pre - Paper Pulp
10924	2009-05-2.	Z	Pre - Werkhoven
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11037	2009-08-0.	F1	Post AD-before Transfer .
11113	2009-09-2.	Z	Pre - Werkhoven
11151	2009-10-1.	A	Pre - Werkhoven AD In f.
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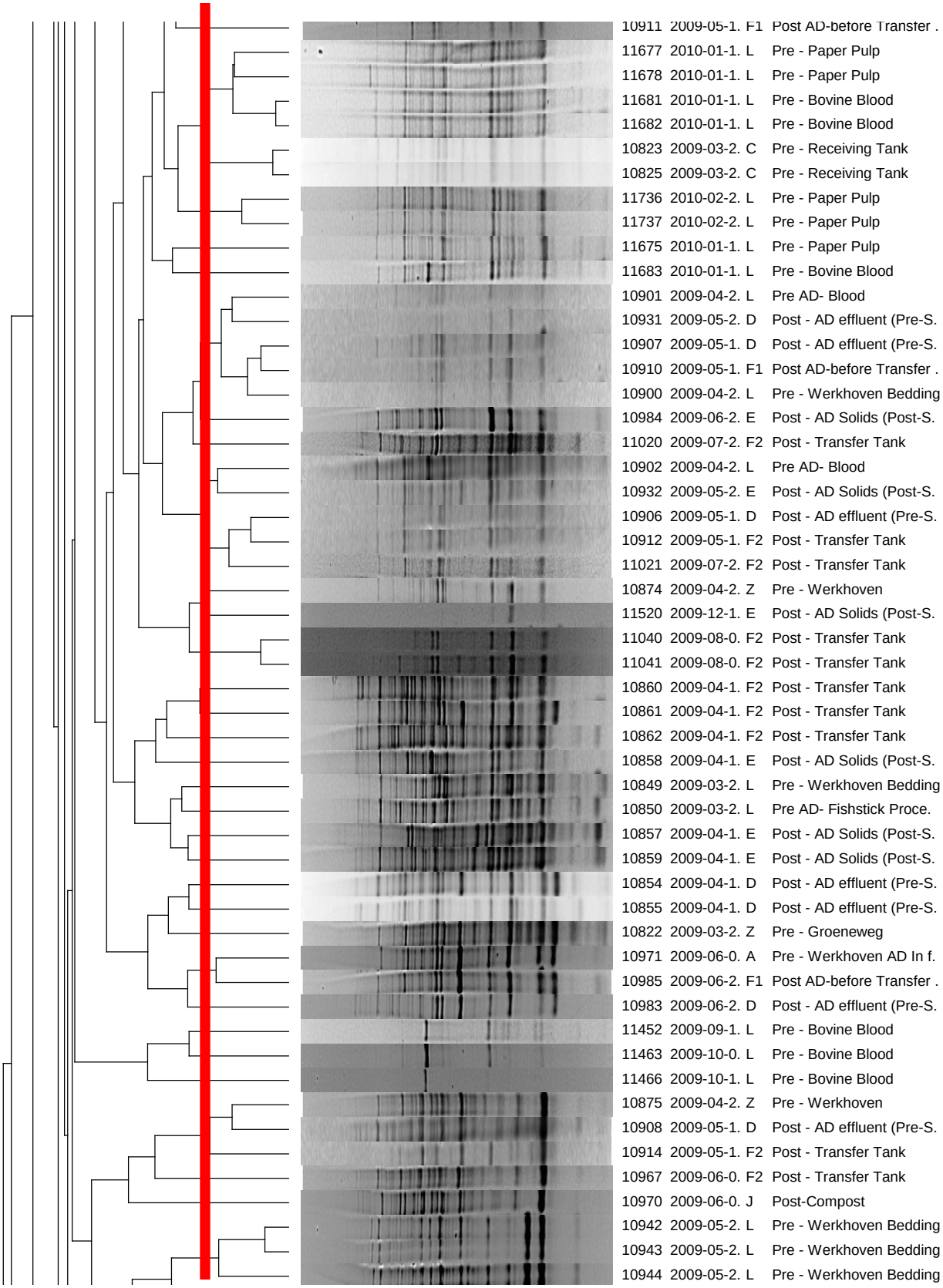
10911	2009-05-1.	F1	Post AD-before Transfer .
11677	2010-01-1.	L	Pre - Paper Pulp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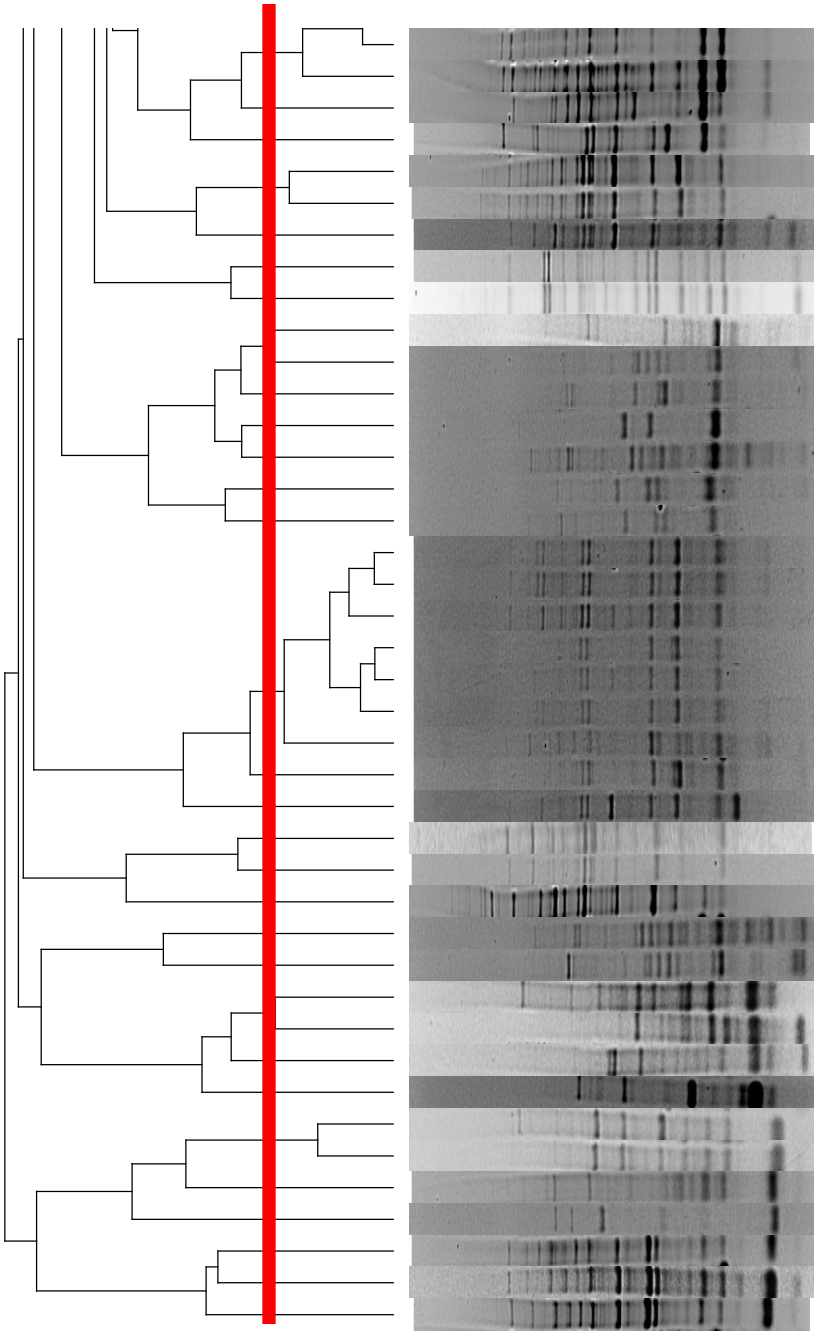
11682 2010-01-1. L Pre - Bovine Blood
10823 2009-03-2. C Pre - Receiving Tank
10825 2009-03-2. C Pre - Receiving Tank
11736 2010-02-2. L Pre - Paper Pulp
11737 2010-02-2. L Pre - Paper Pulp
11675 2010-01-1. L Pre - Paper Pulp
11683 2010-01-1. L Pre - Bovine Blood
10901 2009-04-2. L Pre AD- Blood
10931 2009-05-2. D Post - AD effluent (Pre-S.
10907 2009-05-1. D Post - AD effluent (Pre-S.
10910 2009-05-1. F1 Post AD-before Transfer .
10900 2009-04-2. L Pre - Werkhoven Bedding
10984 2009-06-2. E Post - AD Solids (Post-S.
11020 2009-07-2. F2 Post - Transfer Tank
10902 2009-04-2. L Pre AD- Blood
10932 2009-05-2. E Post - AD Solids (Post-S.
10906 2009-05-1. D Post - AD effluent (Pre-S.
10912 2009-05-1. F2 Post - Transfer Tank
11021 2009-07-2. F2 Post - Transfer Tank
10874 2009-04-2. Z Pre - Werkhoven
11520 2009-12-1. E Post - AD Solids (Post-S.
11040 2009-08-0. F2 Post - Transfer Tank
11041 2009-08-0. F2 Post - Transfer Tank
10860 2009-04-1. F2 Post - Transfer Tank
10861 2009-04-1. F2 Post - Transfer Tank
10862 2009-04-1. F2 Post - Transfer Tank
10858 2009-04-1. E Post - AD Solids (Post-S.
10849 2009-03-2. L Pre - Werkhoven Bedding
10850 2009-03-2. L Pre AD- Fishstick Proce.
10857 2009-04-1. E Post - AD Solids (Post-S.
10859 2009-04-1. E Post - AD Solids (Post-S.
10854 2009-04-1. D Post - AD effluent (Pre-S.
10855 2009-04-1. D Post - AD effluent (Pre-S.
10822 2009-03-2. Z Pre - Groeneweg
10971 2009-06-0. A Pre - Werkhoven AD In f.
10985 2009-06-2. F1 Post AD-before Transfer .
10983 2009-06-2. D Post - AD effluent (Pre-S.
11452 2009-09-1. L Pre - Bovine Blood
11463 2009-10-0. L Pre - Bovine Blood
11466 2009-10-1. L Pre - Bovine Blood
10875 2009-04-2. Z Pre - Werkhoven
10908 2009-05-1. D Post - AD effluent (Pre-S.
10914 2009-05-1. F2 Post - Transfer Tank
10967 2009-06-0. F2 Post - Transfer Tank
10970 2009-06-0. J Post-Compost
10942 2009-05-2. L Pre - Werkhoven Bedding
10943 2009-05-2. L Pre - Werkhoven Bedding
10944 2009-05-2. L Pre - Werkhoven Bedding



