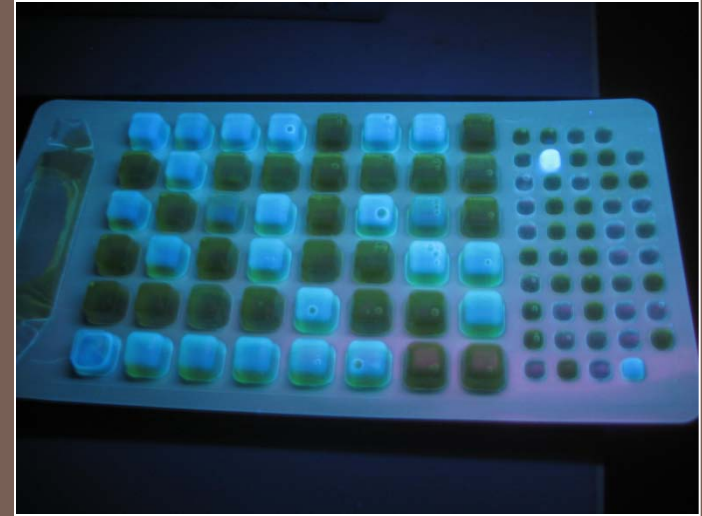




CHARACTERIZING THE SOURCES OF MICROBIAL CONTAMINATION: DNA TESTING OF EXCESSIVE E.COLI IN THE SANTA CRUZ RIVER, TUMACACORI NHP, AZ

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The Problem: Organic Pollution

- Human sewage, animal waste, and plant residue also contain organic material, which can pollute water.
- Bacteria in the water decompose the organic material, producing additional nutrients for plant growth.
- This can cause further decreases in oxygen content in the water.
- Human and animal wastes can also carry harmful bacteria and viruses that can spread diseases caused by **enteric pathogens**.

CONTAMINATION OF SURFACE WATER WITH PATHOGENS



Wastewater



Recreation

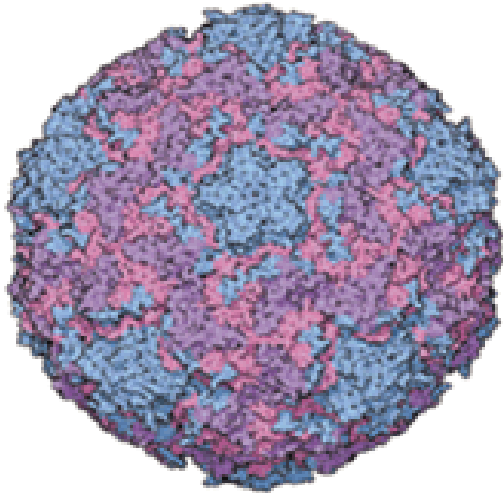


Wildlife

There's *E.coli* in the water, but where did it come from?

