Population Dynamics of *Escherichia coli* and *Enterococcus* spp. in Buffalo Bayou and White Oak Bayou

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A comprehensive, multi-year study of the fate and transport of *E. coli* and *Enterococcus* spp. to address several questions:

1. **Under what conditions can *E. coli* and *Enterococcus* spp. bacteria survive for extended periods in the natural waters and sediment in Buffalo Bayou and White Oak Bayou and in the soils in the watersheds?**

2. **Under what conditions can *E. coli* and *Enterococcus* spp. bacteria replicate (grow) in natural waters and soils in Buffalo Bayou and White Oak Bayou?**
Questions 1 & 2 - Sampled from January to August 2008 to assess influence of temperature and water flows

- Analysis of nutrients in water, sediments (from pore water) and soils

- FISH and Micro-FISH (microautoradiography combined with FISH-determines activity) analysis of all substrates with *E. coli* and *Enterococcus* specific DNA probes *(in progress)*

- Traditional EC detection methods for comparison

- Laboratory incubations using substrates to determine if *E. coli* and *Enterococcus* will replicate or are only viable but non culturable (VBNC) *(in progress and focus of FY2009)*

- Determination of false-positives produced with traditional FC detection methods using DNA probes *(in progress)*

- HFERP fingerprinting to determine ‘naturalized’ indicator bacteria *(in progress)*
3. Under the conditions indicator bacteria are found to replicate in natural waters and sediment, what are they using for growth substrates?

Are they utilizing WWTP effluent derived substrates from chlorine or UV based processes or from other substrates found in the waters and sediments?

- End member chemical characterization of organic sources (awaiting results from contract lab)
- Stable isotopic composition of bacteria that replicate in natural substrates (in progress)
Typical $\delta^{15}N$ values of various nitrogen sources in the environment.
4. *Are these indicator bacteria attached to particulate matter in the watersheds or surviving on biofilms in discharge conveyance systems and are then flushed into bayous during rain events?*

- FISH analysis of biofilms and particulate matter collected during storm events (focus of FY2009)
- Determination of biofilm growth on microscope slides affixed in discharge conveyance systems (in progress)
5. How does the hydrologic cycle influence survival and replication of these indicator bacteria?

- Sampling during storm events.
- Analyses to include nutrients, DOC, FISH, IDEXX
- Comparison of strain genotypes from soils and sediments to cultivated isolates.

Focus of FY2009
WWTP, Water, Sediment & Soil Samples (base flow & rain events)

IDEXX Colilert
EPA Membrane Filter methods

Incubations with filtered water using *E. coli* & *Enterococcus* isolated from same sample

Isolate Cultures

DNA fingerprinting

Carbon, Nitrogen, Sulfur stable isotope analyses of bacteria and substrates

Stormwater biofilm cultivation experiments

Detection & enumeration of *E. coli* and *Enterococcus* with fluorescence *in situ* hybridization (FISH)
2 Control Sites on Lake Creek in the Caney Creek Watershed
In Spring 2008, learned that FY2009 funding would be reduced by more than 50%.

Increased FY2008 budget to include 15 additional sampling sites and QPCR & DNA probing analyses.

Trade offs—more samples but less time in FY2008 for processing and FY2009 reduction in research staff.
Sediment Cores collected Above the Water Line (AWL), at Water Line (WL) & Below Water Line (BWL). Three horizons sub-sampled: top (0-1 cm), mid (variable), bottom (variable).

Highest bacterial concentrations typically in top (0-1 cm) horizon.
Buffalo Bayou (sediment cores- top horizons)

17492 Sediment Core (WL)
MPN/g Wet Weight

17493 Sediment Core (AWL)
MPN/g Wet Weight

8072730 Sediment Core (AWL)
MPN/g Wet Weight

EC ENT TC
Low Flow (3.00 cfs)

17492 Water
MPN/100ml

17493 Water
MPN/100ml

8072730 Water
MPN/100ml

Low Flow (3.00 cfs)
Buffalo Bayou (sediment cores- top horizons)

- **11364 Sediment Core (WL)**
  - MPN/g Wet Weight
  - Low Flow (92 cfs)
  - EC: 4948, ENT: 8469, TC: 8469

- **11362 Sediment Core (WL)**
  - MPN/g Wet Weight
  - Low Flow (227 cfs)
  - Ec: 6065, ENT: 4212, TC: 8469

- **11360 Sediment Core (AWL & WL)**
  - MPN/g Wet Weight
  - Low Flow (202 cfs)
  - EC: 8469, ENT: 8469, TC: 8469

- **11364 Water**
  - MPN/100ml
  - Low Flow (212 cfs)
  - EC: 2420, ENT: 2420, TC: 2420

- **11362 Water**
  - MPN/100ml
  - Low Flow (227 cfs)
  - Ec: 1300, ENT: 2420, TC: 2420