10943 2009-05-2. L Pre - Werkhoven Bedding
10944 2009-05-2. L Pre - Werkhoven Bedding
10958 2009-06-0. C Pre - Receiving Tank
10993 2009-07-0. F1 Post AD-before Transfer .
10941 2009-05-2. A Pre - Werkhoven AD In f.
10969 2009-06-0. J Post-Compost
11031 2009-08-0. D Post - AD effluent (Pre-S.
10819 2009-03-2. Z Pre - Werkhoven
10824 2009-03-2. C Pre - Receiving Tank
11336 2010-10-2. E Post - AD Solids (Post-S.
11339 2010-10-2. F1 Post AD-before Transfer .
11338 2010-10-2. F1 Post AD-before Transfer .
11126 2009-09-2. F1 Post AD-before Transfer .
11337 2010-10-2. E Post - AD Solids (Post-S.
11125 2009-09-2. F1 Post AD-before Transfer .
11335 2010-10-2. E Post - AD Solids (Post-S.
11026 2009-07-2. L Pre - Werkhoven Bedding
11027 2009-07-2. L Pre - Werkhoven Bedding
11024 2009-07-2. A Pre - Werkhoven AD In f.
11007 2009-07-2. C Pre - Receiving Tank
11009 2009-07-2. C Pre - Receiving Tank
11025 2009-07-2. L Pre - Werkhoven Bedding
11006 2009-07-2. Z Pre - Groeneweg
11004 2009-07-2. Z Pre - Werkhoven
11022 2009-07-2. A Pre - Werkhoven AD In f.
10896 2009-04-2. A Pre - Werkhoven AD In f.
10925 2009-05-2. Z Pre - Werkhoven
10957 2009-06-0. C Pre - Receiving Tank
11340 2010-10-2. F1 Post AD-before Transfer .
11553 2009-11-1. L Pre - Egg Waste
11691 2010-02-0. L Pre - Bovine Blood
11696 2010-01-2. L Pre - Bovine Whey
11689 2010-01-2. L Pre - Bovine Blood
11633 2010-01-1. L Pre - Paper Pulp
11354 2009-11-1. Z Pre - Werkhoven
11373 2009-11-1. A Pre - Werkhoven AD In f.
10895 2009-04-2. A Pre - Werkhoven AD In f.
11120 2009-09-2. D Post - AD effluent (Pre-S.
10968 2009-06-0. F2 Post - Transfer Tank
10998 2009-07-0. A Pre - Werkhoven AD In f.
10991 2009-07-0. F1 Post AD-before Transfer .

