



# Use of Source Tracking Markers to Measure Human-Origin Fecal Contamination in the Ohio River

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in collaboration with  
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# Objective

- Measure *E. coli* and microbial source tracking markers in the Ohio River in order to detect reaches where human sources may be contributing high *E. coli*
  - Requires technical improvements to analytical detection protocols for MST markers
  - Requires confirmation of MST marker specificity in fecal sources
  - Requires confirmation that *E. coli* and MST markers do not have different rates of die-off in river water
  - Requires positive-control tests in environmental waters

# Project task – Improve analytical methods

- Introduction of spike-and-recovery controls into samples to control for losses during processing
- Published in Water Research

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## Evaluation of two spike-and-recovery controls for assessment of extraction efficiency in microbial source tracking studies

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# Project Task – Fecal Source Characterization

- Sensitivity – Human sewage sources along the Ohio carry the human-associated MST markers at log(conc) 8.5 to 10.5 copies per g dry wt.
- Specificity – The human-associated MST markers generally are absent in nonhuman fecal material but can be detected in dogs and cattle at low concentrations.
- The ratio between MST marker and *E. coli* is inconsistent, but falls within bounds.

# Project Task – Effect of Aging

- Consistency of relations
  - Human-associated marker decayed more rapid than *E. coli*
    - If human-associated marker is still detected, then human-source *E. coli* of the same age are still present.
  - AllBac general marker decayed slower than *E. coli*
  - Relative decay rates of human-associated marker and *E. coli* remained the same with different applied stressors

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## Relative Decay of *Bacteroidales* Microbial Source Tracking Markers and Cultivated *Escherichia coli* in Freshwater Microcosms<sup>∇</sup>

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# Project Task – Validation in environmental waters

Test to see if MST markers are detected in environmental water samples after a known input of sewage

- Take advantage of known combined sewer overflows or other contamination sources
- Sample upstream and downstream from an active source
- Measure fecal-indicator bacteria and MST markers in the samples

# Results from Task

- Confirmation of fundamental hypothesis:

An increase in fecal-indicator bacteria from human sources will be detected as an increase in MST markers in environmental settings

On the Ohio River (near Cincinnati, during CSO)

On a tributary stream (Tug Fork at Warfield, KY with uncontrolled sewage discharge)

# Main Project Task – Testing Ohio River samples to prioritize human-impacted reaches

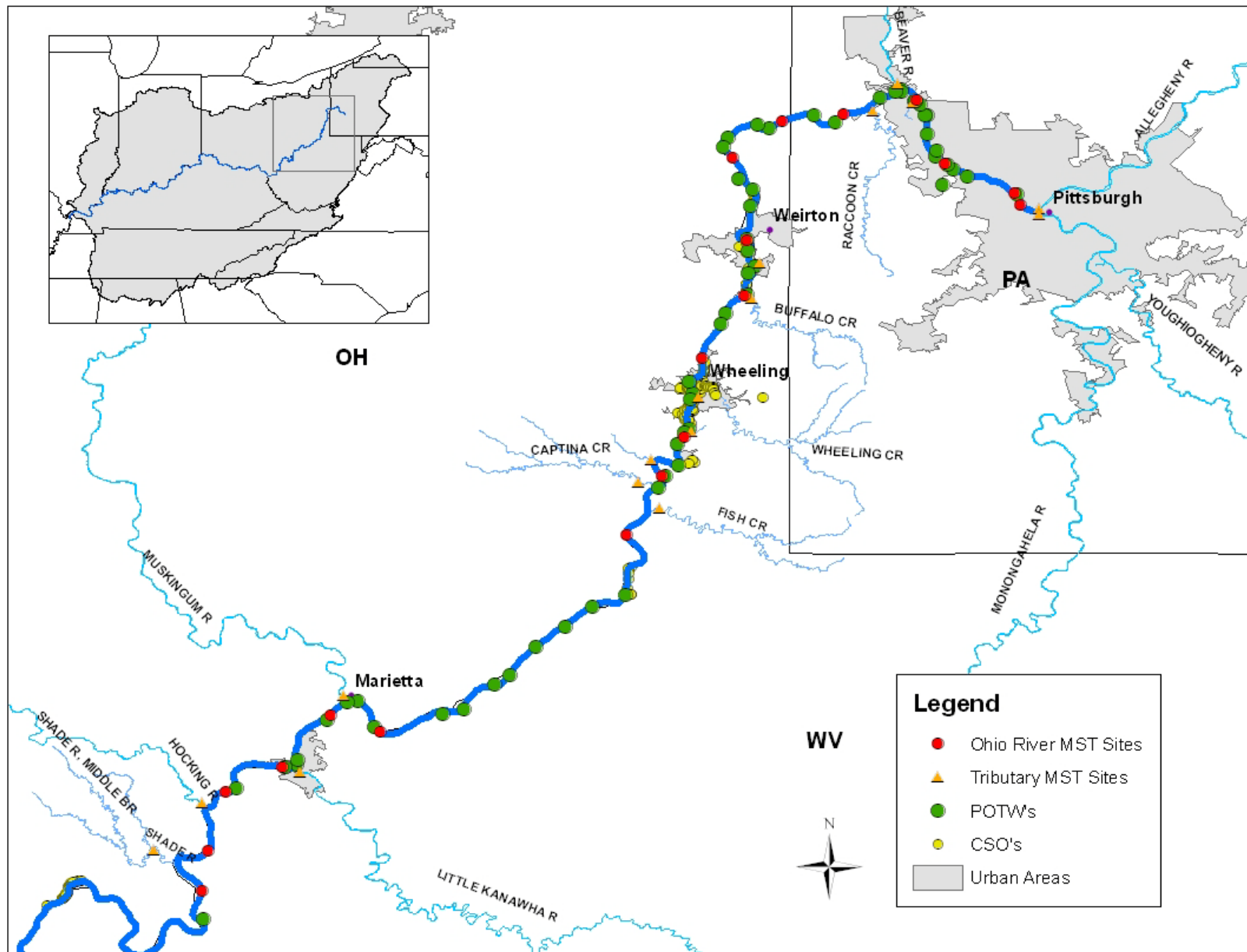
Objective: Collect and test samples from the Ohio River and tributaries for MST markers as a way to understand sources of fecal contamination

