Atlantic Oceanographic & Meteorological Laboratory
National Oceanic & Atmospheric Administration

Oceans and Human Health Center
University of Miami • Rosenstiel School
National Science Foundation • National Institute of Environmental Health Sciences

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

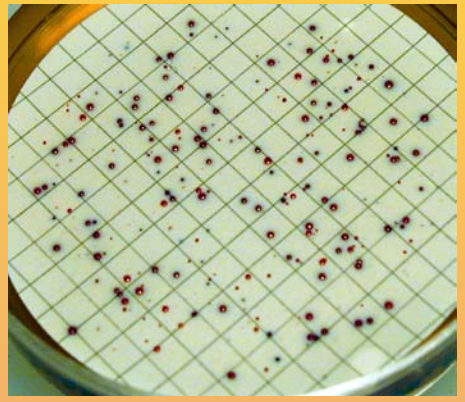
The Specifics?

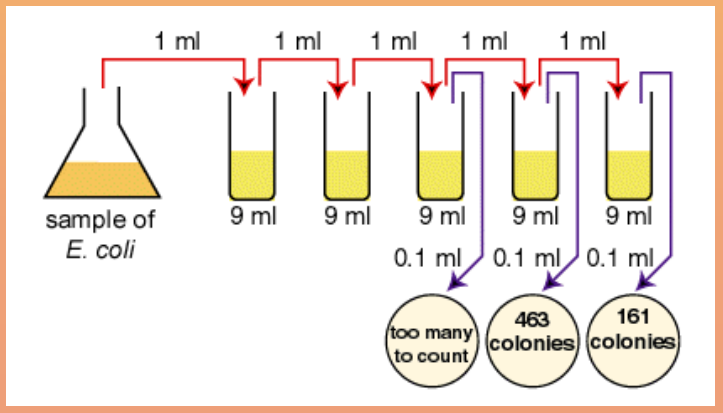
Develop and/or adapt ***molecular assays and sensors*** to detect microbial contaminants in coastal waters by measuring DNA or RNA signatures.

Who are our Customers and Partners?

Public Health Agencies, Fisheries, Tourism Industry, Environmental/Conservation Organizations, Concerned Citizens, Beach Managers, Water Quality Managers, Academic Institutions, Environmental and Public Health Scientists

Traditional assays need improvement

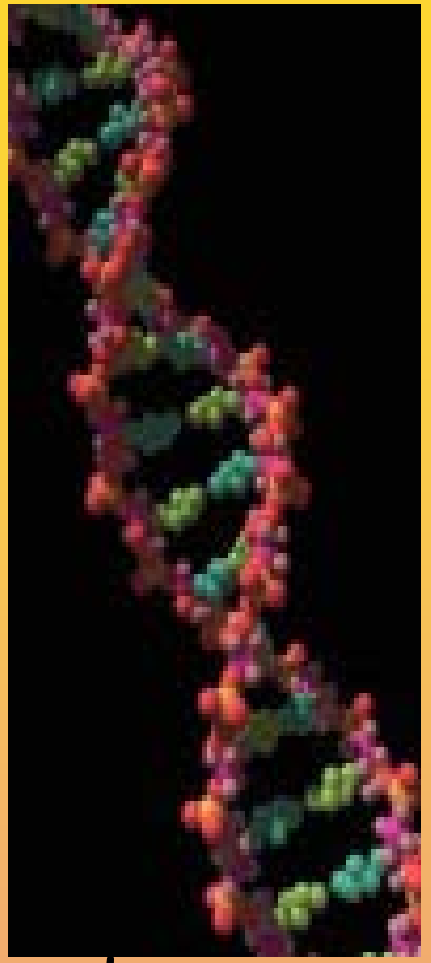




Fecal Indicator Assays:

