

Characterization of Fecal Contamination Targeting 16S rRNA *Bacteroides* Molecular Markers in the Santa Cruz River, Arizona

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Outline:

- Surface water pollution
 - Point source
 - Non-point source
- Objective
- Methods
 - -Microbial
 - Results
 - Molecular
 - Results
- On going work

USEPA Surface water quality standards

- Human sources of fecal pollution represent a serious health threat due to the likelihood of pathogens.
- Water quality standards
 - Partial body contact cannot exceed 575 Colony Forming Units (CFU) per 100 mL *E. coli*
 - Full body contact cannot exceed 235 CFU per 100 mL *E. coli*
 - 8 cases of gastrointestinal illness per 1,000 swimmers per year.



Microbial Source Tracking

- Differentiates between human and nonhuman sources of fecal pollution
- Targeting specific genetic sequences in conjunction with specific primers
- MST provides a more complete picture of the areas surrounding watersheds and the impacts associated with fecal contamination.

Objectives of this study

- To differentiate between human and other sources of fecal contamination by targeting 16S rRNA *Bacteroides* in the Santa Cruz River.

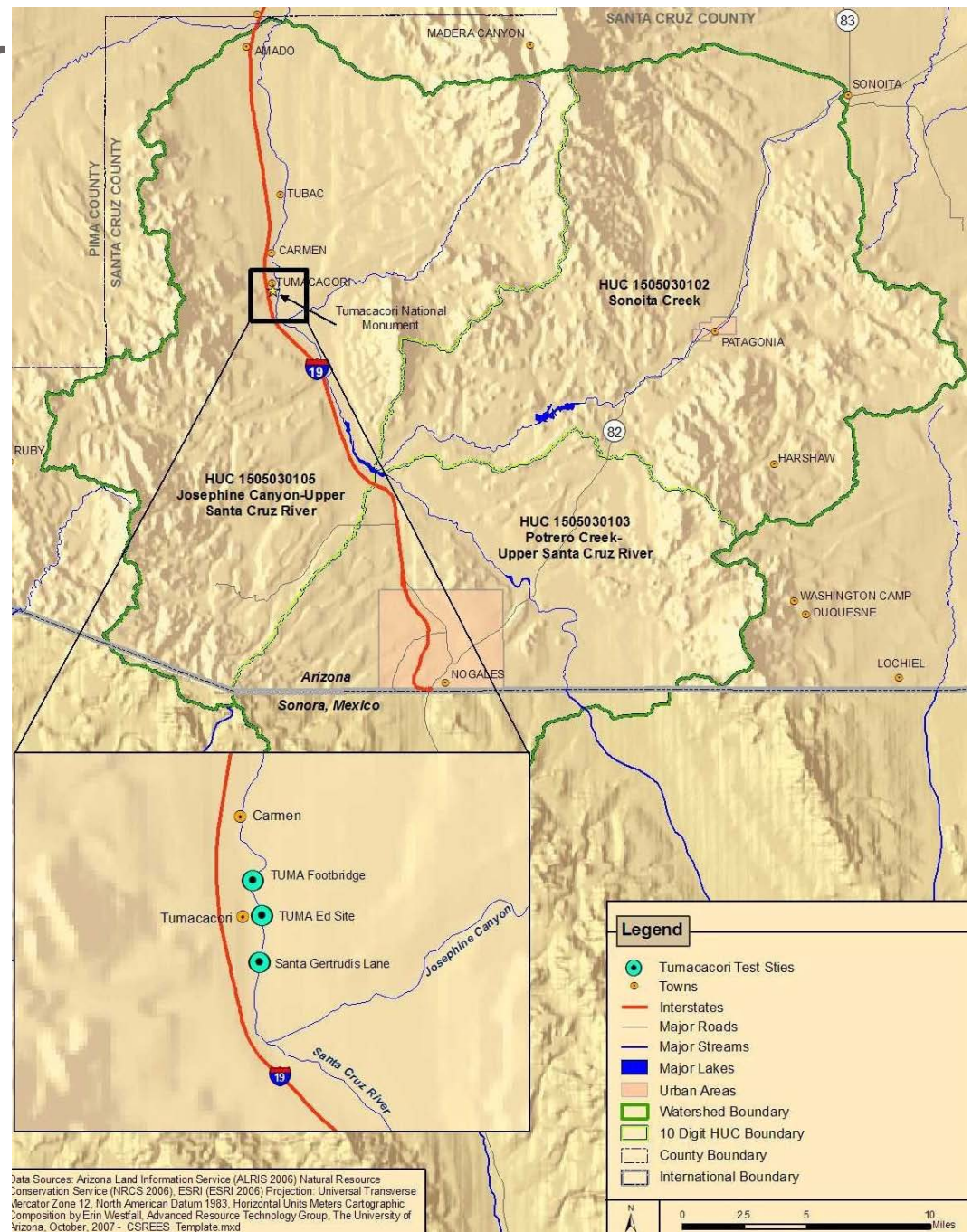
Santa Cruz River

Site 1: Rio Rico Bridge (RRB)

Site 2: Santa Gertrudis Lane (TSG)

Site 3: TUMA Ed Site (TES)

Site 4: TUMA Foot Bridge (TFB)



Materials and Methods

- Duplicate samples were collected in a 1-L sterile container by Tumacacori and U of A personnel.
- Samples were collected from May 26, 2009 through September 27, 2010.
- Samples analyzed seasonally:
 - Spring= March-May
 - Summer= June-August
 - Fall= September-November
 - Winter= December-February
 - *Monsoon= Mid June-late September

Microbial Assay	Target	Method #
Total Coliform	Total coliform group-bacteria possessing the enzyme β -D-galactosidase, which cleaves the chromogenic substrate, resulting in the release of the chromogen.	IDEXX SM #9223B
<i>E. coli</i>	<i>E. coli</i> -bacteria as giving a positive total coliform response and possessing the enzyme β -glucuronidase, which cleaves a fluorogenic substrate, resulting in the release of the fluorogen.	IDEXX SM #9223B
Male-Specific coliphages	RNA or DNA viruses that infect via the F-Pilus of male strains of <i>E. coli</i> .	EPA 1602
Somatic coliphages	DNA viruses that infect host cells via the outer cell membranes.	EPA 1602

Total Coliforms (MPN/100mL)