Approach: Two sample longitudinals, Pittsburgh, PA to Pomeroy, OH

- September 2007 – dry weather flow, samples rarely exceeded 240 MPN/100 mL
- June 2008 – higher flow associated with wet weather, samples frequently exceeded 240 MPN/100 mL



# Area Map

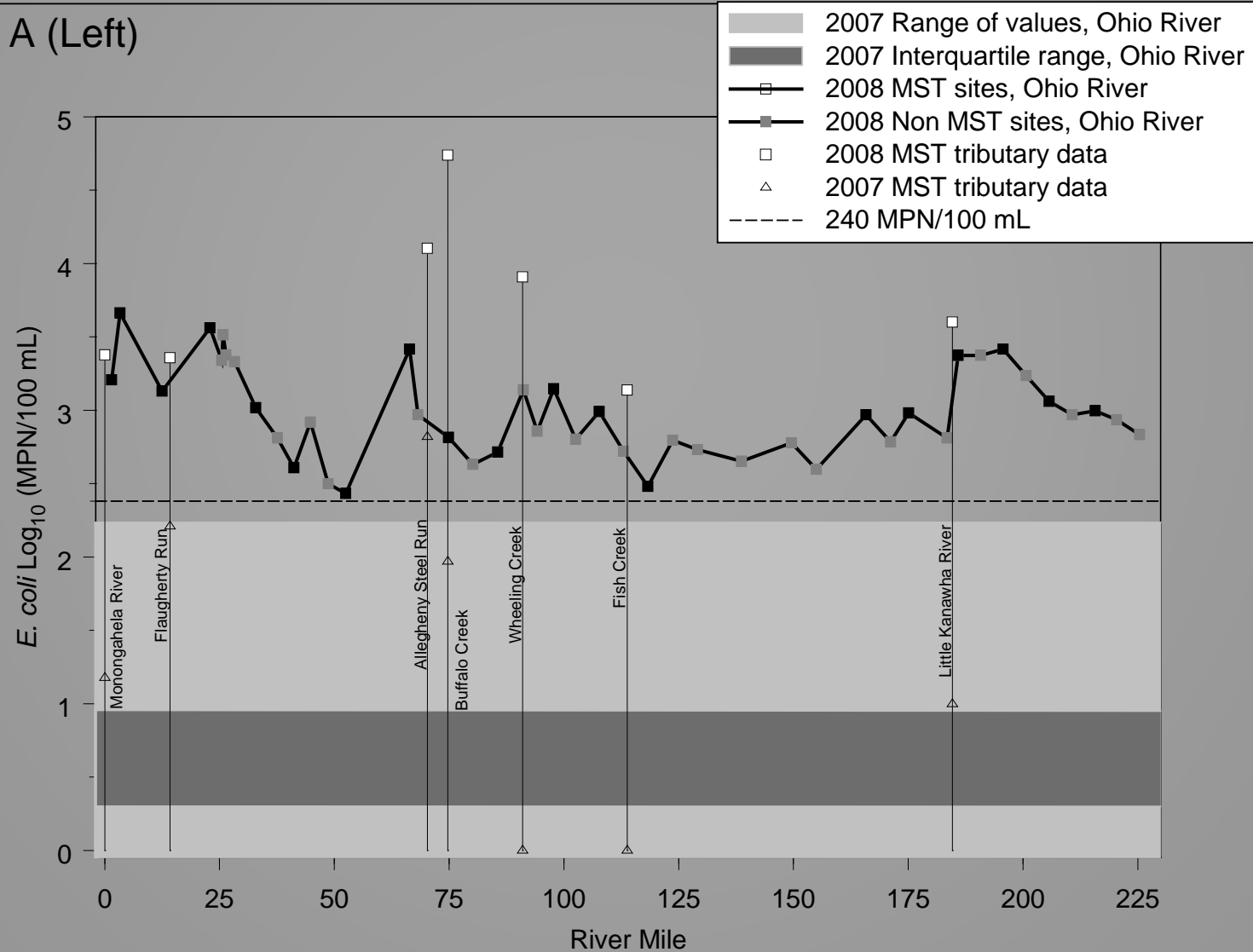


# Provisional data from the Ohio River

## (Left Bank series, 2008)

*E. coli* density (MPN/100 mL)