- 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

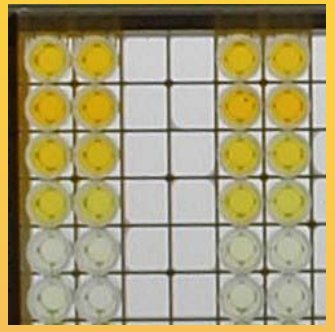
Key Scientific Questions

- 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



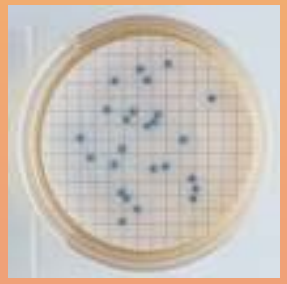
colorimetric microplate hybridization

Luminex suspension array

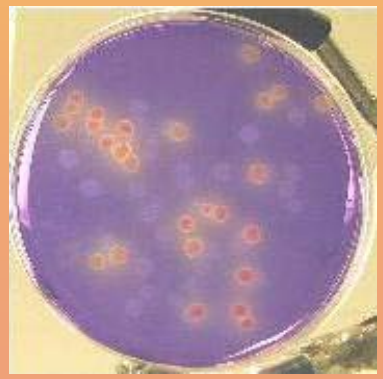
Handheld Electrochemical Biosensor

Traditional Culture-Based

IDEXX EnteroLert™

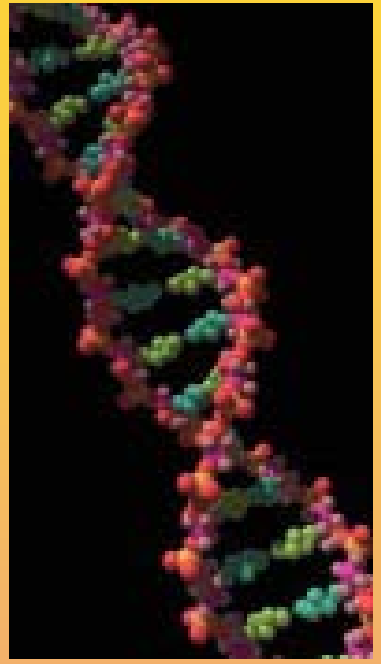


Membrane-Filtration Plate Counts



Alternative Indicators (Clostridium, etc.)

Incorporation of Molecular Assays into Field Programs



Southeast Environmental Research Center
Working to Restore and Protect our Unique Environment
Florida International University
Miami's public research university

Oceans and Human Health Center
University of Miami • Rosenstiel School
National Science Foundation • National Institute of Environmental Health Sciences

NGI
Northern Gulf Institute

CDC
SAFER • HEALTHIER • PEOPLE

National Marine Sanctuaries
Florida Keys

Florida Department of Health

University of Miami
Rosenstiel School

Woods Hole Oceanographic Institution
1930

Georgia College & State University
Georgia's Public Liberal Arts University

HML
Hollings Marine Laboratory
NIST
MUSC
Medical University of South Carolina

Alderon Biosciences Incorporated

Source Molecular

National Marine Fisheries Service
1871
125th Anniversary
1996

USF
University of South Florida

Department of Environmental Protection
Florida

FAU
Florida Atlantic University

Oceans & Human Health Initiative
NOAA

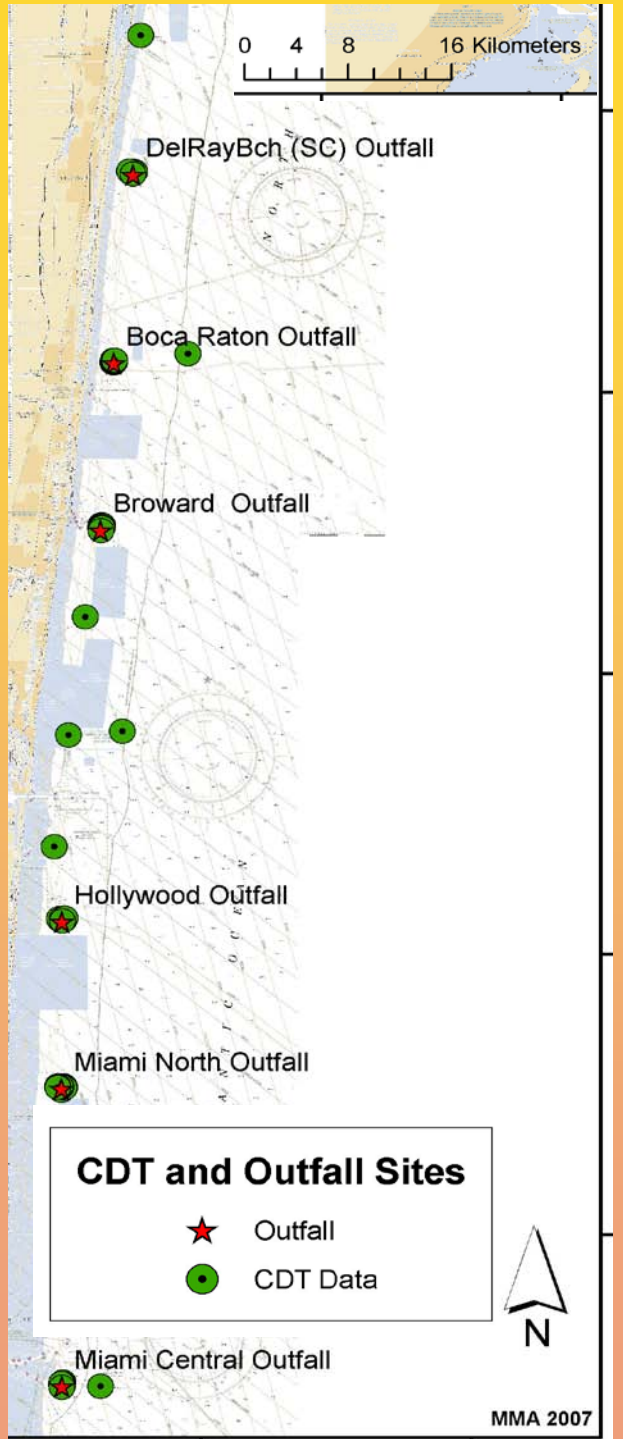
NOAA
Atlantic Oceanographic & Meteorological Laboratory
National Oceanic & Atmospheric Administration