8) Fate of Bacteria upon Lagoon Storage

This component was added in the fall of 2010 after data from this project was presented at the International ASABE Air and Manure Symposium in Dallas, TX in September 2010. A question was asked by NRCS staff at the conference about "what was known about the fate of bacteria from AD manure that had been subsequently stored in a lagoon, would re-growth of bacteria counts occur". Two sets of lagoons located at two dairies that stored either anaerobically digested dairy manure or undigested dairy manure were utilized for this evaluation (2 lagoons with AD manure and 2 lagoons with undigested dairy manure). Samples were taken at ~ 2 week intervals for 7 samplings in the fall of 2010 and early 2011. When possible samples were obtained at 3 depths, bottom (6 ft), mid (3 ft), and top (12 in) of each lagoon.

To determine the fate of bacteria in a static system without inflows, outflows or contribution from weather events, two 5 gallon buckets of AD lagoon content and two of non-AD lagoon content were stored at ambient environmental temperature (6^{o}C to 17^{o}C) and sampled after mixing twice per week for four samplings then every other week for three samplings. The bacteria selected for evaluation were: generic *E. coli*, fecal Enterococcus, and Salmonella for the lagoon samples; and generic *E. coli* and fecal Enterococcus for the bucket study.

Interpretive Statement - Using the GEE population-averaged model, the LogGEC and LogEnterococcus counts were significantly lower in the AD lagoon-stored manure when compared to non-AD lagoon-stored manure than had not been AD treated. Insufficient sample numbers in each level were available to determine if level within the lagoon was a significant factor affecting bacterial concentration. The difference in the number of Campylobacter counts between AD and NonAD systems is statistically significant, with AD manure having lower counts. Depth was not associated with bacterial presence or absence (the term is not statistically significant in a model including it). Salmonella were only isolated from the AD lagoon and analysis of the effect of sample date indicates that the Salmonella concentration differed across the several months of sampling. (table 29 and figures 38 – 41).

Table 29 Summary of enterococccus and generic e-coli in AD and non-AD manure under lagoon storage.

Lagoon System Generic *E. coli* (log CFU/ml)					
Variable	**Level**	**N**	**1st Quartile**	**Median**	**2nd Quartile**
System	AD	67	2.2553	2.4367	3.3010
Depth	Bottom	17	2.3485	2.6368	3.4120
Depth	Middle	25	2.2218	2.3554	3.3282
Depth	Top	25	2.1562	2.4150	3.009
System	NonAD	30	2.7391	3.0412	3.1401
Depth	Bottom	8	2.7258	3.1021	3.3627
Depth	Middle	9	2.9177	3.0792	3.3603
Depth	Top	13	2.6751	2.8151	3.0765
Overall		97	2.3357	2.6690	3.2541