Types of Waterborne Pathogens

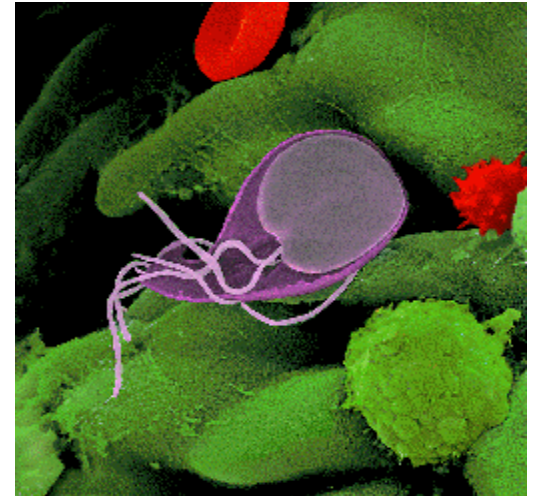
Viruses



Bacteria

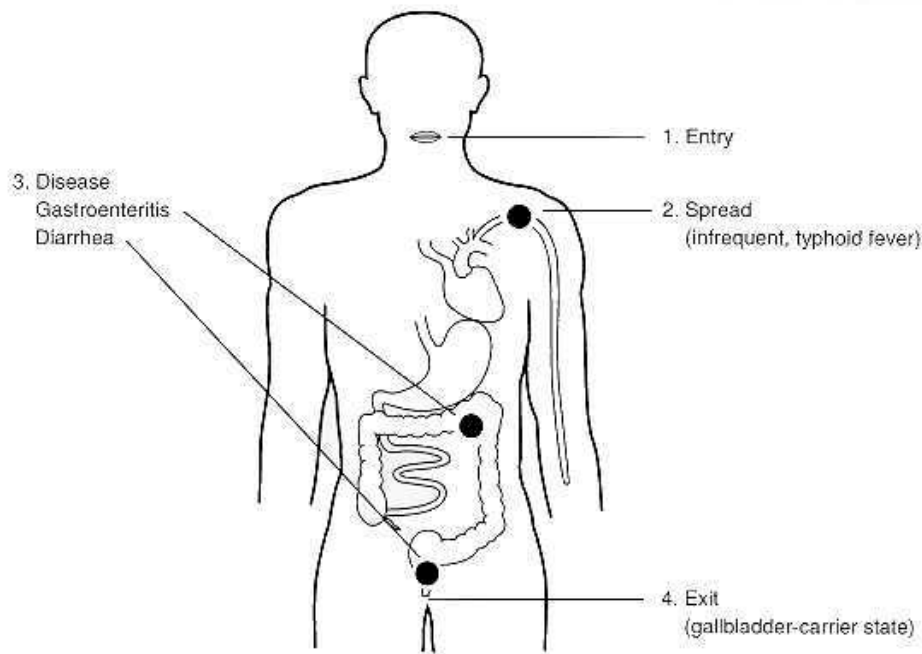


Parasites



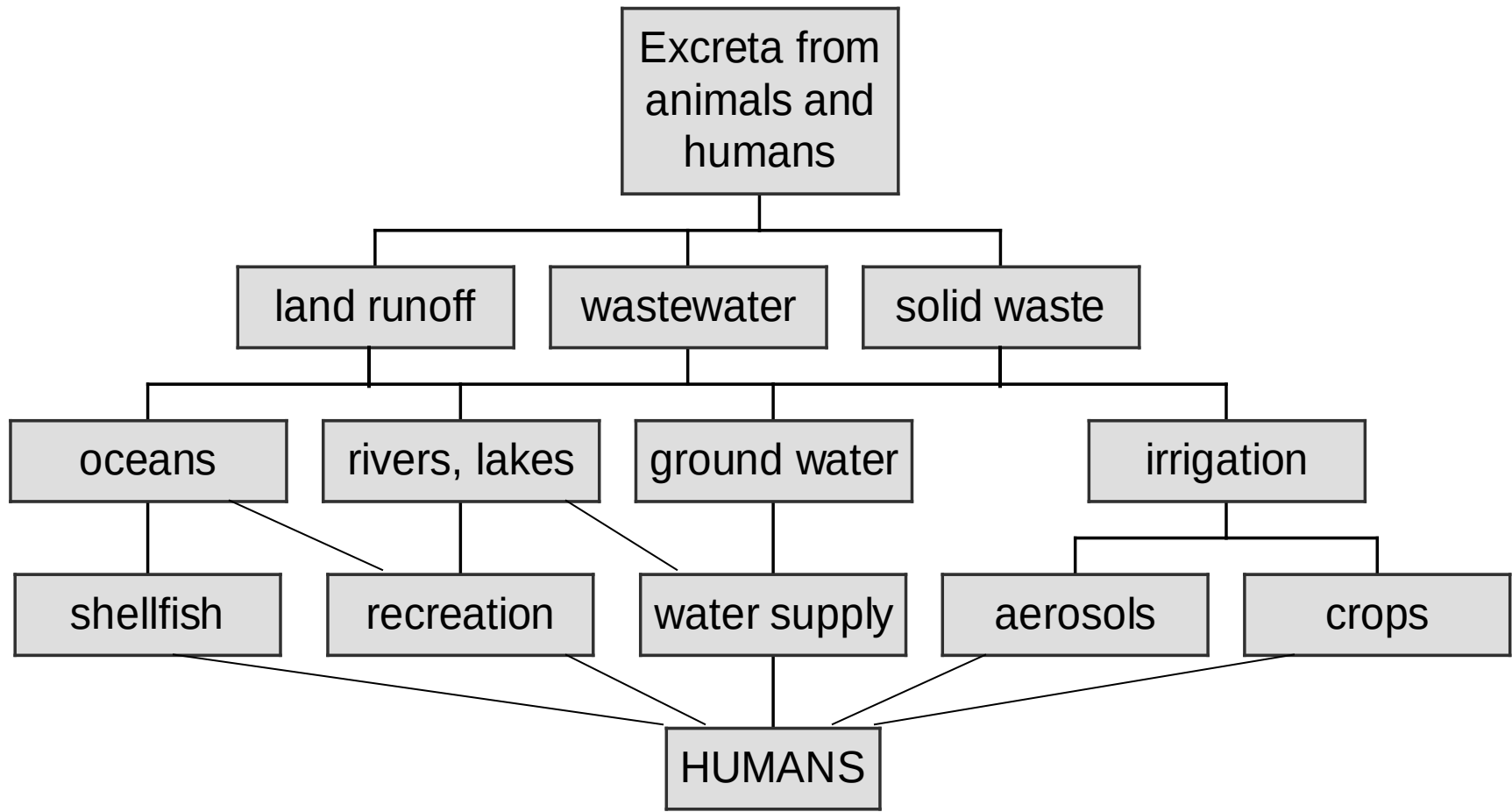
A pathogen is something that causes disease

Enteric Pathogens



- Exposure is via ingestion
- Primary site of infection is gastrointestinal tract
- Gastroenteritis symptoms
 - nausea
 - vomiting
 - diarrhea
 - fever
- May spread to other sites (blood, liver, nervous system)
- Shed in fecal material
- “Fecal-oral” route of transmission

Waterborne Transmission of Organic Pollutants



How can we tell if surface water has
been contaminated by feces?

Indicator Bacteria

Rules for Fecal Indicator Bacteria

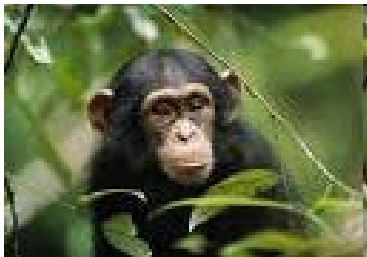
- Fecal Coliforms and *E.coli*
- Bacteria from feces of warm-blooded animals
- Present in higher numbers than pathogens
- Nonpathogenic
- Do not persist longer than the pathogen in the environment

Why not sample for pathogens?

- Few laboratories have the capacity
- It's expensive
- Takes a long time for analysis
- Requires a large volume of water
- Most tests identify only one pathogen
- They are difficult to isolate and identify

What the Heck is *E. coli* ?

- *Escherichia coli* (*E.coli*) is a bacterium naturally found in the intestines and the feces of warm-blooded animals.
- Commonly used as an indicator of **fecal** pollution of water.
- Many different types of *E. coli*, most harmless, but some may cause illness (e.g. hamburger restaurant outbreaks caused by *E. coli* O157:H7)

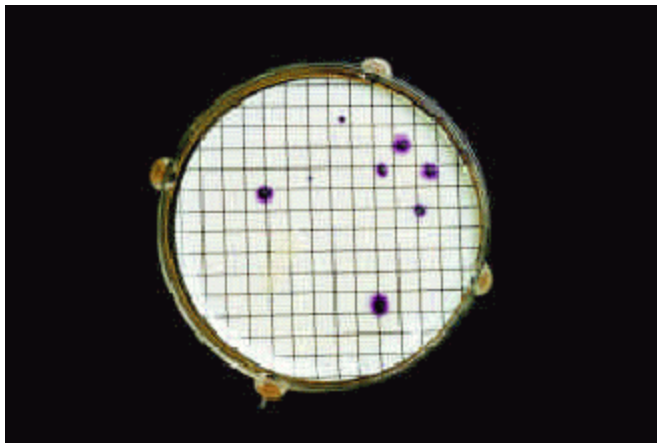
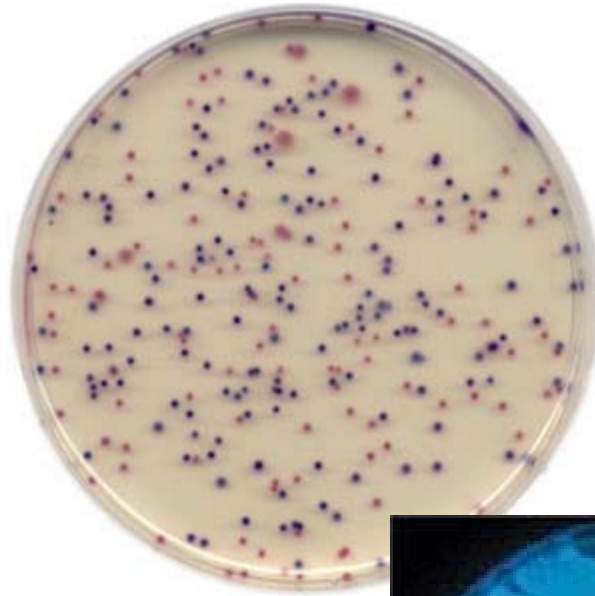
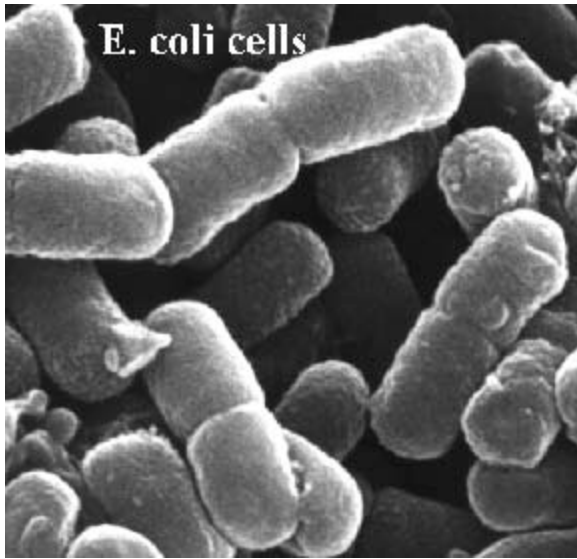