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.

} standard fecal indicators

Bacteroides Fragilis Group
Bacteroides distasonis

} alternative fecal indicators

enterococci human marker
Bacteroides human markers
Bacteroides dog marker

} source tracking markers

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

} pathogens intestinal & dermal bacteria and virus

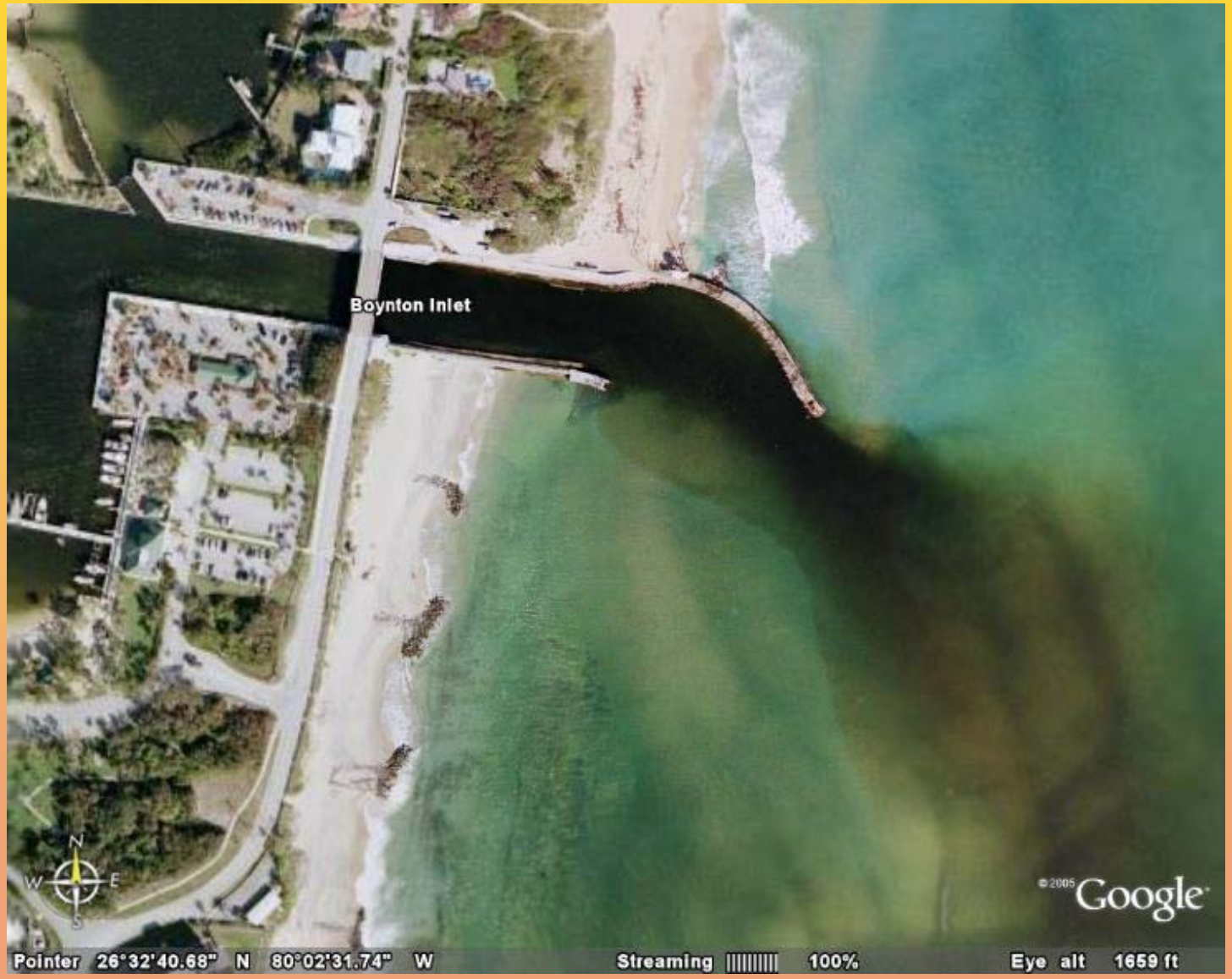
= Luminex + = qPCR & EC

Indicators and Pathogens in Wastewater Outfall Boils

assay	HWD	HWD	HWD btm	MC	MC btm	NMB	NMB btm
enterococci	+	+	+	-	+	-	+
human *Bacteroides* (HF8)	-	-	+	-	-	-	-
S. aureus	-	-	-	+	-	-	+
adenovirus	-	-	+	+	-	-	-
Norovirus	-	-	+	+	-	-	+