Lagoon System Enterococcus (log CFU/ml)					
Variable	**Level**	**N**	**1st Quartile**	**Median**	**2nd Quartile**
System	AD	67	1.7270	2.0792	2.3291
Depth	Bottom	17	1.9205	2.0792	2.5946
Depth	Middle	25	1.7782	2.0792	2.2922
Depth	Top	25	1.5229	2.0000	2.1663
System	NonAD	30	1.7526	2.2550	2.6164
Depth	Bottom	8	1.9273	2.2284	2.7887
Depth	Middle	9	1.6645	2.3802	2.5642
Depth	Top	13	1.3010	2.2389	2.6276
Overall		97	1.7526	2.1027	2.4573

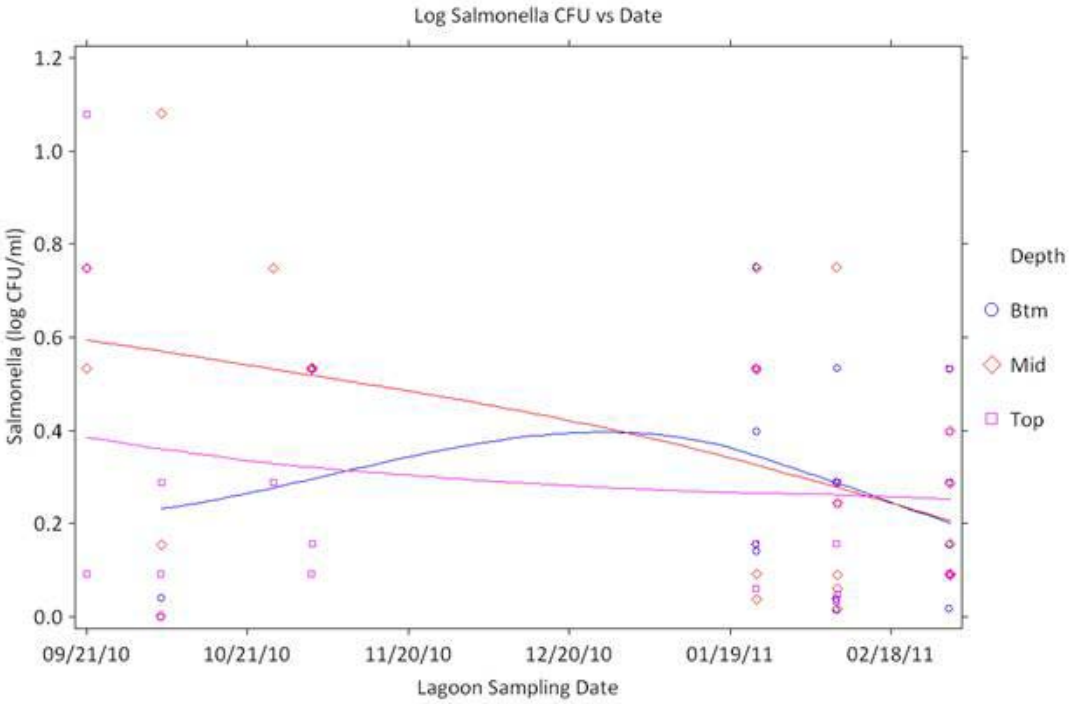


Figure 38 – Concentration of salmonella in manure from the AD lagoon.

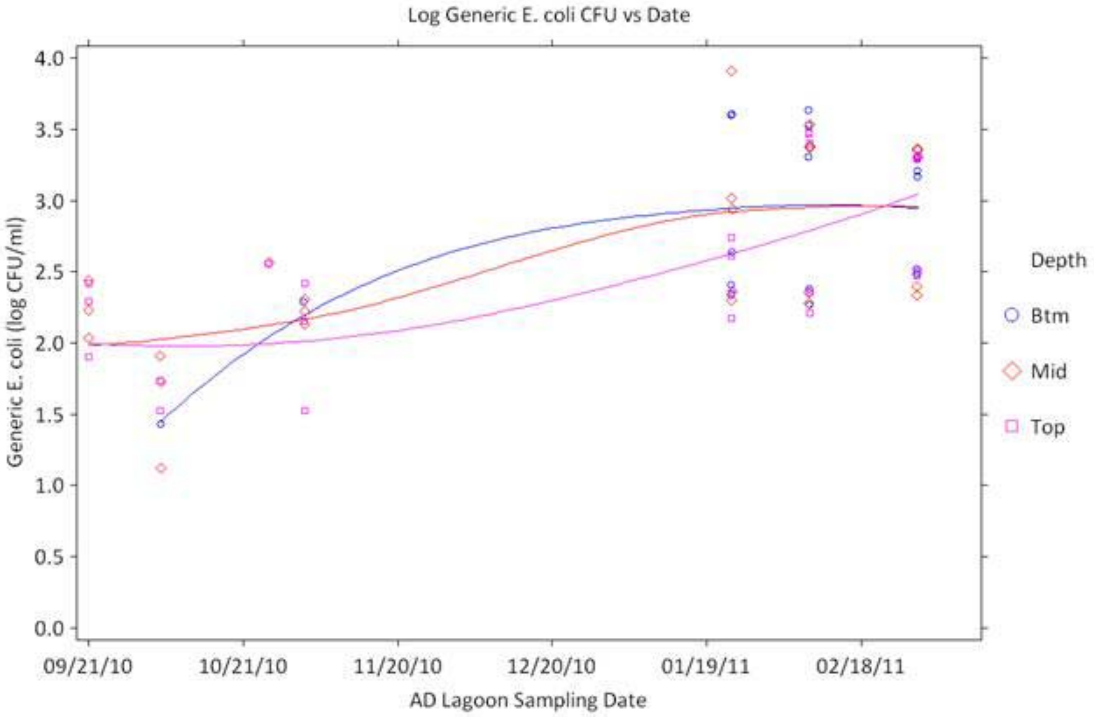


Figure 39 – Concentration of generic *E. coli* in manure from the AD lagoon.