E.coli are used as indicators because they:



- Indicate fecal contamination
- Suggest the presence of pathogens
- Are easy to collect and analyze
- Are relatively safe to handle and generally harmless

What Does *E.coli* Look Like?

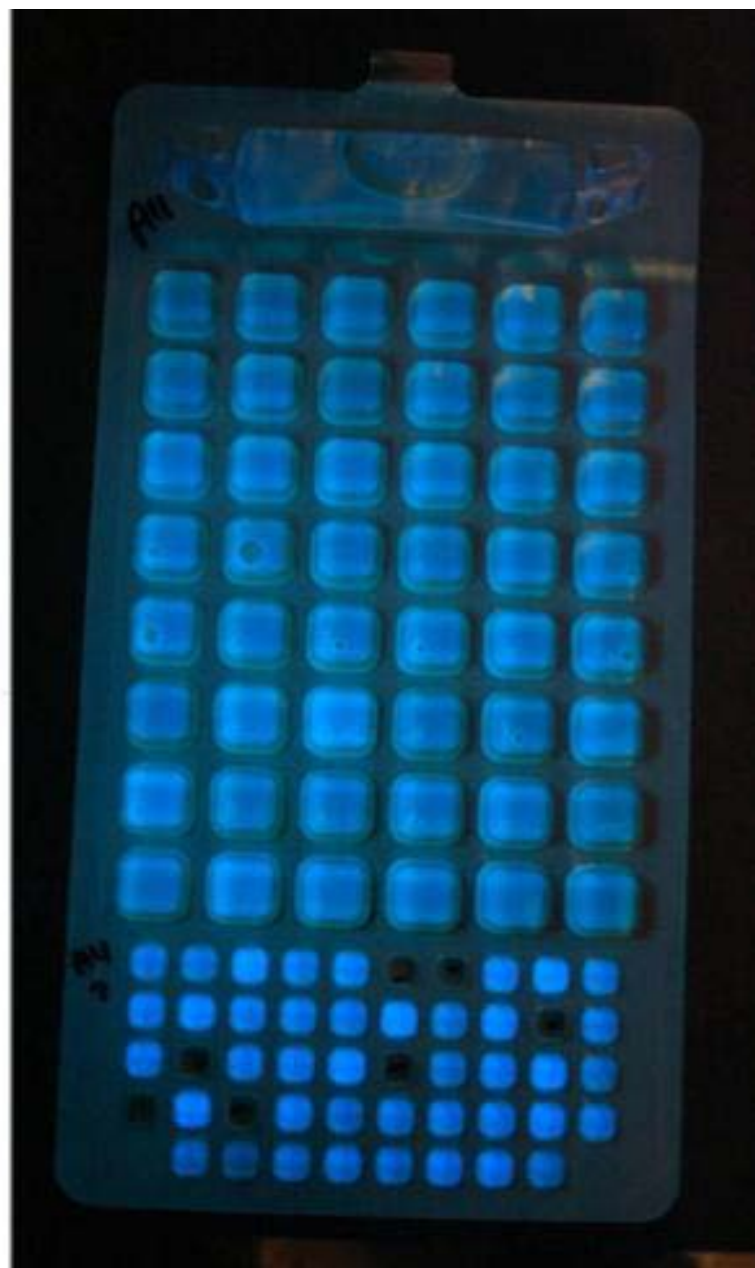


Isolating *E. coli* from water to determine fecal pollution

- Laboratory tests can distinguish one strain from another
- Cultural Methods:
 - Colilert: Enzyme Reaction – color change
 - Membrane filtration – bacteria absorb nutrients from the agar and multiply, forming cells which can be visualized as a “colony” and counted.
- Molecular Methods:

Polymerase chain reaction

Presence (+) Absence (-); does not determine if living.



Environmental Impact

- The presence of *E.coli* bacteria in aquatic environments indicates that the water has been contaminated with the fecal material of man or other animals.
- If *E.coli* is present, the source water may have been contaminated by pathogens or disease producing bacteria or viruses which can also exist in fecal material.
- The presence of fecal contamination is an indicator that a potential health risk exists for individuals exposed to this water.

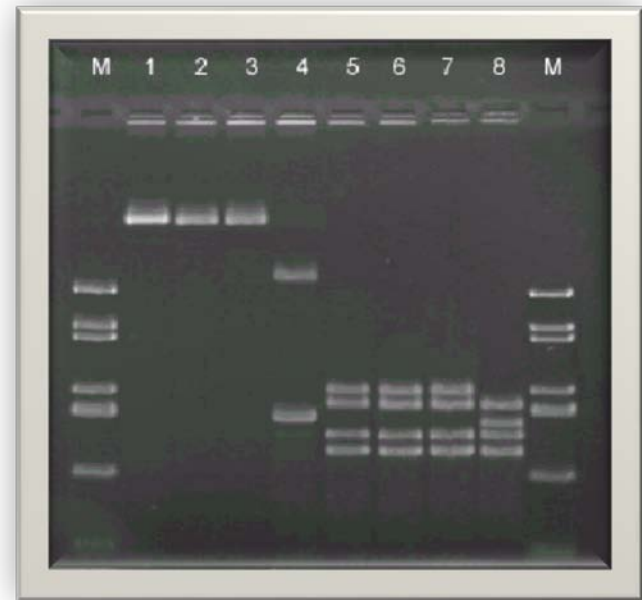
There's *E.coli* in the Water, But Where Did it Come From?

