Evaluation of Methods for Enumerating Enterococci in Beach Sand

John Griffith

Southern California Coastal Water Research Project

Background

- Recent International interest in beach sand as a source or reservoir of fecal bacteria
- Many published protocols for enumerating bacteria in sand
- No standard method
  - Prevents assessment of problem
  - Limits comparability of data between beaches
- Goal: Identify a standard method for measuring enterococci in beach sand

Approach

- Bring together leading researchers in field
- Process a common set of samples

Participants

Jenny Jay – UCLA
Richard Whitman – USGS
Helena Solo-Gabriel - U of Miami
Ali Boehm - Stanford
Tom Edge- Environment Canada
Marty Getrich - OCPHL

Details

- 21 permutations of 4 treatments
- Four replicates processed by each method
  - Duplicates by each of two researchers
- Three sand samples
  - One sample processed each day
- Analyze for enterococci using EPA Method 1600

Treatments

- Hand Shaking
- Mechanical Shaking
- Sonication
- Blending

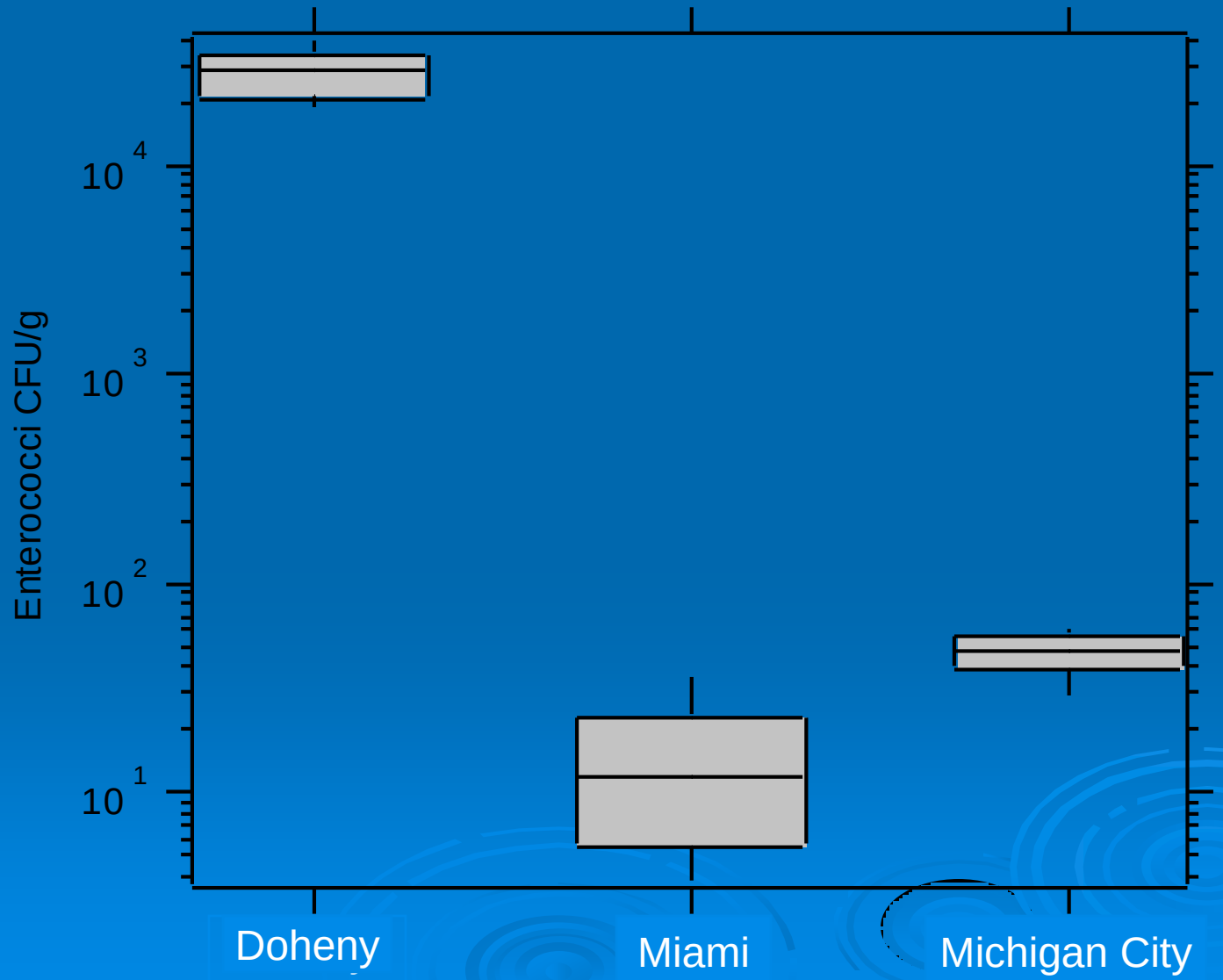
Variables

- Mass of sand
- Duration of shaking
- Settling time
- Number of rinses
- Eluant (PBS or DI water)
- Surfactant (Tween 80 or metaphosphate)
- Eluant collection (decant, pipette, vacuum)

Sand Sources

- Doheny State Beach, Dana Point, CA
- Hobie Cat Beach, Miami, FL
- Washington Park Beach, Michigan City, IN

Enterococcus Concentration



Results

- Blending produced lower numbers than sonication or shaking
- Lower eluant to sand ratio led to reduced recovery
  - Mechanical shaking produced lower numbers than hand shaking
- No effects noted for shaking time, sonication, number of rinses, or settling time

Bottom Line

- Hand-shaking performed as well or better than more complex treatments
  - Simple
  - Inexpensive
  - Consistent

Method Recommended by Experts

- 10g sand
- 60ml PBS
- 2 min. hand shake
- Settle 30s
- Decant eluant
- 40ml PBS rinse
- Settle 30s
- Decant eluant

What Next?

- Laboratory Intercalibration Exercise
  - Conducted November 2008
- Test method on more challenging sands
  - High silt
  - High organic content

Instructional Video