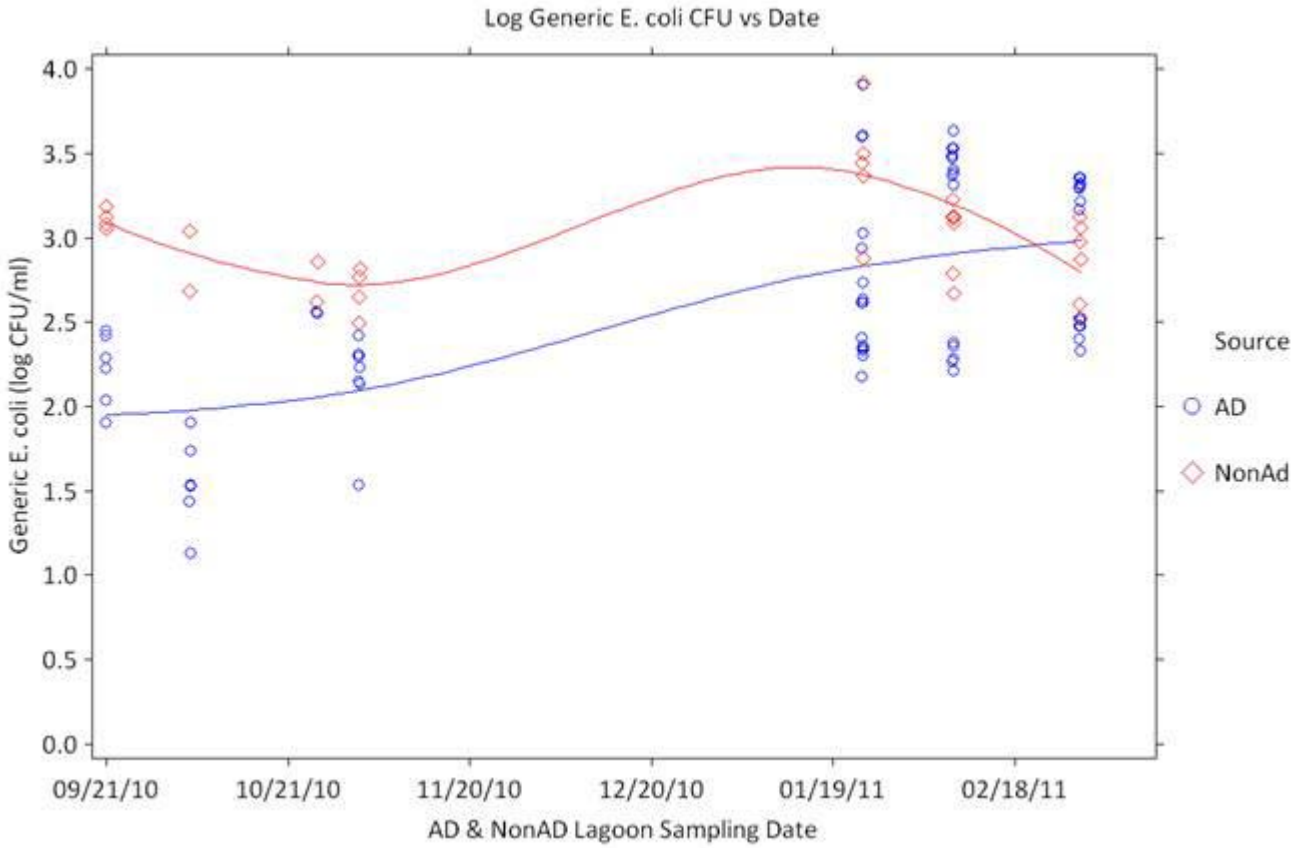


Figure 40 – Concentration of generic *E. coli* in manure from the AD lagoon and non-AD lagoon.

Cross Tabulation of Depth by Campylobacter
Controlling for System = AD

	Campylobacter		
Depth	**0**	**1**	**Total**
Bottom	8	4	12
Row %	66.7	33.3	23.1
Middle	17	3	20
	85.0	15.0	38.5
Top	15	5	20
	75.0	25.0	38.5
Total	40	12	52

Cross Tabulation of Depth by Campylobacter
Controlling for System = NonAD

	Campylobacter		
Depth	**0**	**1**	**Total**
Bottom	4	3	7
Row %	57.1	42.9	29.2
Middle	4	2	6
	66.7	33.3	25.0
Top	5	6	11
	45.5	54.5	45.8
Total	13	11	24

Cases Included 76 Missing Cases 0

Generalized linear models
Optimization : ML

Deviance = 10.45962537
Pearson = 11.80501041

Variance function: V(u) = u
Link function : g(u) = ln(u)

Log likelihood = -26.32447417

No. of obs = 12
Residual df = 9
Scale parameter = 1
(1/df) Deviance = 1.162181
(1/df) Pearson = 1.311668

[Poisson]
[Log]

AIC = 4.887412
BIC = -11.90453

	OIM				
count	Coef.	Std. Err.	z	P>|z|	[95% Conf. Interval]
System	.7731899	.2467741	3.13	0.002	.2895216 1.256858
state	-.8347977	.2496922	-3.34	0.001	-1.324185 -.34541
_cons	1.719	.2176619	7.90	0.000	1.292391 2.14561

The difference in the number of Campylobacter counts between AD and NonAD systems is statistically significant but depth is not a factor (the term is not statistically significant in a model including it).

