INVESTIGATING SOURCES OF FECAL CONTAMINATION IN ANTELOPE CREEK

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Outlook

- Background

- Motivation for the study

- Microbial Community Analysis **(V4 region of 16s rRNA gene)**
  - Introduction
  - Results
  - Preliminary findings

- Human host specific method **(HF183 16s rRNA Gene cluster)**
  - Introduction
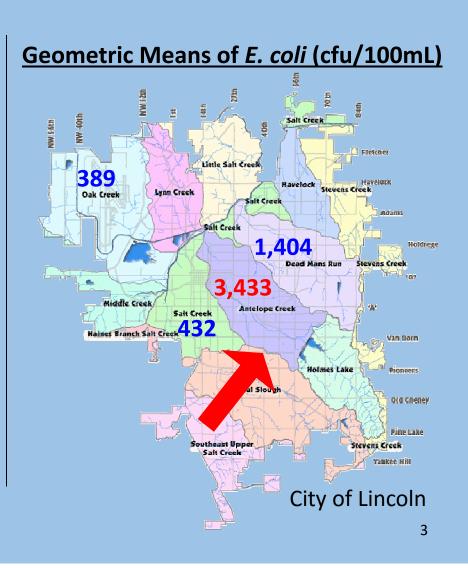 - Results
  - Preliminary findings
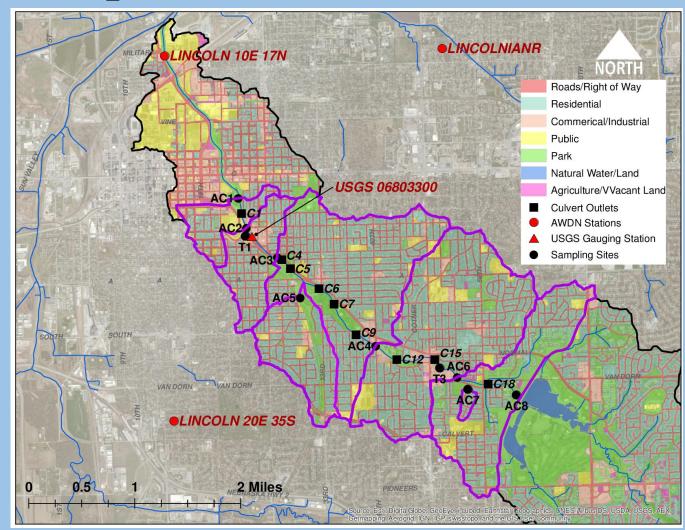
Background