*E. coli* exceeded the standard of 240 MPN/100 mL in 54 of 55 samples overall

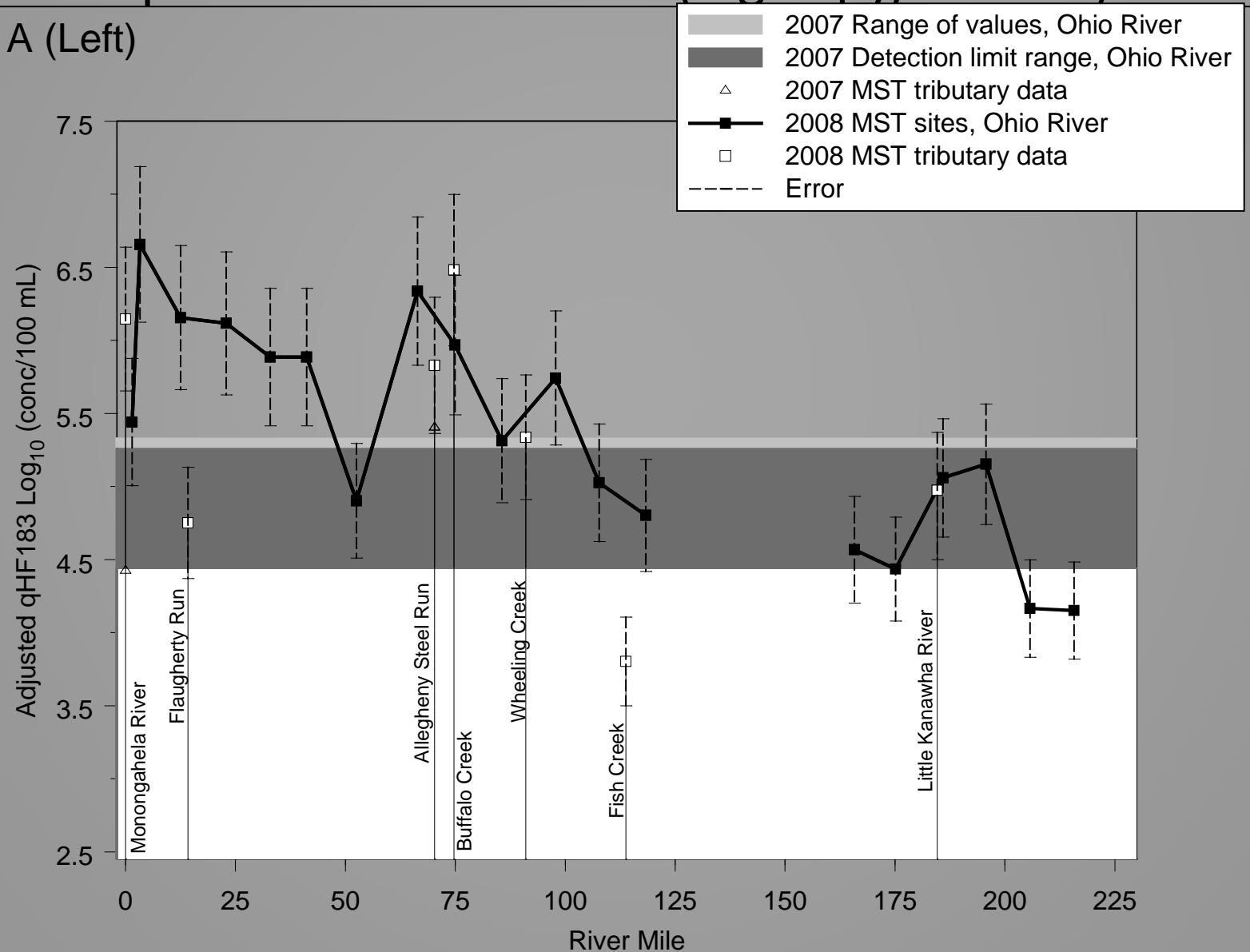