Depth = Top

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Wilcoxon Rank Sum Test for LgGEC by System

System	Rank Sum	N	U Stat	Mean Rank
AD	245.00	20	35.000	12.3
NonAD	251.00	11	185.00	22.8
Total	496.00	31		

Normal Approximation with Corrections for Continuity and Ties 3.078
Two-tailed P-value for Normal Approximation 0.0021

Total number of values that were tied 7
Maximum difference allowed between ties 0.00001

Cases Included 31 Missing Cases 0

Statistix 9.0 LagoonData315, 3/25/2011, 2:57:45 PM

Wilcoxon Rank Sum Test for LgEntero by System

System	Rank Sum	N	U Stat	Mean Rank
AD	291.50	20	81.500	14.6
NonAD	204.50	11	138.50	18.6
Total	496.00	31		

Normal Approximation with Corrections for Continuity and Ties 1.158
Two-tailed P-value for Normal Approximation 0.2468

Total number of values that were tied 15
Maximum difference allowed between ties 0.00001

Cases Included 31 Missing Cases 0

Depth = Middle

Statistix 9.0 LagoonData315, 3/25/2011, 2:59:12 PM

Wilcoxon Rank Sum Test for LgGEC by System

System	Rank Sum	N	U Stat	Mean Rank
AD	228.00	20	18.000	11.4
NonAD	123.00	6	102.00	20.5
Total	351.00	26		

Exact Permutation Test Two-tailed P-value 0.0082

Normal Approximation with Corrections for Continuity and Ties 2.527
Two-tailed P-value for Normal Approximation 0.0115

Total number of values that were tied 6
Maximum difference allowed between ties 0.00001

Cases Included 26 Missing Cases 0

Statistix 9.0 LagoonData315, 3/25/2011, 2:58:52 PM

Wilcoxon Rank Sum Test for LgEntero by System

System	Rank Sum	N	U Stat	Mean Rank
AD	264.00	20	54.000	13.2
NonAD	87.000	6	66.000	14.5
Total	351.00	26		

Exact Permutation Test Two-tailed P-value 0.7335

Normal Approximation with Corrections for Continuity and Ties 0.335
Two-tailed P-value for Normal Approximation 0.7374

Total number of values that were tied 12
Maximum difference allowed between ties 0.00001

Cases Included 26 Missing Cases 0

Depth = Bottom

Statistix 9.0 LagoonData315, 3/25/2011, 3:00:49 PM

Wilcoxon Rank Sum Test for LgGEC by System

System	Rank Sum	N	U Stat	Mean Rank
AD	114.00	12	36.000	9.5
NonAD	76.000	7	48.000	10.9
Total	190.00	19		

Exact Permutation Test Two-tailed P-value 0.6504

Normal Approximation with Corrections for Continuity and Ties 0.465
Two-tailed P-value for Normal Approximation 0.6420

Total number of values that were tied 0
Maximum difference allowed between ties 0.00001
Cases Included 19 Missing Cases 0

Statistix 9.0 LagoonData315, 3/25/2011, 3:02:36 PM

Wilcoxon Rank Sum Test for LgEntero by System

System	Rank Sum	N	U Stat	Mean Rank
AD	115.00	12	37.000	9.6
NonAD	75.000	7	47.000	10.7
Total	190.00	19		

Exact Permutation Test Two-tailed P-value 0.6960

Normal Approximation with Corrections for Continuity and Ties 0.381
Two-tailed P-value for Normal Approximation 0.7033

Total number of values that were tied 6
Maximum difference allowed between ties 0.00001

Cases Included 19 Missing Cases 0

Crosstabulations AD = Qualco sites, NonAD = anything else:

Salmonella – don't have pair-wise since non-ad did not have salmonella.