Phage MS2	+	-	-	+	-	+	+
Crytpo/100L	55	17		236		8	
Giardia/100L	68	119		246		120	

Human enterococci, *Salmonella*, enterovirus: all negative

Tidal Discharges from Coastal Inlets



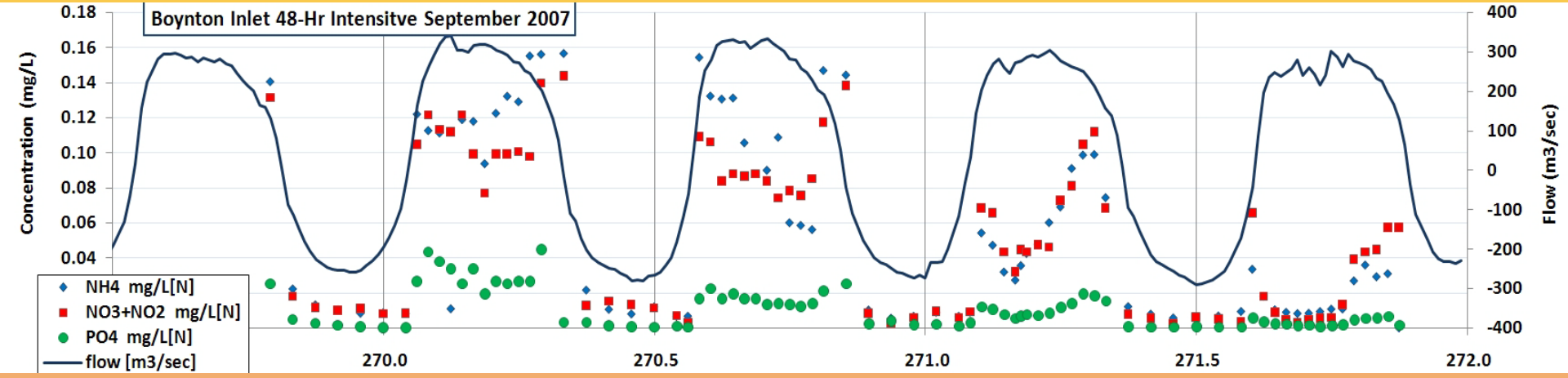
Satellite Photo Boynton Inlet

Export of microbial contamination with outgoing tide, Boynton Inlet 1st 48-hour intensive study, June 2007

assay	0400	1000	1600	2200	0400	1000	1600	2200	1400
human enterococci (esp)	+	-	-	-	+	-	-	-	+
human *Bacteroides* (HuBac)	+	-	-	-	+	-	+	+	+
S. aureus	+	-	-	-	+	-	+	+	+
adenovirus	+	-	-	+	-	-	+	-	+
norovirus	+	-	-	-	-				+
enterovirus	-	-	-	-	+				+
Crytpo/100L			6.3	<1	2.4				24.9
Giardia/100L			4.2	<1	1.2				19.4