RRB

TSG

TES

TFB

1.00E+07

1.00E+06

1.00E+05

1.00E+04

1.00E+03

1.00E+02

1.00E+01

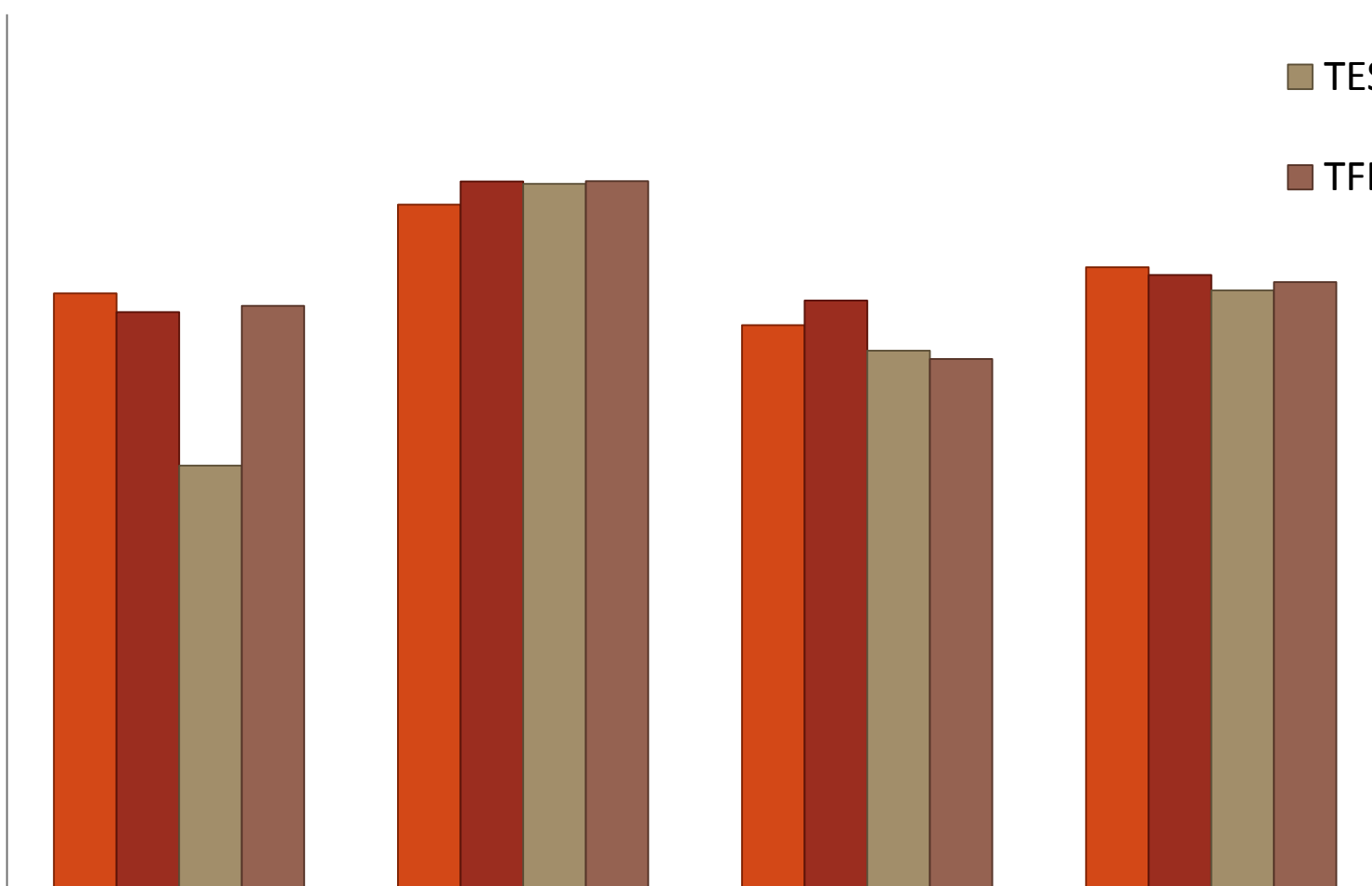
1.00E+00

spring

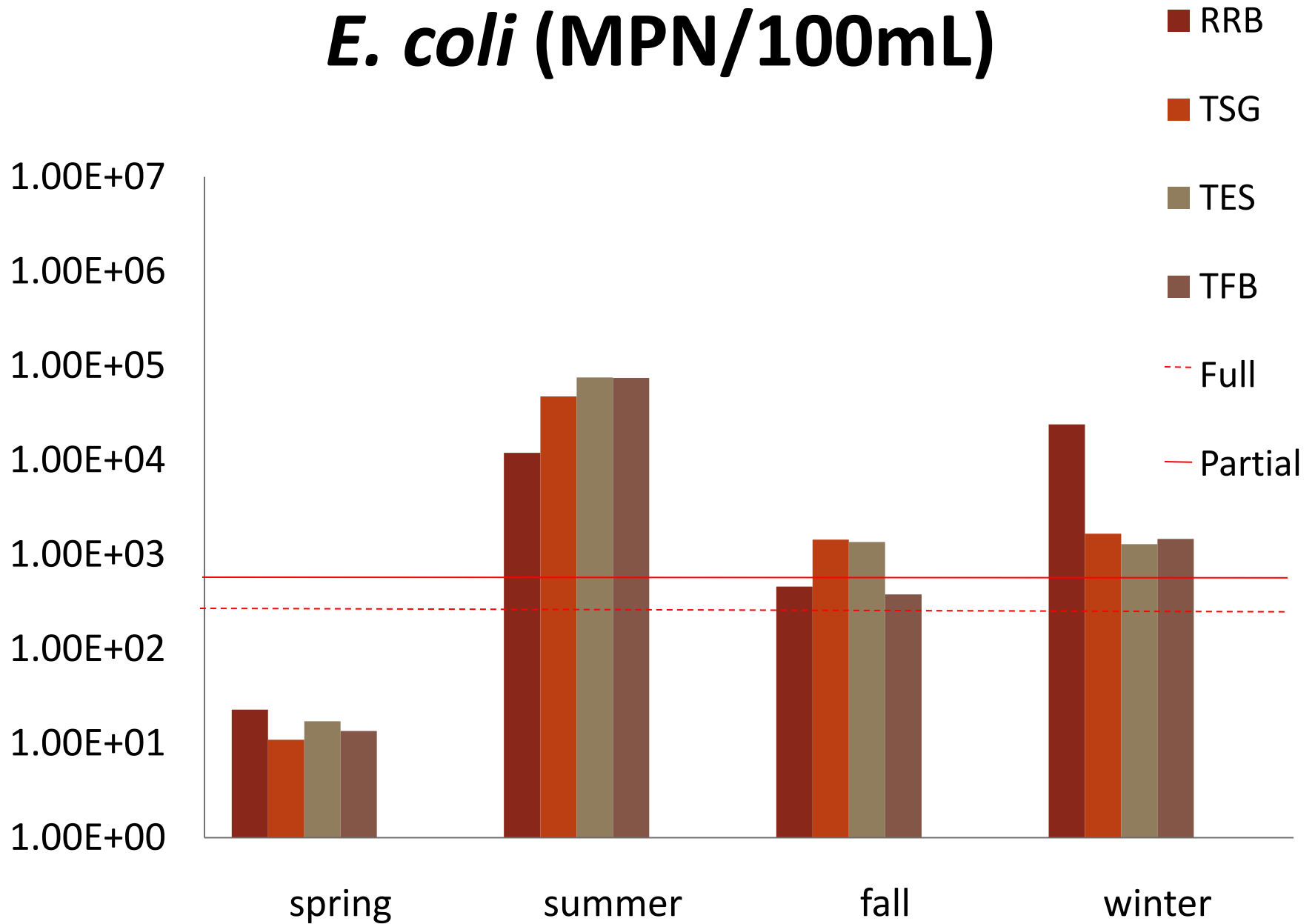
summer

fall

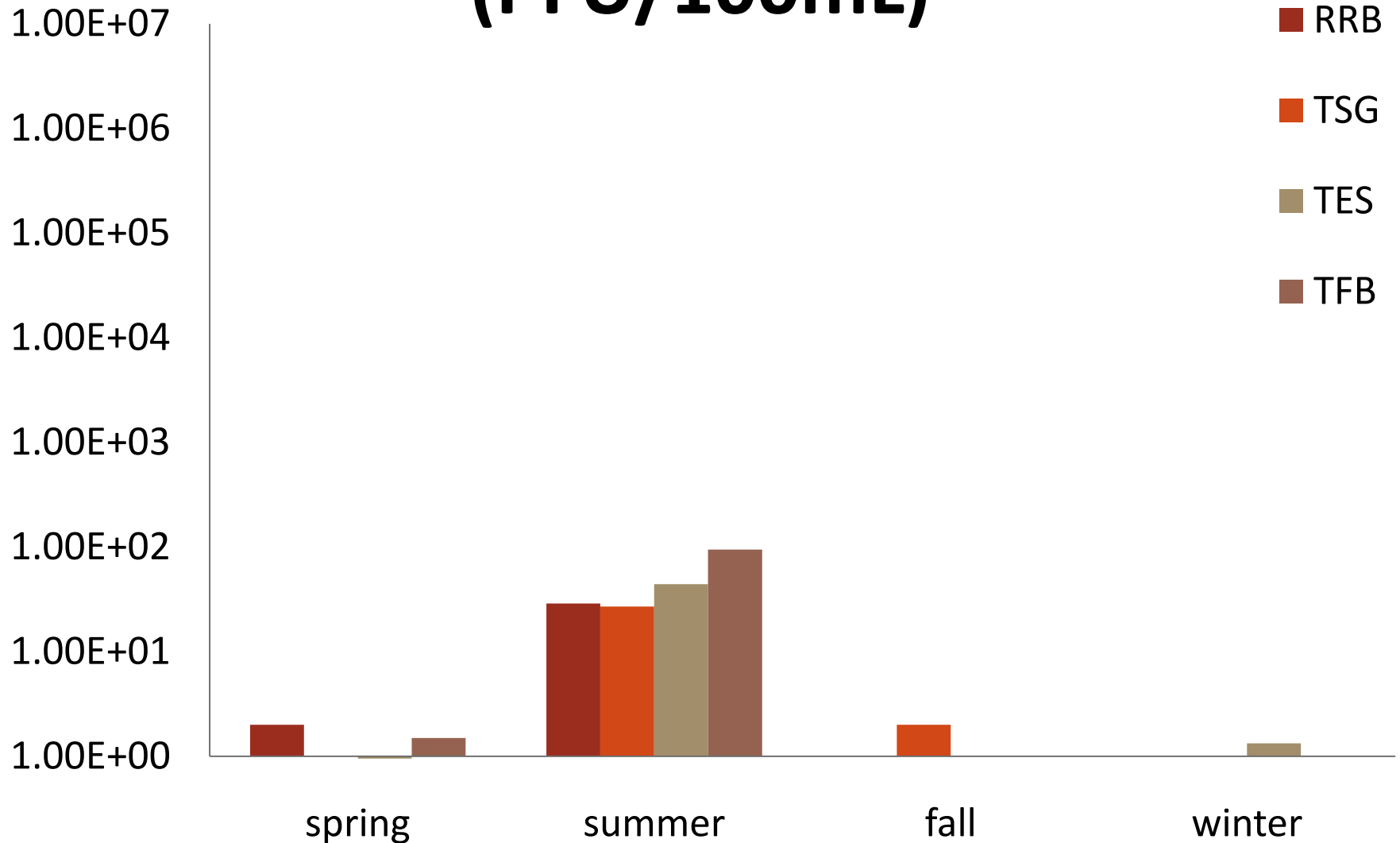
winter



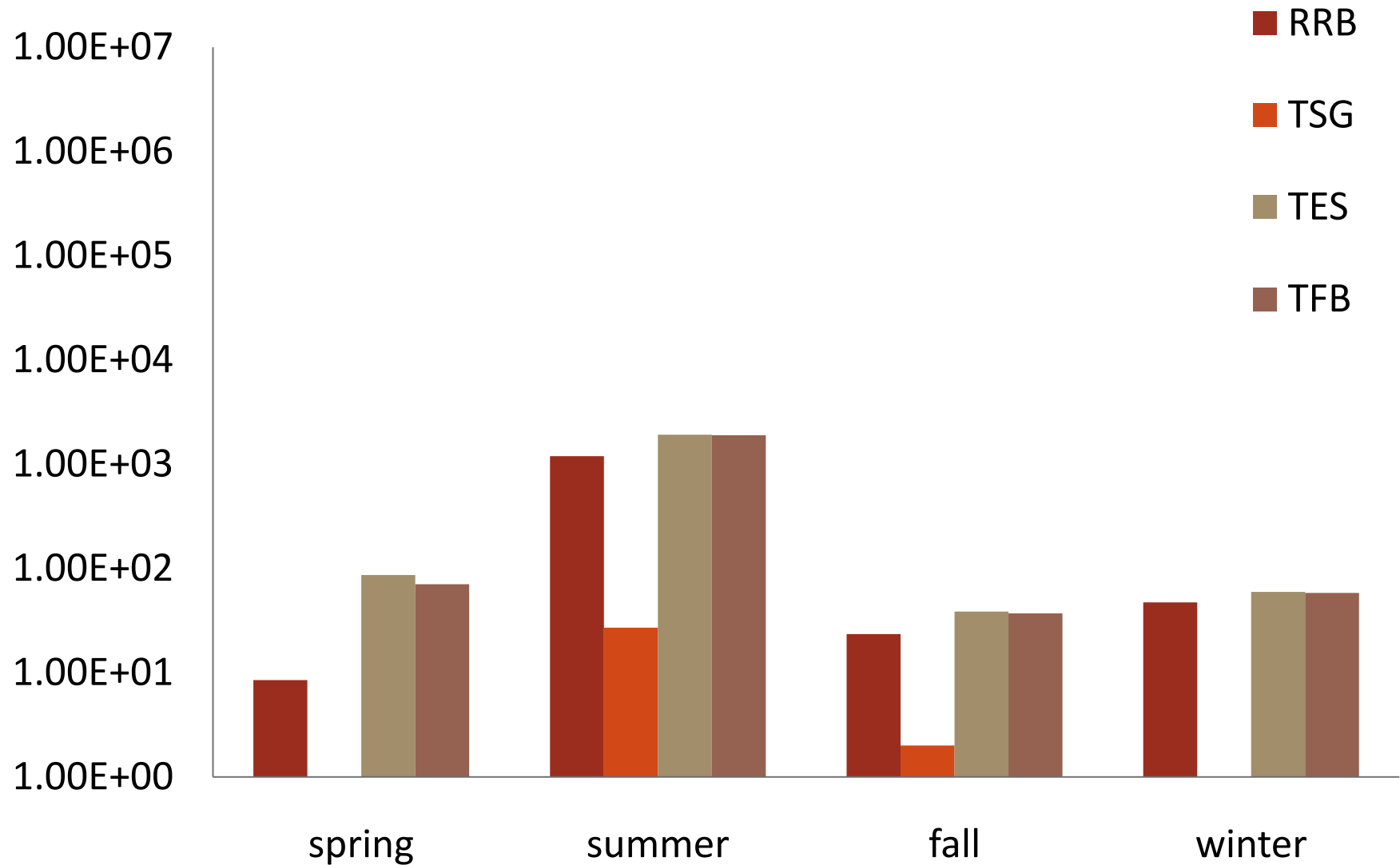