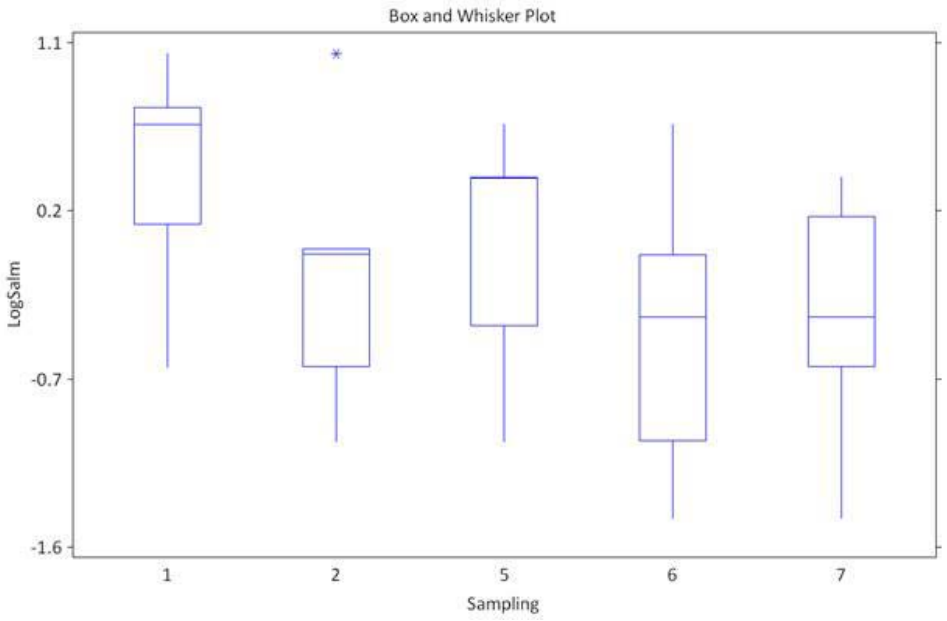

Figure 41 – Box – Whisker plots of concentration of salmonella in manure from the AD lagoon over the 7 sampling times.

```
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Tukey HSD All-Pairwise Comparisons Test of LogSalm by Sampling

Sampling      Mean  Homogeneous Groups
       1    0.4619  A
       3    0.3156  AB
       5  8.75E-03  AB
       2   -0.1473  AB
       7   -0.2900  AB
       6   -0.4762   B

Alpha             0.05     Standard Error for Comparison  0.2120
TO 0.4741
Critical Q Value  4.178    Critical Value for Comparison  0.6264
TO 1.4006
There are 2 groups (A and B) in which the means
are not significantly different from one another.
```

In addition, 5 gallon buckets of manure were stored at ambient temperature and sampled at was twice per week for four samplings then every other week for three samplings to determine the fate of bacteria. Three samples from each of the 2 buckets of AD Lagoon material and 2 buckets of NonAD Lagoon material. The bacteria selected for evaluation were generic e-coli and eneterococcus.

Interpretive Statement - The regression analyses of the die-off data for generic *E. coli* indicates that those in the non-AD lagoon manure began at a higher concentration ($\log_e$ CFU/ml) and died-off faster than those from the AD lagoon manure (see figure 42). The generic *E. coli* in the AD lagoon manure did not increase in concentration initially and died-off at a rate slower than generic *E. coli* from the non-AD lagoon manure. Data on fecal Enterococcus were not sufficient to characterize their die-off differences.

Below is the regression comparing AD material GEC dieoff to NonAD GEC material dieoff. (the enterococcus results were too few to be useful).
The intercept for the NonAD lagoon material is the constant or 6.66720. The intercept for the AD lagoon material is Const – ADConst or 5.1179.
The dieoff curve in logs per day for the NonAD lagoon material is -0.04853. The dieoff in logs per day for the AD lagoon material is Days + ADSlope or -0.01349. All these terms are significant, the most important term being ADSlope, meaning that the dieoff rate of AD Lagoon material is statistically significantly slower than for the NonAD lagoon material (a negative value is faster, a positive value slower). This is the important point.

Least Squares Linear Regression of LnGEC

Predictor Variables	Coefficient	Std Error	T	P	VIF
Constant	6.66720	0.22966	29.03	0.0000	0.0
Days	-0.04853	0.01167	-4.16	0.0004	2.0
ADConst	-1.54921	0.32478	-4.77	0.0001	2.1
ADSlope	0.03504	0.01650	2.12	0.0442	3.1

R-Squared	0.6273	Resid. Mean Square (MSE)	0.35732
Adjusted R-Squared	0.5807	Standard Deviation	0.59776
AICc	-20.405		
PRESS	11.725		

Source	DF	SS	MS	F	P
Regression	3	14.4349	4.81165	13.47	0.0000
Residual	24	8.5756	0.35732		
Total	27	23.0105			
Lack of Fit	10	4.85125	0.48513	1.82	0.1476
Pure Error	14	3.72433	0.26602		