# Provisional data from the Ohio River

(Left Bank series, 2008)

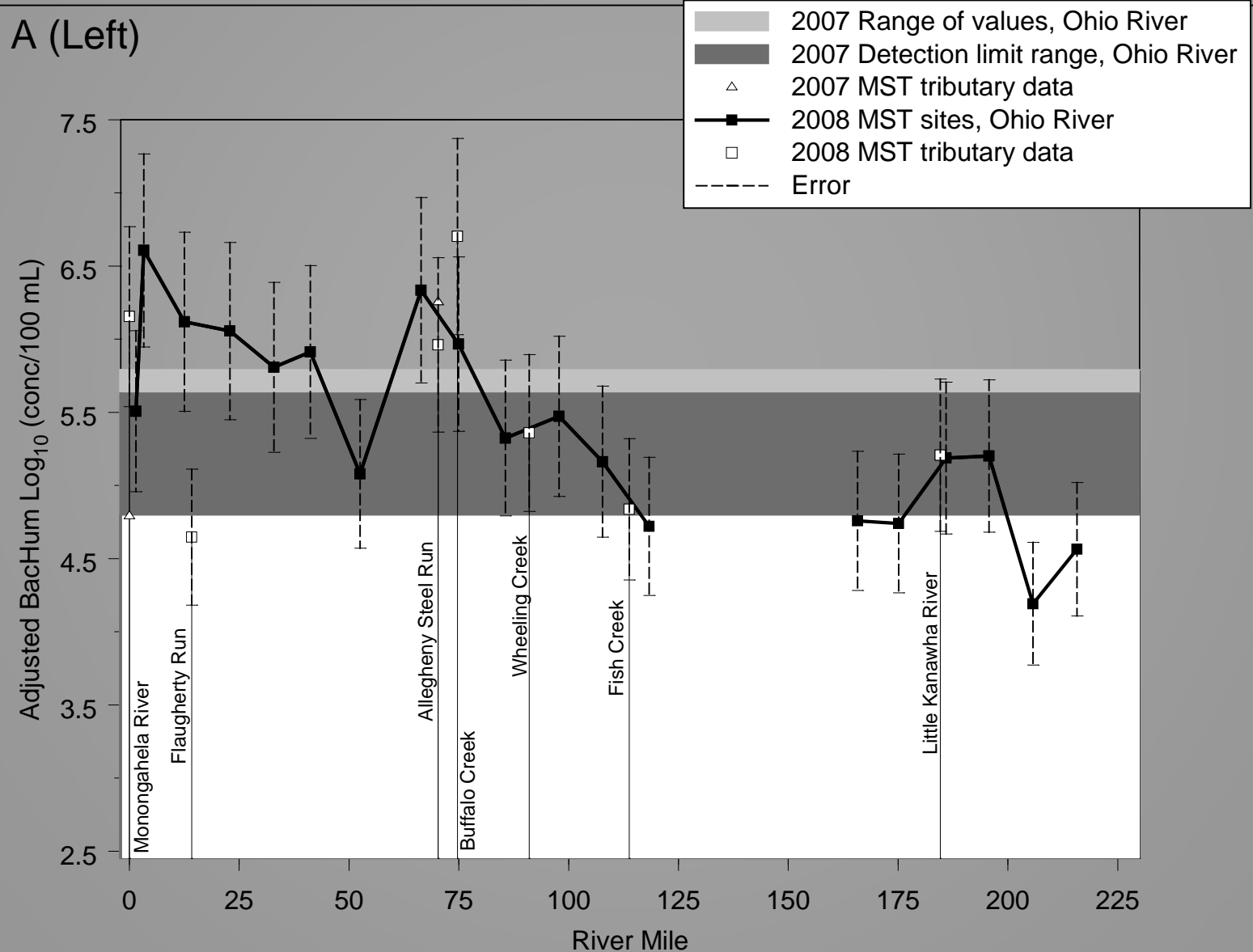
qHF183 concentration (log copy/100 mL)