E. coli (MPN/100mL)



Male-Specific Coliphage (PFU/100mL)



Somatic Coliphage (PFU/100mL)



Microbial Results

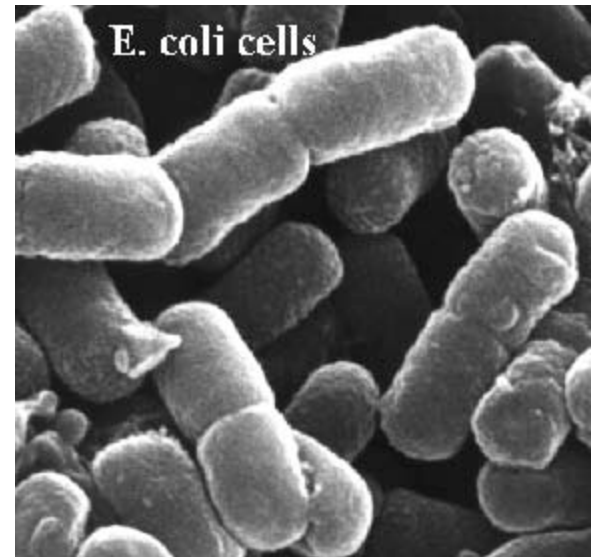
- Throughout the initial study period, all four organisms have been detected within the study area indicating fecal contamination within the watershed.
- Microbial levels seem to fluctuate throughout the watershed indicating that potentially little die-off is occurring.
- Slight increases in microbial parameters are seen among the selected sites indicating non-point source contributions to water quality deterioration.
- Initial analysis suggests that these fluctuations coincide with extreme storm events and thus are a result of increased overland flow.

So what is next

- Although these methods have proven to be useful in indicating presence of *E. coli*, we still do not know the source of contamination.