- **Nebraska Surface Water**
  - *E. coli* – 96 impairments
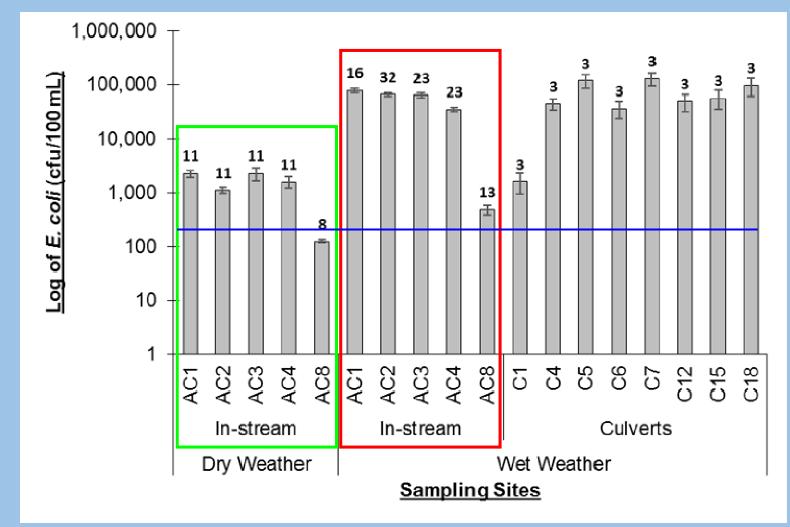 - Nitrogen – 66 impairments
- **Antelope Creek (AC)**
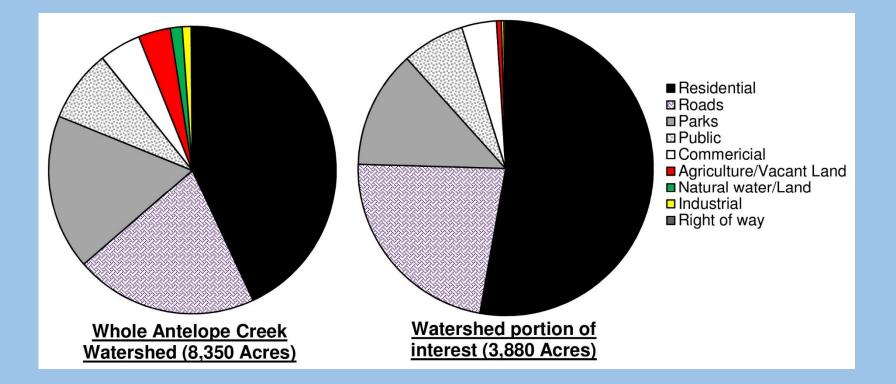 - Impaired due to *E. coli*.
  - TMDL: 113 cfu/100 mL

Geometric Means of *E. coli* (cfu/100mL)

City of Lincoln

Antelope Creek Watershed

E. coli in Antelope Creek Water

Antelope Creek Watershed Land Use

Whole Antelope Creek Watershed (8,350 Acres)

Watershed portion of interest (3,880 Acres)

Legend:
- ■ Residential
- ▨ Roads
- ▨ Parks
- ▨ Public
- ☐ Commericial
- ■ Agriculture/Vacant Land
- ■ Natural water/Land
- ■ Industrial
- ■ Right of way
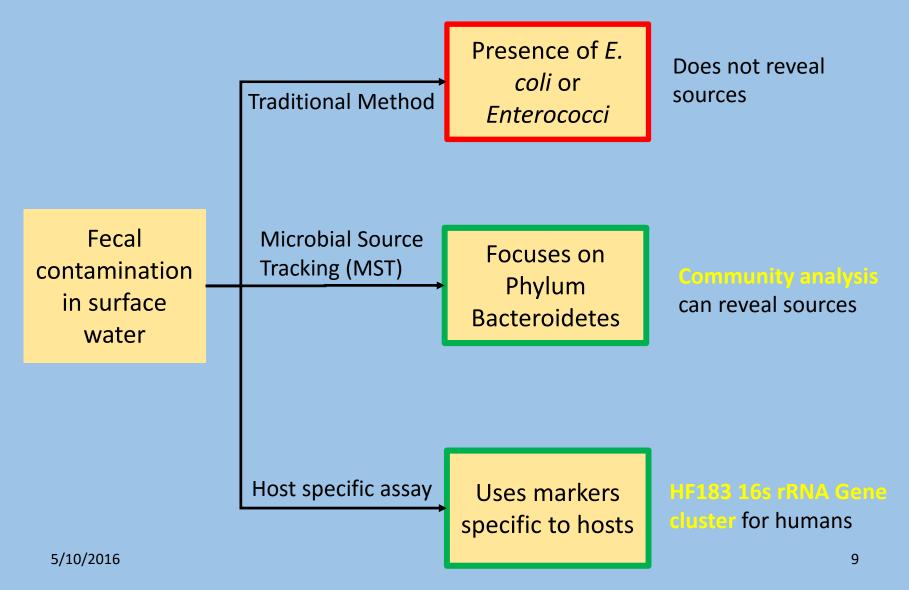
Study Area: Antelope Creek

Objectives

1. **To investigate the sources of fecal contamination to the Antelope Creek**
   - Stream Sediments

   - Eroded Soils

   - Animal Droppings

   - Sanitary Sewage Exfiltration, etc.