- Microbial Source Tracking (MST) uses laboratory tests to determine if *E.coli* (or other fecal bacteria) in water samples came from animal or human feces



Polymerase Chain Reaction

- ❑ Polymerase Chain Reaction (PCR) amplifies the genetic material or DNA/RNA of the bacteria of interest



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

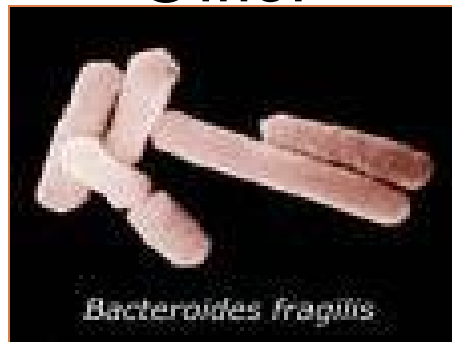
Alternatives to *E.coli* : *Bacteroides*

- 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 of the fecal bacterial population
- Have little potential for re-growth in the environment
- Have a high degree of host specificity that likely reflects differences in host animal digestive systems

Microbial Source Tracking

- These methods are designed to target specific DNA sequences present in feces from different animals
- Specific Primers and Probes are designed to “bind” only to the DNA/RNA from the organism of interest (lock and key)

Other



Human



Bovine



GOAL:

Determine the potential human or animal sources of *E.coli* contamination of water to aid development of TMDLs and watershed protection strategies

Project Objectives

- Tumacacori National Historical Park (NHP) is responsible for management of its water resources for the benefit of the park and for the health of its visitors.
- The existence of microbial contaminants in the park poses risks that must be considered in park planning and operations.
- An assessment of potential risks in this area will provide needed information for public and private decision making.

Approach

- Microbial Indicators
 - ▣ Total Fecal Coliform
 - ▣ *E.coli*
 - ▣ Bacteriophage (virus)
 - ▣ *Bacteroides*
- Physical and Chemical Analysis
 - ▣ pH, Temperature, Turbidity, Residual Chlorine
- Molecular confirmation of isolates by Polymerase Chain Reaction



Sample Collection

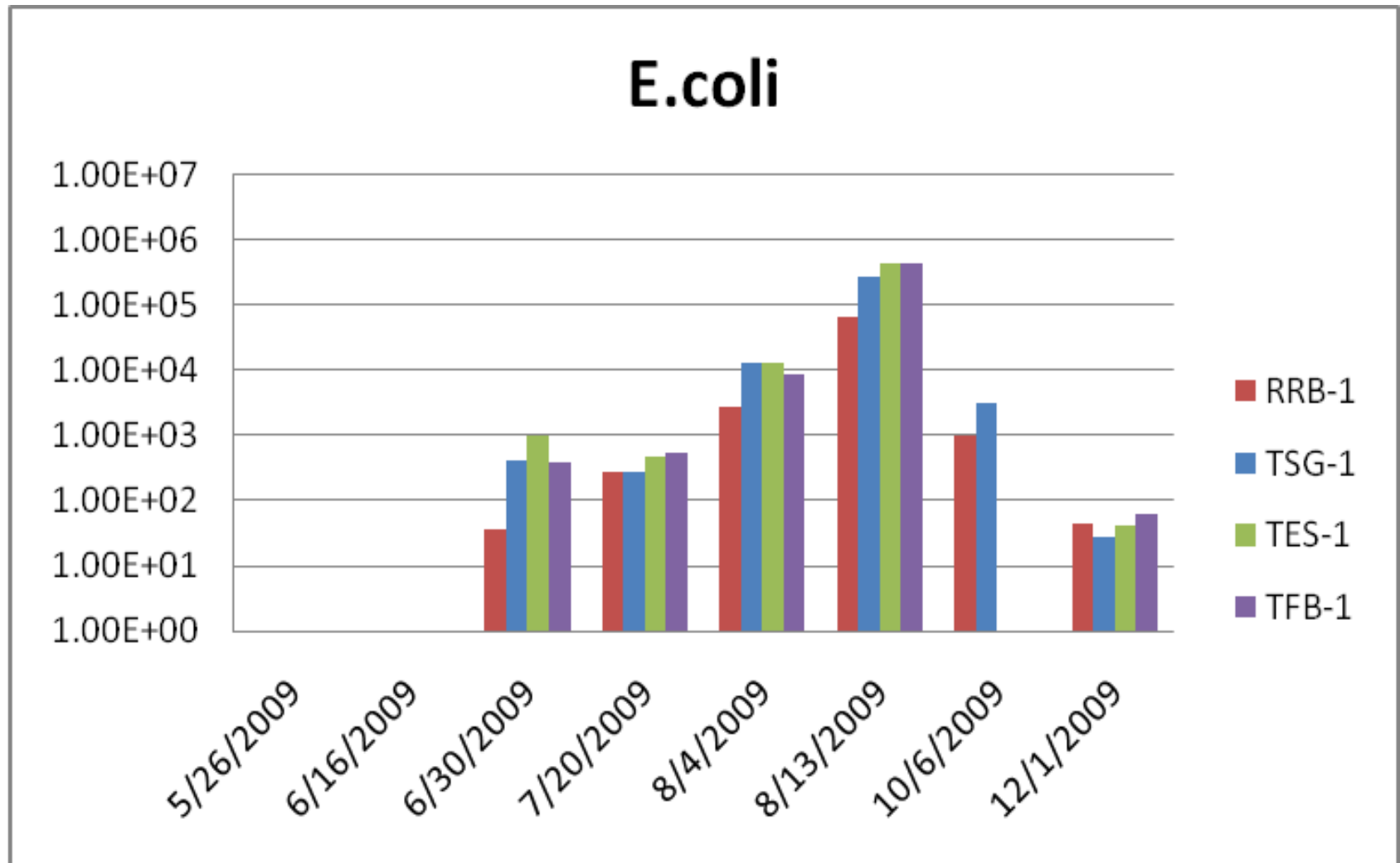
- Duplicate grab water samples were collected and 1 L of each sample was filtered onto a 0.45um filter by membrane filtration technique
- Total DNA/RNA was extracted from the bacteria collected on each filter using the Qiagen Fecal extraction kit
- Real-Time PCR was preformed using the AllBac, the HuBac, and the BoBac primer and probe pairs