inlet, outgoing tide; *Salmonella*, 0157:H7, *C. jejuni*: negative

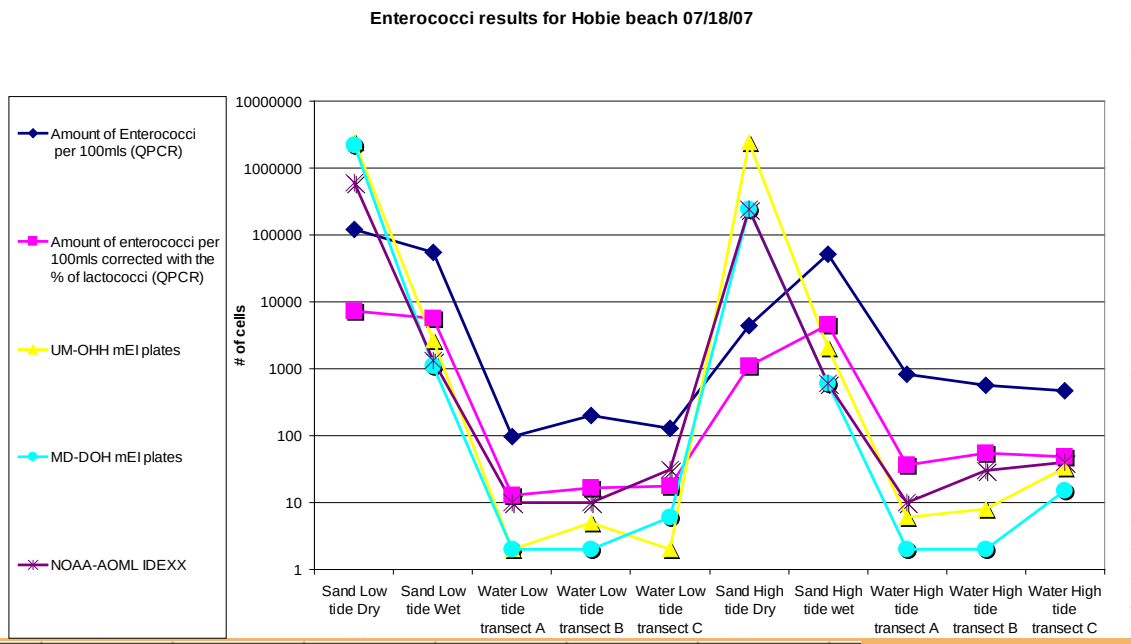
Trends in elevated microbial contamination on outgoing tides correspond to trends in elevated nutrient levels