2. To determine the proportional contribution of Nitrate (NO_3) from various sources

Investigation of Fecal Contamination

Fecal contamination in surface water

Traditional Method →
Presence of *E. coli* or *Enterococci*

Does not reveal sources

Microbial Source Tracking (MST) →
Focuses on Phylum Bacteroidetes

Community analysis can reveal sources

Host specific assay →
Uses markers specific to hosts

HF183 16s rRNA Gene cluster for humans

Microbial Community Analysis

- **Genomic microbial source tracking (MST) Technique**
- Based on **V4 region of 16S rRNA** gene for prokaryotes (~250 base pair long)
- Sequences reveal microbial community in each sample
- Comparative analysis of microbial community is used for MST
- Used successfully in urban and agricultural settings
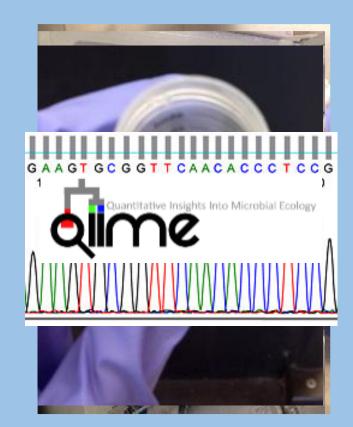 - Dubinsky et al. (2012), Newton et al. (2013), Cao et al. (2011), etc.

| 0 | 100 | 200 | 300 | 400 | 500 | 600 | 700 | 800 | 900 | 1000 | 1100 | 1200 | 1300 | 1400 | 1500 bp |

V1 V2 V3 V4 V5 V6 V7 V8 V9

CONSERVED REGIONS: unspecific applications
VARIABLE REGIONS: group or species-specific applications

Alimetrics.net

Bacteroidetes as a fecal signature

- Obligate anaerobes

- Survival outside animal gut is limited

- More abundant in feces than *E. coli*

- Includes host specific marker groups
    - Order > Bacteroidales
    - **Genus > *Bacteroides***

- Has been used as an indicator of fecal contamination
    - Unno et. al 2012, Dubinsky et al. 2012, etc.

MST Workflow

- Sample Collection
- Preparation
  - Filtration of water through 0.45 μm membrane filters
- Storage at -20°C
- DNA extraction and Purification
- Sequencing
  - Identifying exact sequence of nucleotides
- Bio-informatic Analysis

Sampling (General Overview)

- **In 2013, 2014, and 2015 summer (JUN – SEP)**

- **Dry Weather**
  - when it hasn't rained for at least 48 hours
  - water and sediment from **5 sites** in the creek itself

- **Wet Weather Water**
  - During rain, at multiple stages
    - Rising Limb (RL), Peak Flow (PF), Declining Limb (DL), Post-declining Limb (PDL)
  - **5 sites** in the creek it self
  - Culvert Outlets (Storm drain outlets)
  - Contributing smaller areas
  - Miscellaneous areas (roofs, parking lots, etc.)

Sampling (General Overview)

- **Fecal Samples**
  - Pigeons, Swallows, Ducks, Geese, Other birds, Dogs, Horses, Small mammals, untreated sanitary sewage , etc.