Problems with *E. coli*

- High degree of genetic diversity not attributed to a specific host animal source
- Potential for *E.coli* to replicate outside of the host
- Geographic and temporal variability



Alternative to *E. coli*: *Bacteroides*

- Bacteria belonging to the genus *Bacteroides* have been suggested as alternative fecal indicators to *E.coli* or fecal coliforms
- They make up a significant portion (30%) of the fecal bacterial population
- Have little potential for growth in the environment
- Have high degree of host specificity that reveal differences in host digestive systems.

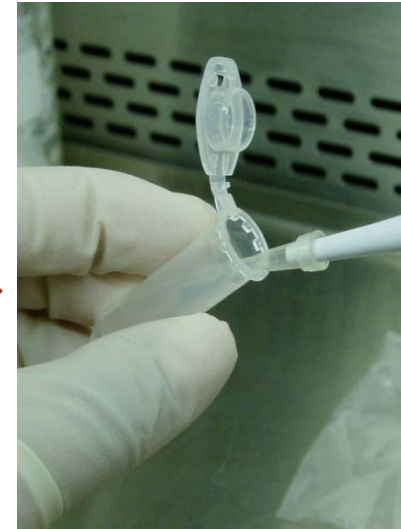
Methods:



Samples collected in
1-L sterile bottles



Samples were concentrated
onto a filter

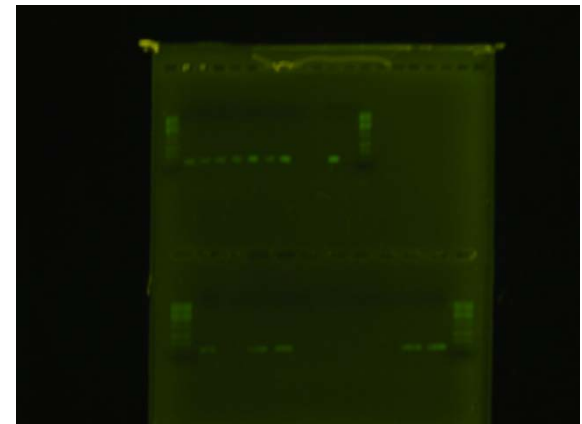


DNA extracted

Primer	Sequence	BP size	Annealing Temp (°C)	Reference
HF 183 f	5'-ATCATGAGTTCACATGTCCG-3'	82	60°C	Seurinck et al, 2005
Newly developed reversed	5'-TACCCCGCCTACTATCTAATG-3'	82	60°C	Seurinck et al, 2005
Allbac 296f	5'-GAGAGGAAGGTCCCCCAC-3'	106	60°C	Layton et al, 2006
Allbac 412r	5'-CGCTACTTGGCTGGTTCAG-3'	106	60°C	Layton et al, 2006