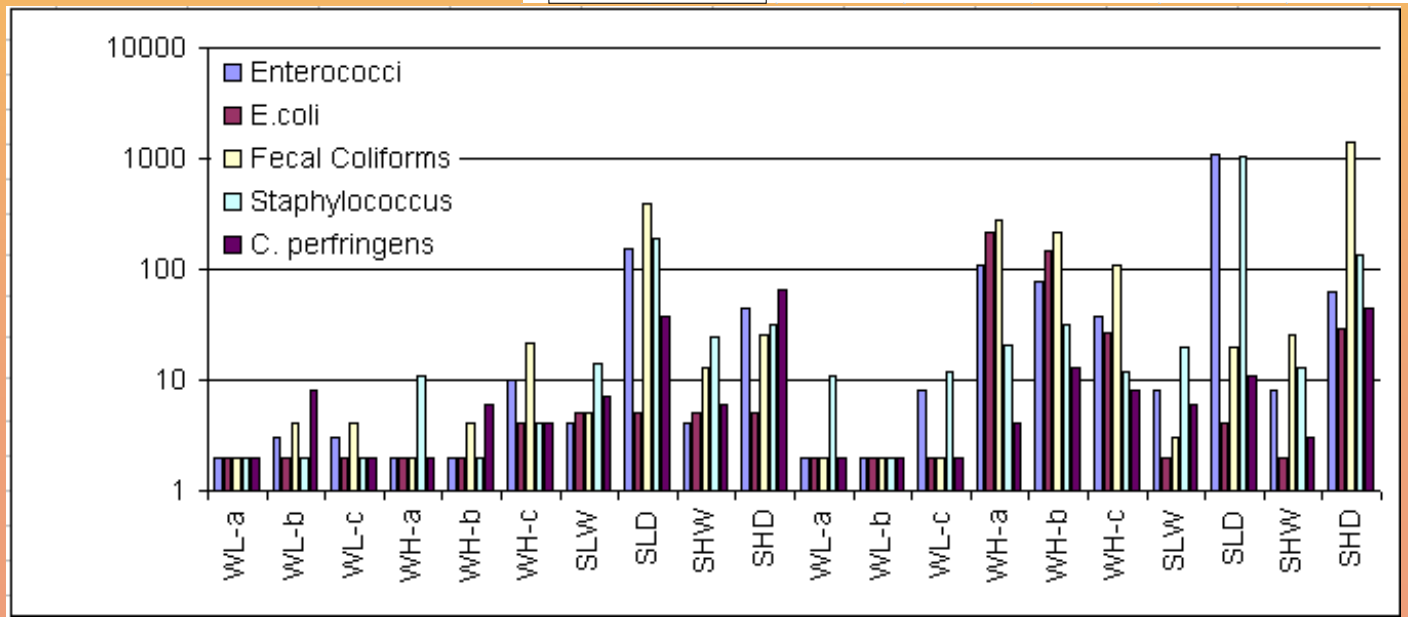


Nutrients and Flow, Boynton Inlet 2nd 48-hour Intensive study, September 2007

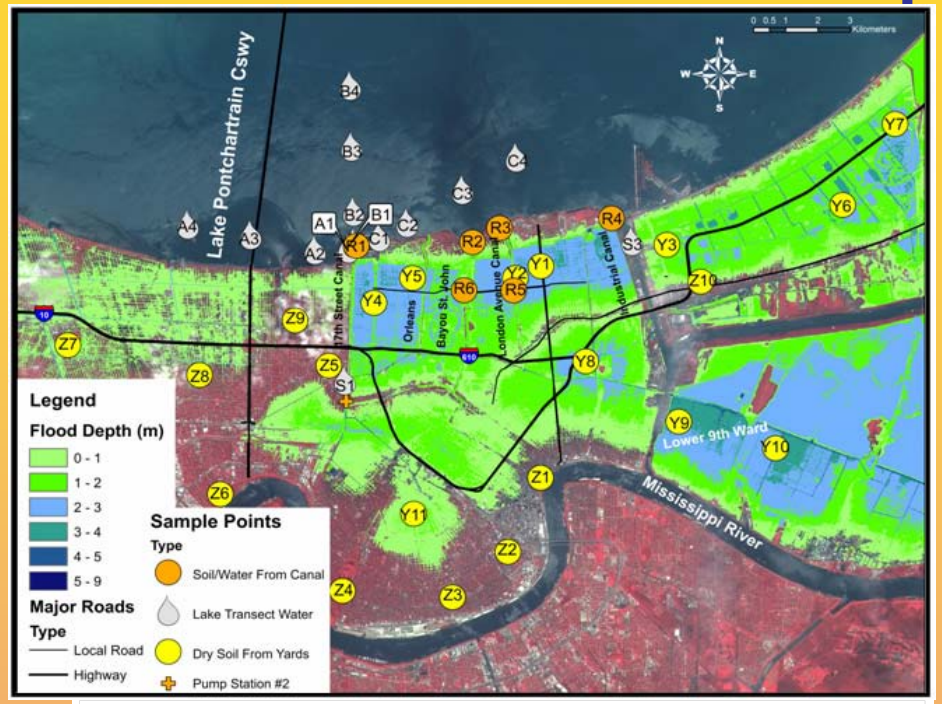
(Zheng et al.)

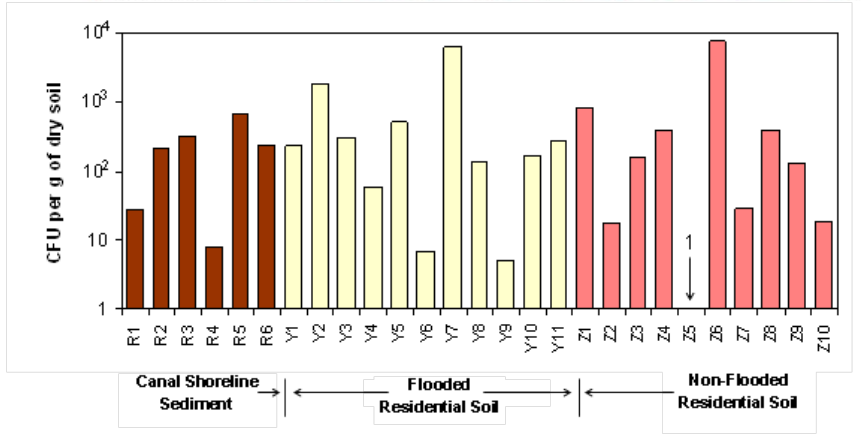
Beach Environmental Assessment and Characterization of Human Exposure Study (BEACHES)