- **Soil**
  - Throughout the watershed
  - Creek embankments

- **Miscellaneous**
  - Street Sweepings
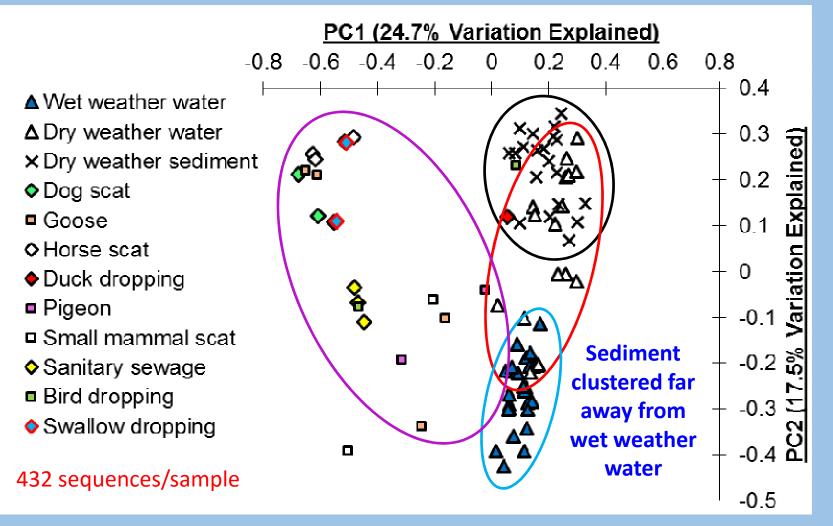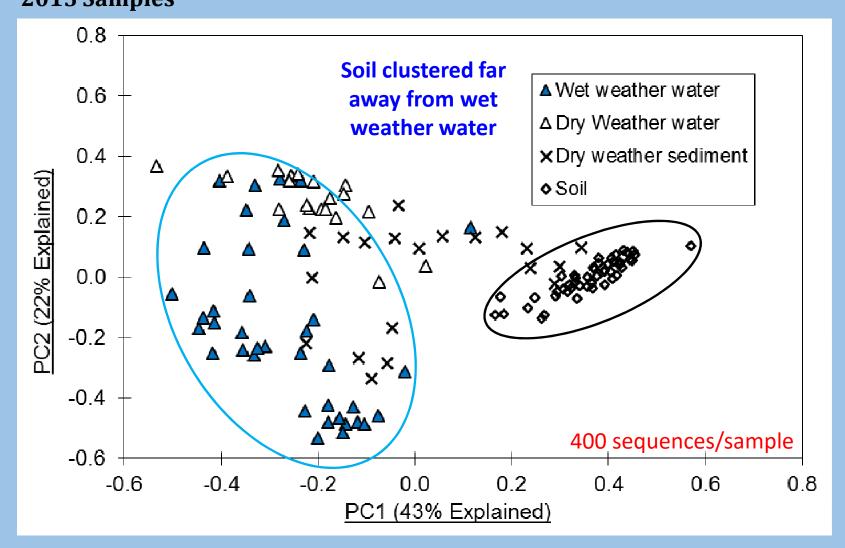 - Rain water
  - Tap water
  - Sump pump water

PCoA (Bacteroidetes)

2013 & 2014 Samples

432 sequences/sample

PCoA (Bacteroidetes)
2015 Samples

Preliminary Findings

Community Analysis

- None of the sources among the ones tested is the sole contributor

- Sediment is not a major source/repository of FIB

- Soil is not a major source/repository of FIB

- Horse, dog, and swallow scat less likely to be major source of fecal contamination

- Sanitary sewage, ducks, small mammals, and birds should be further investigated
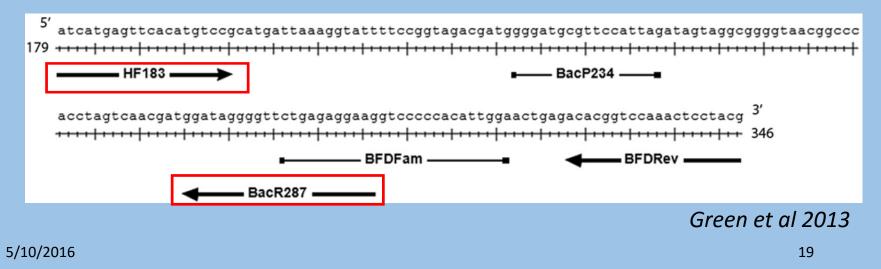
Human host specific assay

- Fecal contamination from **human and cattle sources are likely to pose more human risk** due to the presence of enteric pathogens (Gomi et al. 2014; Harwood et al. 2000).