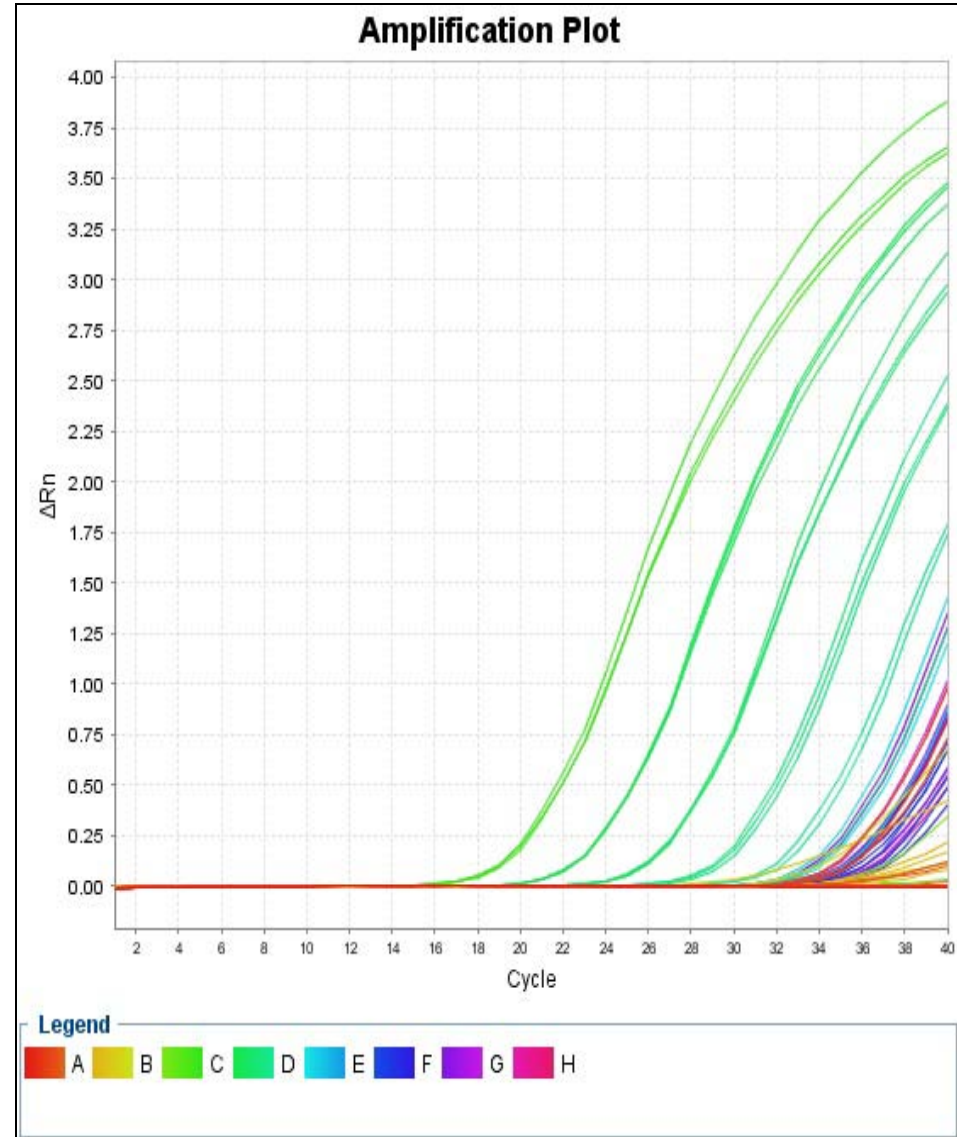
Molecular Methods:

- Conventional Polymerase Chain Reaction (PCR)
 - Was used as initial screening to see if the markers were present.
 - Used specific primer sets
 - Human (HF183f/newly developed reverse)
 - Allbac (Allbac296f/Allbac412r)



Molecular Methods cont.

- Real-Time quantitative PCR:
 - Specific Primers (short pieces of DNA) designed to “bind” only to the DNA sequence from the organism of interest (lock and key).
 - Special dyes such as SYBR green are used to indicate if the Primers “bind” to DNA in the sample.
 - If the binding occurs the dyes emit a fluorescence signal that is then detected by a machine in real time.
 - This signal increases in direct proportion to the amount of DNA in a reaction.



Quality Control

- Standards are used that contain known amount of DNA for the target of interest (ex: human)
- Only if results are the same for all three replicates will an unknown sample be called positive (+) or negative (-)
- Each sample is run in triplicate (3 times)

Molecular Results

- Of the total 58 samples that were analyzed using molecular methods, 52% were positive for Allbac molecular markers.
- Seasonal impacts were apparent. Summer had the highest number of positive Allbac molecular markers for all 4 sites.

Molecular Results

- Of the 58 samples that were analyzed using molecular methods 29% were positive for Human molecular markers.
- The presence of Human molecular markers indicate that possibly failing septic systems and residual bacteria from the NIWTP are currently impacting water quality in the river in the locations mentioned above.

Recommendations

- The study can be used as a tool for community outreach.
 - Teach the community about what is in their watershed and how they can minimize contamination.
- Development of Best Managements Practices with park personnel.

Ongoing Work

- Additional sample analysis will evaluate different environmental conditions and chemical parameters in order to better understand the variations (natural or other) that may be evident in this system.
- Compare other primer sets to assess efficiency and specificity.
 - Pig
 - Bovine
 - Poultry
 - Wildlife

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Questions?