Sample Locations

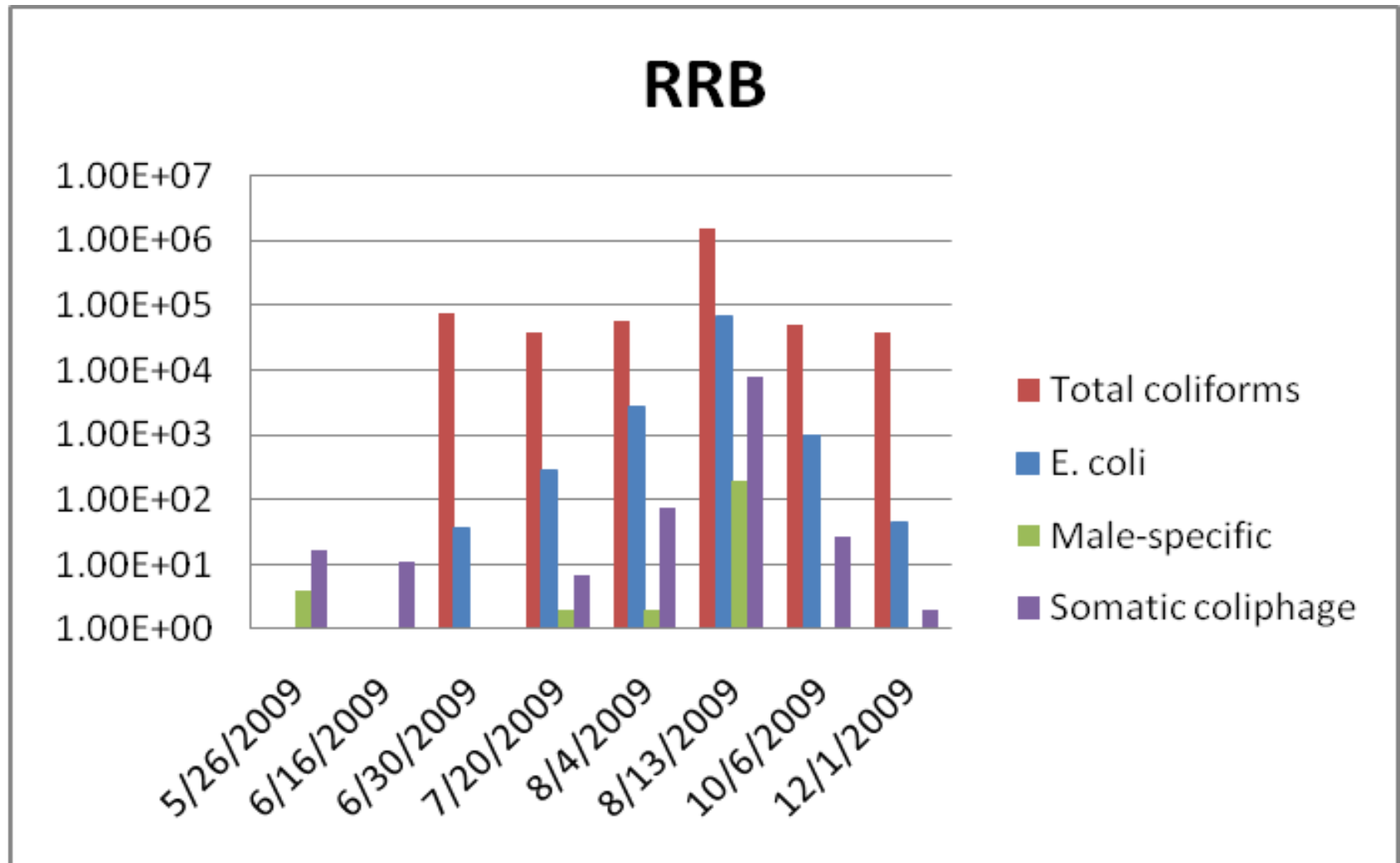
- Rio Rico Bridge
- Santa Gertrudis Lane
- TUMA Ed Site
- TUMA Foot Bridge