- Cattle not expected to be a likely source of fecal contamination to the Antelope Creek

- Host specific method for this study will focus on examining human fecal contamination.
  - **HF183 16s rRNA Gene cluster**

Library Independent Method
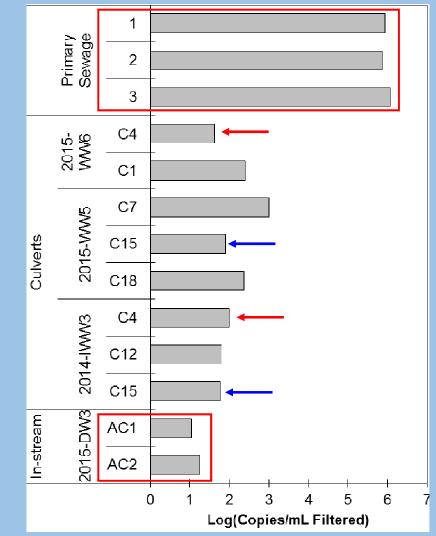
- Targets **HF183 16s rRNA gene cluster**
  - specific to the genus *Bacteroides*
  - Known to be associated with human hosts (*Green et al 2013*)
- Quantitative Polymerase Chain Reaction (qPCR)
  - Primers target the HF183 gene cluster in 16s rRNA
  - Fluorescence due to probes corresponds to amplification
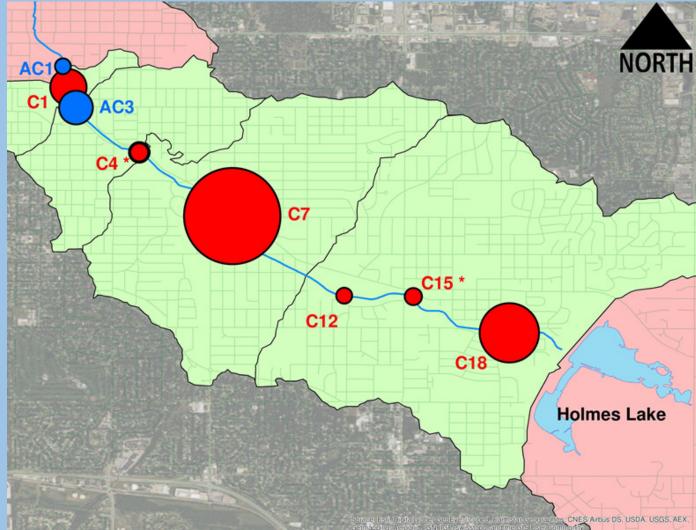 - Comparison against standard will reveal quantity

Green et al 2013

qPCR Results

HF183/BacR287 Assay

Samples	Samples tested	Positive samples	% positive
Dry weather sediment (in-stream sites)	10	0	0%
Wet weather water (in-stream sites)	19	0	0%
Embankment soil	32	0	0%
Sump pump water	2	0	0%
Sediment deposited inside culverts	9	0	0%
Street sweepings	4	0	0%
Dry weather water (in-stream sites)	10	2	20%
Wet weather water (culvert outlets)	25	8	32%
Primary influent sewage	3	3	100%

qPCR Results

qPCR results

Preliminary Findings

- There may be hot spots of human fecal contamination but not necessarily of *E. coli*

- Dry weather sediment, embankment soil, sump pump water, and street sweeping samples **tested negative**
  - not a likely source or a repository of human fecal contamination for Antelope Creek.

- Positive detection in dry weather water but negative detection in wet weather
  - Maybe dilution of contamination during wet weather flows

- Will investigate further

THANK YOU!

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