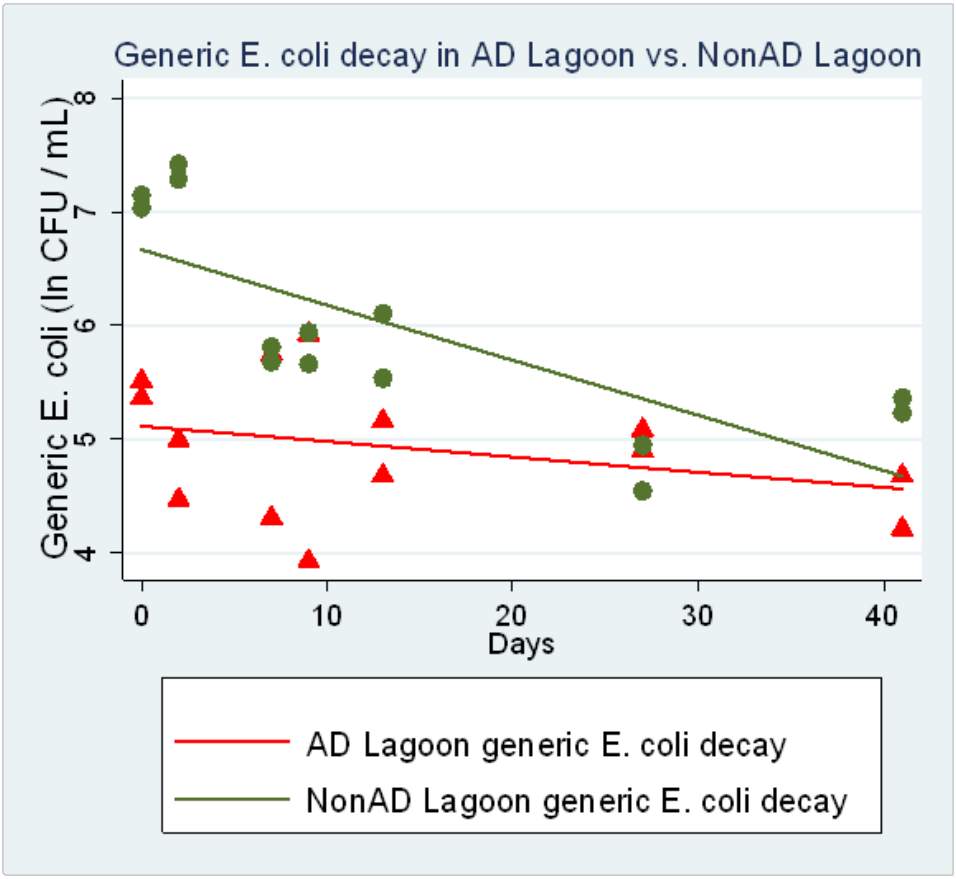

Figure 42 Generic E.coli decay in bucket storage of AD and non-AD manure.

E. Outreach Effort

Field Days

An information Field Day was held July 30, 2010 at the Qualco Anaerobic Digester facility in Monroe, WA. The field day was designed as a round-robin tour style with 6 tours stops.

Tour Stops

Compost and Biosolids – Andy Bary – WSU and Steve Peerce – Daritech

Struvite production – Keith Bowers, Multiform Harvest

Pathogens – Joe Harrison, John Gay, and Russ McClanahan, WSU

Economics - Shannon Neibergs, WSU

Nutrient – Olivia Saunders, Craig Cogger, Ann Marie Fortuna, WSU and Art Groenewg, Hollandia Dairy

Generator building – Andy and Jim Werkhoven

Approximately 50 attendees representing allied ag industry, environmental agencies, dairy producers, and legislative representatives were present. The attendees were asked to complete a post-event on-line survey (see complete survey results in separate document). Responses indicated that the attendees gained an increase in knowledge and that the information was presented at an appropriate technical level.

Webpage

Information from this project was instrumental in developing web presence through the National Livestock and Poultry Environmental Learning Center. The web link is http://www.extension.org/pages/30309/pathogen-reduction-in-anaerobic-digestion-of-manure.

Conference Papers and Presentations

Preliminary reporting of project data was made at conferences, field days, and industry education events on nine occasions in 2009, 2010, and 2011.

Harrison, J.H., Gay, J.M., Davidson, D., Whitefield, E.M., Saunders, O., McClannahan, R., & Fortuna, A. (2010). Effect of Anaerobic Digestion of Dairy Manure on Bacterial Survival after Land Application. *International Symposium on Air Quality and Manure Management for Agriculture Proceedings. ASABE Publication 711P0510cd.* International Symposium on Air Quality and Manure Management for Agriculture, Texas.

Harrison, J H., J M Gay, R McClanahan, E Whitefield, O Saunders, and A M Fortuna. 2011. Managing manure to minimize environmental impact. Proceedings of the 2011 Midwest Manure Summit. Green Bay, WI. Feb 15-16, 2011.

Presentations

Harrison, J H, J M Gay, R McClanahan, E Whitefield, O Saunders, and A M Fortuna. 2011. Managing manure to minimize environmental impact. 2011 Midwest Manure Summit. Green Bay, WI. Feb 15-16, 2011.

Harrison, J.H., Gay, J.M., Davidson, D., Whitefield, E., Saunders, O., McClanahan, R., & Fortuna, A. (2010, September 13). Anaerobic Digestion of Dairy Manure on Bacterial Survival after Land Application. ASABE Air and Manure Conference, Dallas, TX.

Harrison, J.H., & Whitefield, E.M. (2010). Anaerobic Digestion of Manure - Fate and Transport of Bacteria. WSDA Anaerobic Digester Workshop, Mt Vernon, WA.

Harrison, J.H., Gay, J.M., Whitefield, E., & McClanahan, R. (2010, July 30). Fate and Transport of Pathogens. Qualco Field Day, Monroe, WA.

Harrison, J.H., Whitefield, E., Gay, J.M., & McClanahan, R. (2010, April 28). Effect of co-digestion of dairy manure and pre-consumer food wastes on fate of bacteria in post anaerobic digested liquids and solids. EPA AgStar Conference, Green Bay, WI.

Harrison, J.H., Whitefield, E.M., & Saunders, O. (2010, May 19). Pathogen and Nutrient Fate with Anaerobic Digestion. EPA Continuing Education, Monroe, WA.

Harrison, J.H. (2009). December. Role of Anaerobic Digesters in Manure Management. WA DOH Meeting, Olympia.

Harrison, J.H. (2009). Fate of bacteria after anaerobic digestion of manure. WA DOE Meeting, On line (ADOBE).

E.M. Whitefield, J.H Harrison, A.I Bary, C.G Cogger, A. Fortuna, J. Gay, R. McClanahan. 2009. “Overview of Anaerobic Digesters: Nutrient and Pathogen Update”. October 5, 2009. Department of Health: Joint Health Conference. Yakima, WA.

Webcast May 2011

A National webcast summarizing portions of this project is planned for May of 2011 via the National Livestock and Poultry Environmental Learning Center http://www.extension.org/animal+manure+management

Thesis chapter

Olivia Saunders, MS, 2011. Part of this project served as graduate thesis study of Olivia Saunders, WSU Crops and Soils. ENVIRONMENTAL BENEFITS AND CONSEQUENCES OF FIELD APPLIED ANAEROBICALLY DIGESTED DAIRY MANURE FOR FORAGE PRODUCTION.