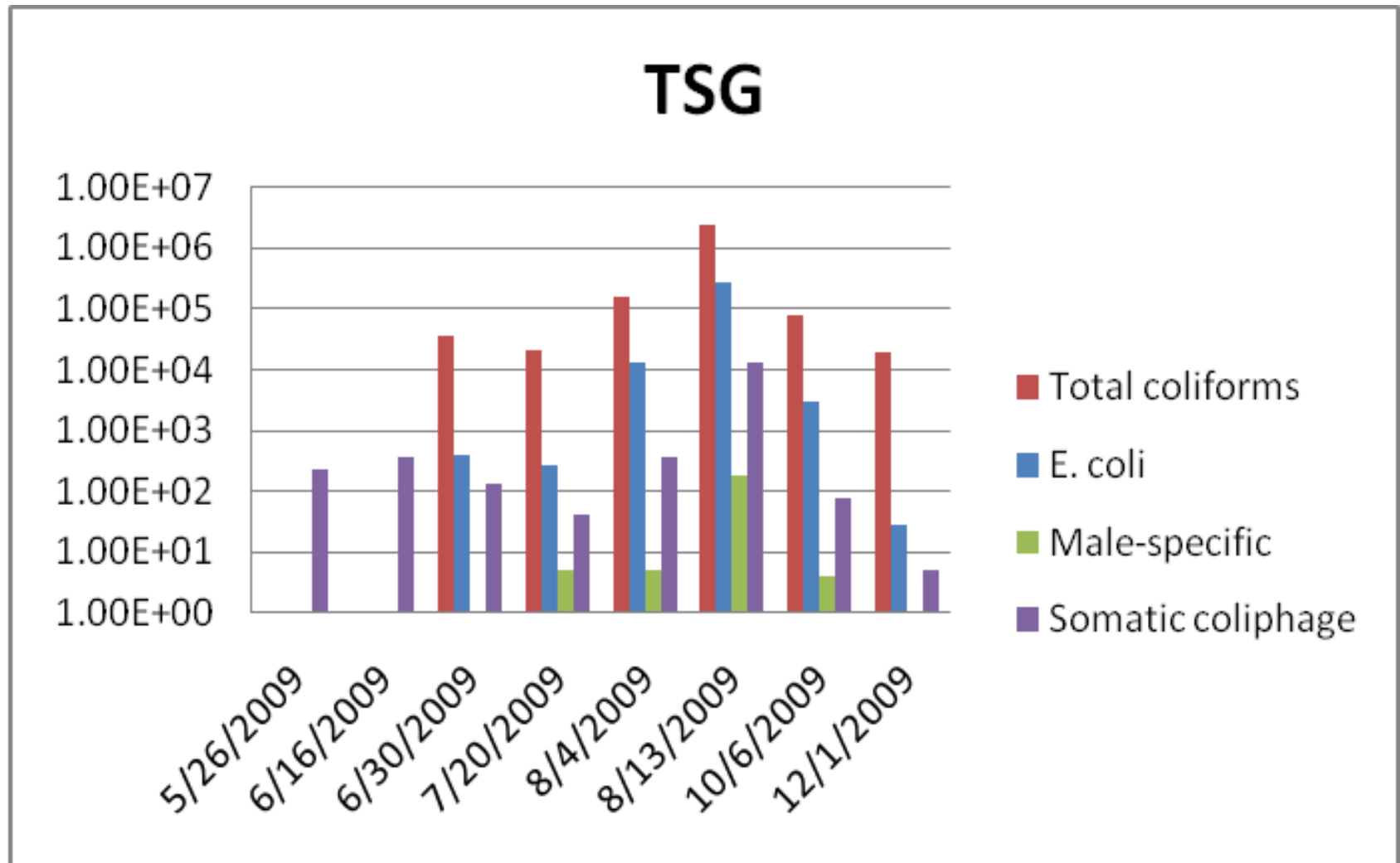
E.coli Results



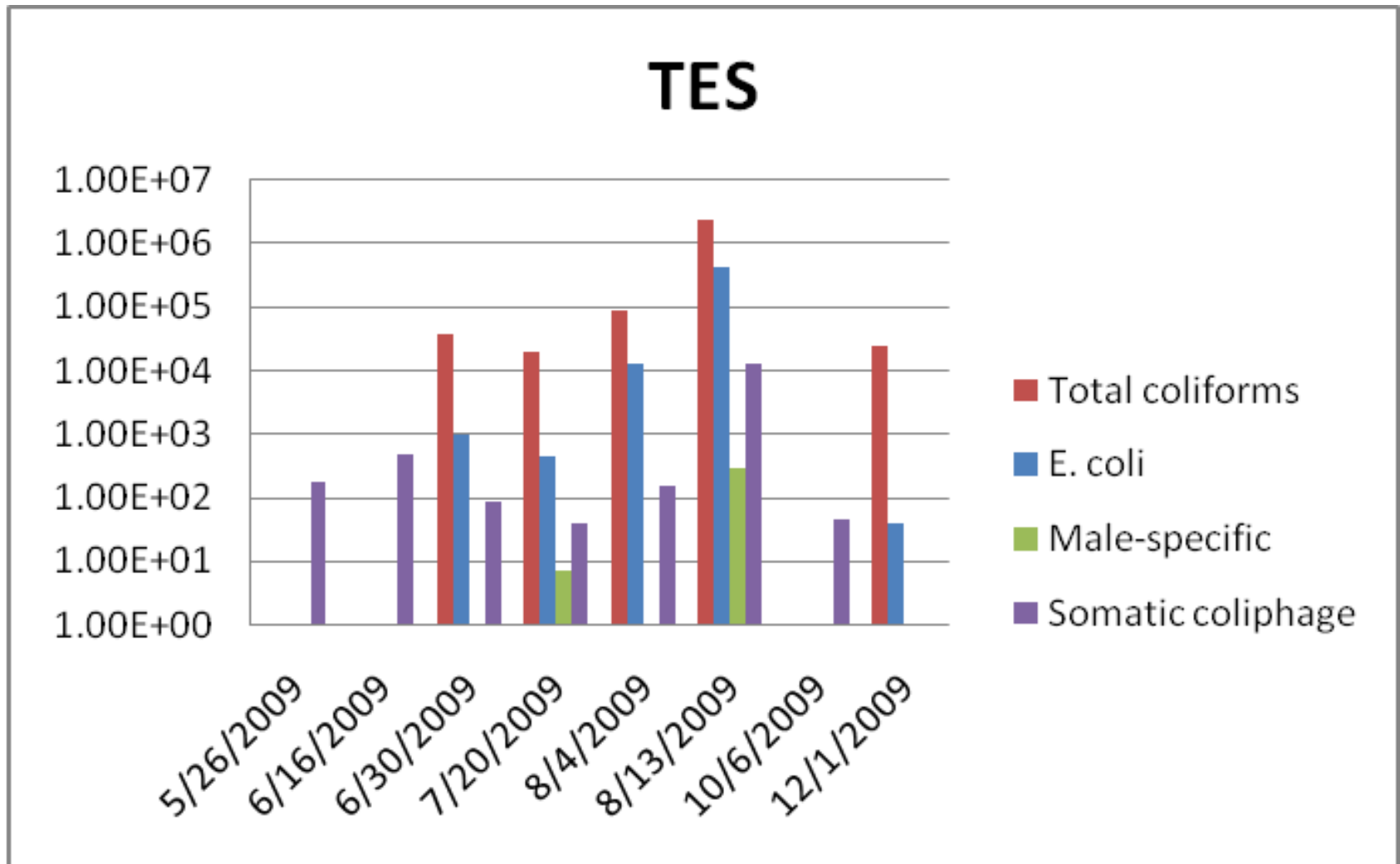
Rio Rico Bridge



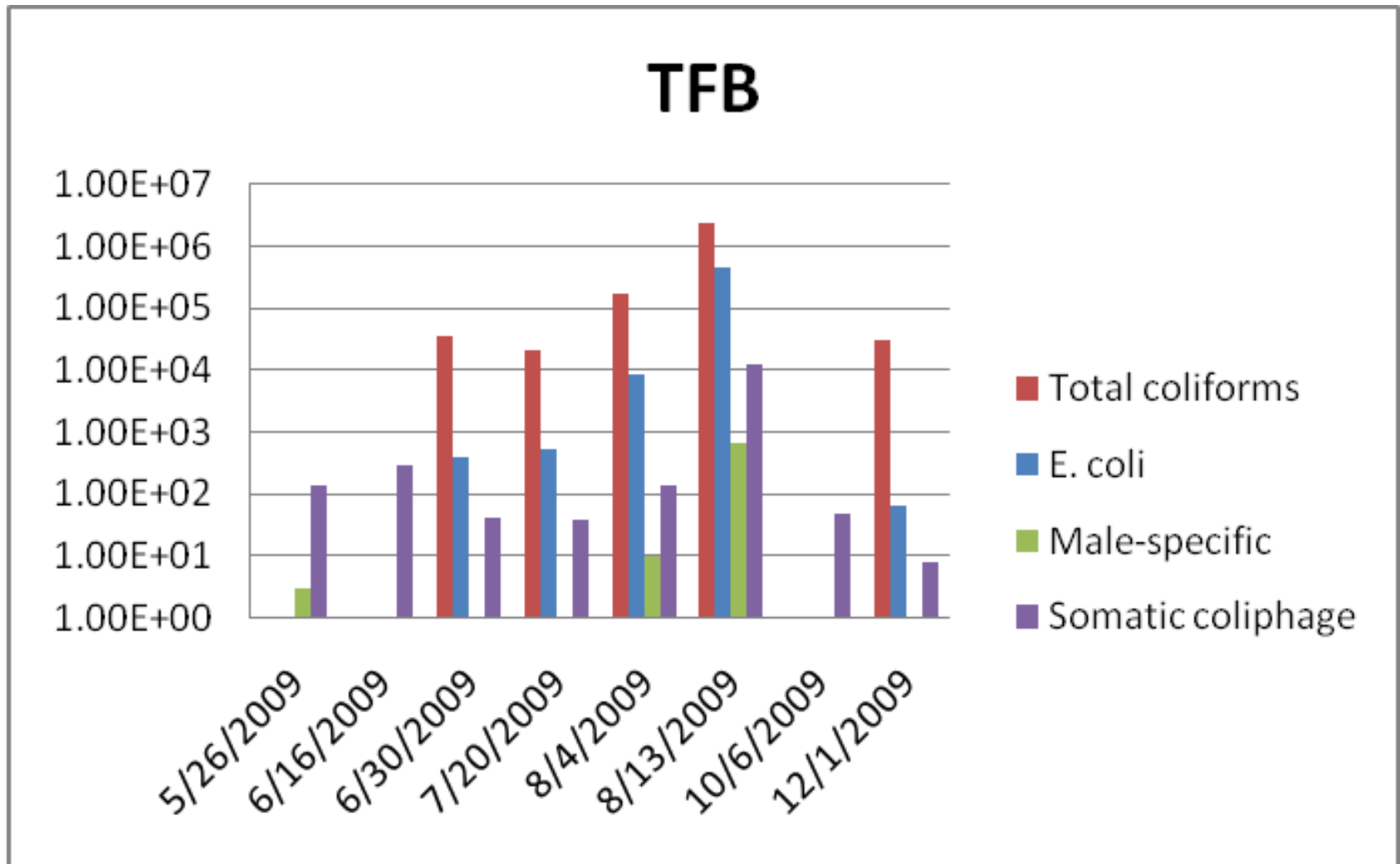
Santa Gertrudis Lane



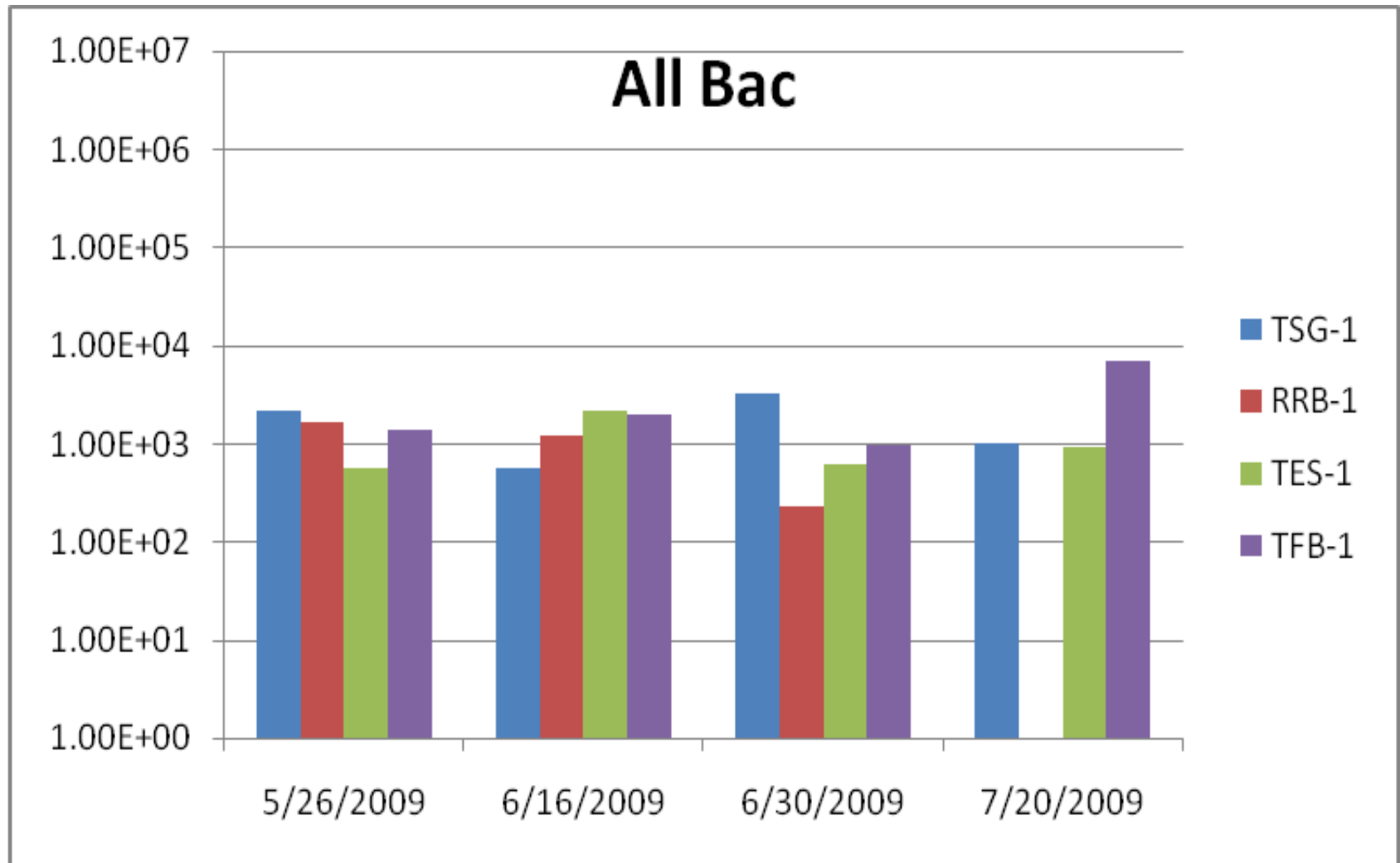
TUMA Education Site



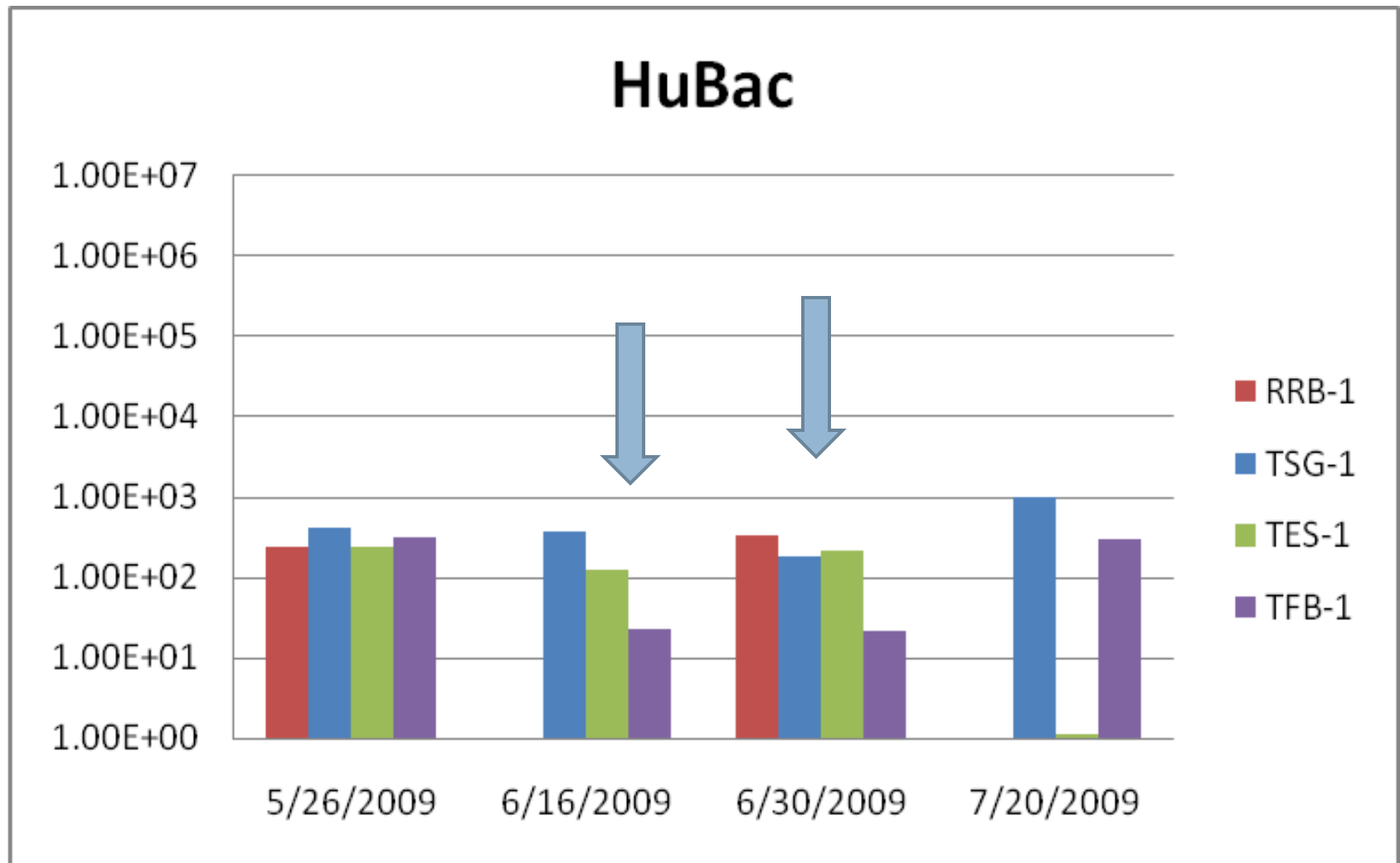
TUMA Foot Bridge



Total Molecular Contribution



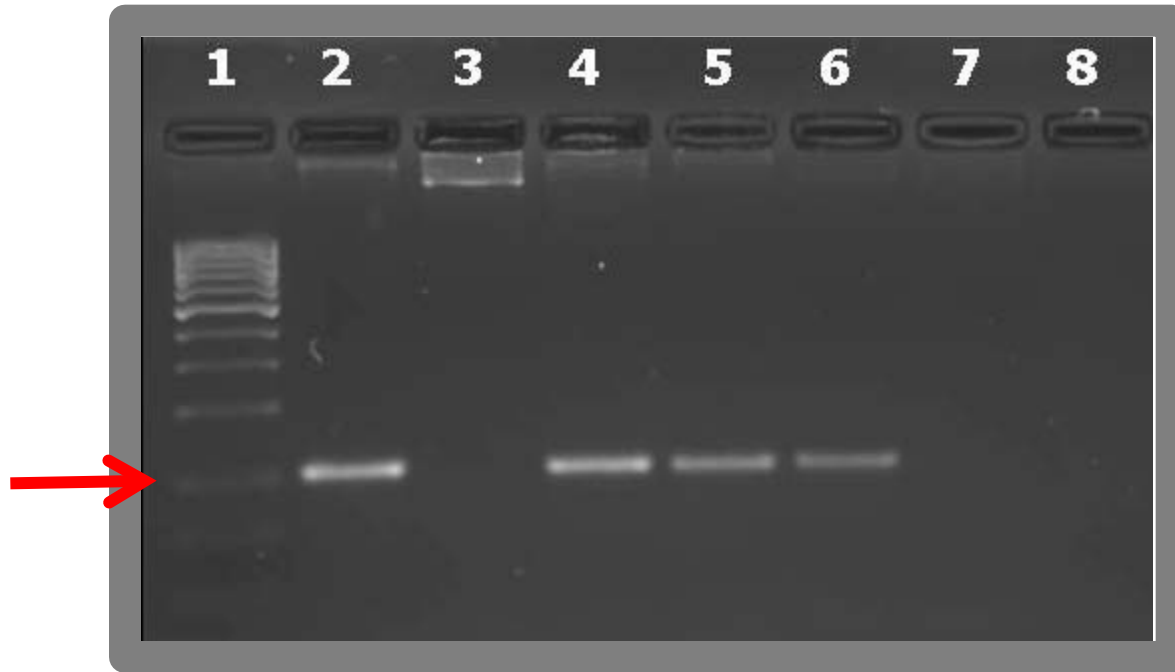
Human Molecular Contribution



Bacteroides Results

- Comparing the average quantity of *bacteroides* detected using the AllBac and the HuBac primer and probes indicates that up to ~26% of the *bacteroides* collected was of human origin
- Detection limits are currently being evaluated using the Santa Cruz River environmental samples
- BoBac assays still need to be confirmed in order to validate these findings

PCR Confirmation of Isolates



Gene target codes for protein sequence common to all known *Bacteroides* found in cattle

Initial Findings

- All four organisms have been detected at each site.
- Seasonal results indicate slight fluctuations in concentrations occurred during the warmer summer months.
- It is also apparent that microbial levels seem to be relatively consistent throughout the park and that little die off is occurring.
- However, slight increases are seen after Rio Rico Bridge indicating non-point source contribution to water quality deterioration within the park.

Initial Findings Continued

- Total concentration of *E.coli* may be increasing as water moves through the park.
- However, human contribution seemed to decrease.
- Thus, indicating that other non-point sources may be contributing to the pollution problems.

Acknowledgements

- Tumacacori National Historical Park
- National Park Service
- DSCESU Cooperative Agreement Number: H1 200050003
- Jeremy Moss
- Kelley Riley
- Arizona Department of Environmental Quality

