The AOML Environmental Microbiology Program:

Collaborative Inter-Disciplinary Research for Molecular Assessment of Microbial Water Quality In Coastal Ecosystems

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NOAA AOML:
The Environmental Microbiology Program

What do we do?
*Molecular Microbiology* to make coastal water quality assessment faster, easier, cheaper, and more accurate

Why do we do it?
To better protect the public health, ecosystems, and economy from: *Sewage Pollution, Human Pathogens, Harmful Algae*

The Specifics?
Develop and/or adapt *molecular assays and sensors* to detect microbial contaminants in coastal waters by measuring DNA or RNA signatures.

Who are our Customers and Partners?
Traditional assays need improvement

Fecal Indicator Assays:

• too slow (>18 hr)
• it is unclear if the correct thing is being monitored (axioms of fecal indicator theory violated)
• no pathogen detection
• no ability to source track (needed to guide remediation)
The Potential of Biotechnology

- species-specific ID
- rapid
- sensitive
- high-throughput
- allows sample storage
- culture & microscope independent
- simultaneous information on multiple targets
- Source-tracking of fecal indicator hosts
Key Scientific Questions

• Can molecular analysis be used to quickly and reliably identify human pathogens in coastal waters?

• Can molecular microbial source tracking aid in the reliable discrimination of input sources of microbial contamination to coastal waters?

• What are the sources of nutrients, pathogens, and fecal indicators in coastal waters?

• Is there a linkage between sources of nutrients and microbial contamination in coastal waters?
NOAA AOML
Tools for Environmental Microbiology:

Molecular-Based
- Real-Time quantitative PCR
- Colorimetric microplate hybridization
- Luminex suspension array

Traditional Culture-Based
- IDEXX EnteroLert™
- Membrane-Filtration Plate Counts
- Alternative Indicators (Clostridium, etc.)

Handheld Electrochemical Biosensor
Incorporation of Molecular Assays into Field Programs
Florida Area Coastal Environment (FACE)

Water discharges to coastal waters including outfalls, inlets, & upwelling characterized for nutrient concentrations & microbiological water quality
Six treated wastewater outfalls off the Southeast Florida Coast

Molecular Microbiology Sampling aboard NOAA Ship Nancy Foster
Multiple Assays Incorporated

- **enterococci**
- **Escherichia coli / Shigella spp.**
- **Bacteroides Fragilis Group**
  - **Bacteroides distasonis**
- **enterococci human marker**
- **Bacteroides human markers**
- **Bacteroides dog marker**

**standard fecal indicators**

- **alternative fecal indicators**

**source tracking markers**

**pathogens**

- **intestinal & dermal bacteria and virus**

- **E. coli O157:H7**
- **Campylobacter jejuni**
- **Salmonella spp.**
- **Staphylococcus aureus**
- **adenovirus**

= Luminex = qPCR & EC
Human enterococci, *Salmonella*, enterovirus: all negative
Tidal Discharges from Coastal Inlets

Satellite Photo Boynton Inlet
### Export of microbial contamination with outgoing tide, Boynton Inlet 1<sup>st</sup> 48-hour intensive study, June 2007

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<th>1000</th>
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</tbody>
</table>

Inlet, outgoing tide; *Salmonella, 0157:H7, C. jejuni*: negative
Trends in elevated microbial contamination on outgoing tides correspond to trends in elevated nutrient levels.

Nutrients and Flow, Boynton Inlet 2nd 48-hour Intensive study, September 2007 (Zheng et al.)
Epidemiological study of risk exposure to non-point-source beach contamination

Investigate correlations between detection of microbial contaminants to actual health outcomes

Multiple assays for traditional and alternative indicators, source tracking markers, and pathogens

Beach Environmental Assessment and Characterization of Human Exposure Study (BEACHES)

Enterococci results for Hobie beach 07/18/07

Amount of Enterococci per 100mls (QPCR)

Amount of enterococci per 100mls corrected with the % of lactococci (QPCR)

UM-OHH mEI plates

MD-DOH mEI plates

NOAA-AOML IDEXX

# of cells

Amount of Enterococci per 100mls (QPCR)

UM-OHH mEI plates

MD-DOH mEI plates

NOAA-AOML IDEXX

Enterococci results for Hobie beach 07/18/07

Enterococci
E.coli
Fecal Coliforms
Staphylococcus
C. perfringens

Sand Low tide Dry
Sand Low tide Wet
Water Low tide transect A
Water Low tide transect B
Water Low tide transect C
Sand High tide Dry
Sand High tide wet
Water High tide transect A
Water High tide transect B
Water High tide transect C

No data available for low tide samples.
Impact of Hurricanes Katrina and Rita on the Microbial Landscape of New Orleans:

- Reported in: Sinigalliano et al., 2007, PNAS 104:9029-9034

Traditional & Non-Traditional Indicators

Non-Traditional Indicators

Potential pathogens or related
- Acinetobacter spp. 13.3%
- Arcobacter spp. 1.27%
- Clostridium spp. 1.27%
- Micrococcineae environmental isolate 1.27%
- Acidovorax spp. 4.88%
- Aeromonadaceae 3.25%
- Novosphingobium spp. 2.44%
- Shigella spp. 0.81%
- Flavobacterium spp. 0.81%
- Sphingomonas spp. 0.81%

Other
- Sludge-wastewater
- Potential pathogen or related Manure-associated
- Chemically-contaminated

Microbial Assemblages
Portable & In-Situ Biosensors
Prototype handheld automated multiwell PCR analyzer and electrochemical detector, with PC interface.

- 8-well chip
- Peristaltic pump
- Heat block (1 of 3), with 8 tubes, ca. 15uL each
- Double lumen fluidic port (1 of 8)

**Graph:**
- µA vs. Sample Type
- Enterococcus faecium (esp)
- Bacteroides fragilis cluster
- Escherichia coli (0157:H7)
- Human Adenovirus
- Salmonella species
- Staphylococcus aureus
- Enterococcus species

- No template
- Target DNA
- Beach water
- Contaminated beach water
The Product: biotechnological innovations to produce timely & accurate reporting of ecosystem health and environmental safety.

Health risks?
Questions?

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