A (Left)



# Provisional data from the Ohio River (Left Bank series, 2008)

## BacHum concentration (log copy/100 mL)



## Areas where increased *E. coli* was matched by increased human-associated markers

- In 19 of the 54 cases of *E. coli* above standard in 2008, all three MST markers were higher than background levels from 2007 and indicated some level of human fecal contamination.
- Samples where high concentrations of human-associated marker were detected all were upstream from mile 74.9. Human fecal contamination appeared to have lesser impact at sites downstream from mile 74.9.

# Specific areas

- Large increases in *E. coli* were apparent across ten different reaches.
  - Of the ten reaches, six showed either a decrease or a stabilized level of human-associated marker.
  - Four reaches showed significant increases in human-associated marker, indicating inputs of human fecal contamination.
    - LDB 3.3 just downstream from the ALCOSAN POTW.
    - RDB 22.9 just downstream from Elkhorn Run and Moon Run POTW and Conway POTW.
    - LDB 66.4 downstream from Weirton POTW, across the river from Steubenville.
    - RDB 74.9 downstream from Wellsburg POTW and off the shoreline of Brilliant, Ohio.

# Bonus!

- The LDB 66.4 reach where potential human fecal contamination was indicated by our study was recently in the news!
- **Weirton raw sewage still emptying into Ohio River**
  - By Keri Brown, West Virginia Public Broadcasting  
March 23, 2010
  - “Raw sewage from about 1,000 buildings in the city is flowing into the Ohio River, without disinfection.”



# Tributaries

- *E. coli* densities exceeded 240 MPN/100 mL in every sampled tributary stream.
  - In 5 of the 14 tributaries sampled, elevated *E. coli* were associated with higher-than-background levels of human associated markers.
    - Monongahela River
    - Allegheny River
    - Beaver River
    - Allegheny Steel Run
    - Buffalo Creek
  - Samples collected below the input of these tributaries did not show exceedances of human-associated marker.
    - The tremendous volume of the Ohio River seems to dilute the effects of tributary streams on the main stem concentrations.



# Conclusions

- In this study, four reaches were found to have potential human fecal contamination.
  - Results are from one “sweep” of the Ohio River
  - More samples collected over time would help make evidence stronger.
- Study showed that methods can work in a large river setting with multiple types of inputs and sources.
- Final manuscript covering Ohio River samples is in preparation.

# Future Application of MST

- MST can be useful to develop pathogen TMDLs
  - TMDL plan entails
    - Quantification of exceedance
      - Done by careful monitoring
    - Allocation of exceedance to sources
      - POTW leaks?
      - Animal production facilities?
      - nonpoint sources?
        - » Wildlife, septic systems, land-applied manure
  - Plan to reduce load from each source
    - Load reduction plan dependant on participant cooperation



# Role of MST in TMDL

- Continuing disparities after sanitary survey results?
  - MST can be used to rule out stretches, specific point sources and/or tributaries.
  - MST can be used to rank stretches and/or tributaries according to *E. coli* and marker concentration.
- Effort involved?
  - Varies depending on goals of study
  - Must sample a few points above and below the input “regularly” to get good spatial data.

# MST markers available

- Human
- Ruminant (cow, deer, etc)
- Dog
- Chicken
- Gull



## Limitations of the science

- **Presence** of an MST marker is **one line of evidence, but not proof**, that the source contributed.
  - Will not be able to give percent contributed
    - Some cross-reactivity of markers
  - Can add second line of evidence with other analyses to help make conclusions stronger:
    - wastewater organics, optical brighteners, etc
- **Absence** of a marker is strong evidence that the **source did not contaminate**.

# Questions?