- **11360 Water**
  - MPN/100ml
  - Low Flow (202 cfs)
  - EC: 1986, ENT: 921, TC: 2420
Buffalo Bayou (sediment cores - top horizons)

- **11353 Sediment Core (AWL)**
  - MPN/g Wet Weight
  - Low Flow: EC, ENT, TC
  - High Flow: EC, ENT, TC

- **15844 Sediment Core (BWL)**
  - MPN/g Wet Weight
  - Low Flow: EC, ENT, TC
  - High Flow: EC, ENT, TC

- **11351 Sediment Core (BWL)**
  - MPN/g Wet Weight
  - Low Flow: EC, ENT, TC
  - High Flow: EC, ENT, TC

- **11353 Water**
  - MPN/100ml
  - Low Flow: EC, ENT, TC
  - High Flow: EC, ENT, TC

- **15844 Water**
  - MPN/100ml
  - Low Flow: EC, ENT, TC
  - High Flow: EC, ENT, TC

- **11351 Water**
  - MPN/100ml
  - Low Flow: EC, ENT, TC
  - High Flow: EC, ENT, TC

BB at 610

BB at Shepherd
Buffalo Bayou (sediment cores- top horizons)

16675 Sediment Core (WL)
MPN/g Wet Weight

15843 Sediment Core (BWL)
MPN/g Wet Weight

11347 Sediment Core (WL)
MPN/g Wet Weight

16675 Water
MPN/100ml

15843 Water
MPN/100ml

11347 Water
MPN/100ml

BB at Main St.
White Oak Bayou (sediment cores- top horizons)

18588 Sediment Core (WL)
MPN/g Wet Weight

8074150 Sediment Core (BWL)
MPN/g Wet Weight

18577 Sediment Core (BWL)
MPN/g Wet Weight

18588 Water
MPN/100ml

8074150 Water
MPN/100ml

18577 Water
MPN/100ml

Low Flow (0 cfs)
White Oak Bayou (sediment cores - top horizons)

15831 Sediment Core (BWL) MPN/g Wet Weight

15831 Water MPN/100ml

15829 Water MPN/100ml

11390 Water MPN/100ml

Low Flow: (27.13 cfs)
**White Oak Bayou (sediment cores- top horizons)**

- **15827 Water**
  - MPN/100ml
  - High Flow
  
- **11387 Water**
  - MPN/100ml
  - Low Flow (35.70 cfs)

**Little White Oak Bayou (sediment cores- top horizons)**

- **11148 Sediment Core (AWL)**
  - MPN/g Wet Weight
  - Low Flow (0 cfs)

- **16648 Sediment Core (WL)**
  - MPN/g Wet Weight
  - High Flow
  - Low Flow (3.00 cfs)
Buffalo Bayou below confluence with White Oak Bayou
Control Sites- MPN values in sediment and water

Control Site 1 Sediment Core (AWL)
MPN/g Wet Weight

Control Site 2 Sediment Core (BWL)
MPN/g Wet Weight

Control Site 1 Water
MPN/100ml

Control Site 2 Water
MPN/100ml
Station 11353 Buffalo Bayou at 610- Cross section analysis of sediments

6/4/08 -11353: Sediment Core Stream Cross Section

Dry rim of bayou   Midway in bayou
Station 11351 Buffalo Bayou at Shepherd - Cross section analysis of sediments
‘Rake Study’ Disturbed sediments at 11351 particle size fractionation & transport analysis
Sediment analysis after Hurricane Eduard

Eduard - 8/6/08-11353: Soil MPN/g Wet Weight According to Soil Horizon

Eduard - 8/6/08-11347: Soil MPN/g Wet Weight According to Soil Horizon
Total Suspended Solids (63-25 μm) and Dissolved Organic Carbon

TSS higher in Buffalo Bayou- carbon and stable isotope analysis of particle size Fractionation in progress.
Percent sediment moisture vs MPN/g Wet Weight

3/13/08 - 11347: % Moisture vs. MPN/g Wet Weight

MPN/g Wet Weight

% Moisture

Soil Horizon

AWL-Top  AWL-Mid  AWL-Bot  WL-Top  WL-Mid  WL-Bot  BWL-Top  BWL-Mid  BWL-Bot  Long-Bot

E. coli MPN/g wet wt
Enterococci MPN/g wet wt
% Moisture
White Oak Bayou: Biofilm MPN/g Wet Weight

- 081108-16648-Biofilm wood
- 081108-16648-Biofilm plastic
- 081108-16648-Biofilm glass

MPN/g Wet Weight

- 081108-16648-Biofilm wood
- 081108-16648-Biofilm plastic
- 081108-16648-Biofilm glass
E. Coli Incubation Experiments in bayou water

Very preliminary – need to be repeated and confirmed with FISH-DNA probing analysis

Sediment incubation studies in progress using gamma irradiated samples from 6 sites.
FY2009 Focus on storm drains, biofilms, and incubation studies