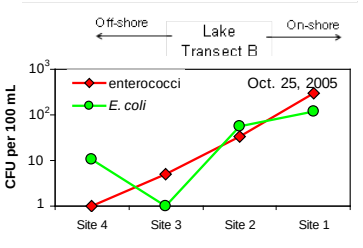


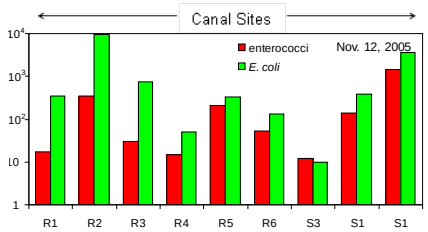
Impact of Hurricanes Katrina and Rita on the Microbial Landscape of New Orleans:





Traditional & Non-Traditional Indicators

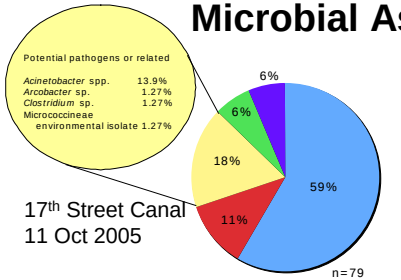




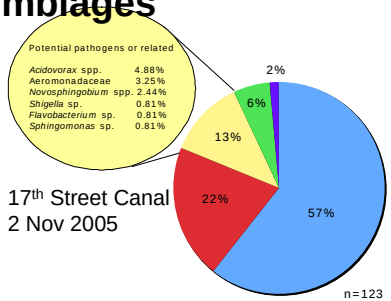
-	-	+	+	Bifido	+	-?	+	-	+	+	+	+	+	
-	-	+	+	Bacteroidetes										
<1	<1	<1	<1	FRNA coliphage										

Non-Traditional Indicators

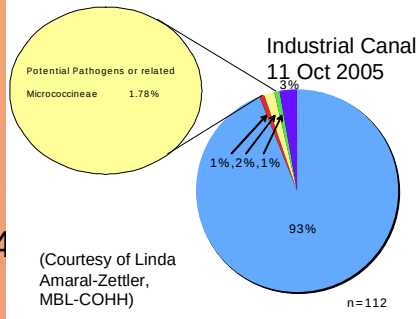
Microbial Assemblages



17th Street Canal
11 Oct 2005

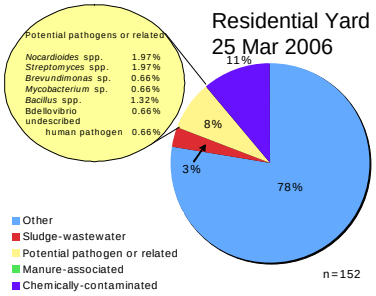


17th Street Canal
2 Nov 2005



Industrial Canal
11 Oct 2005

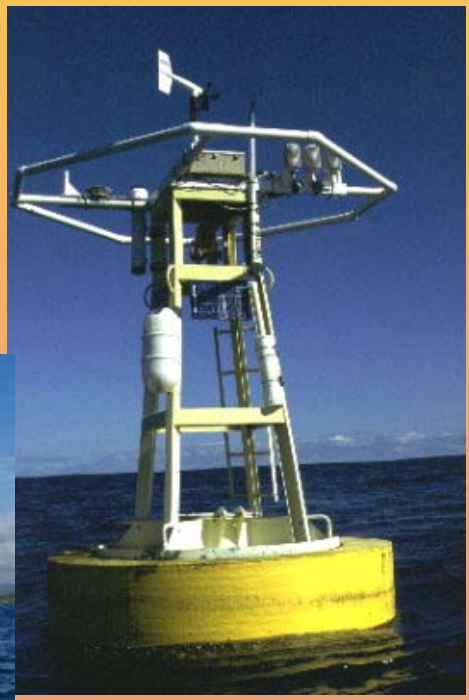
(Courtesy of Linda Amaral-Zettler, MBL-COHH)



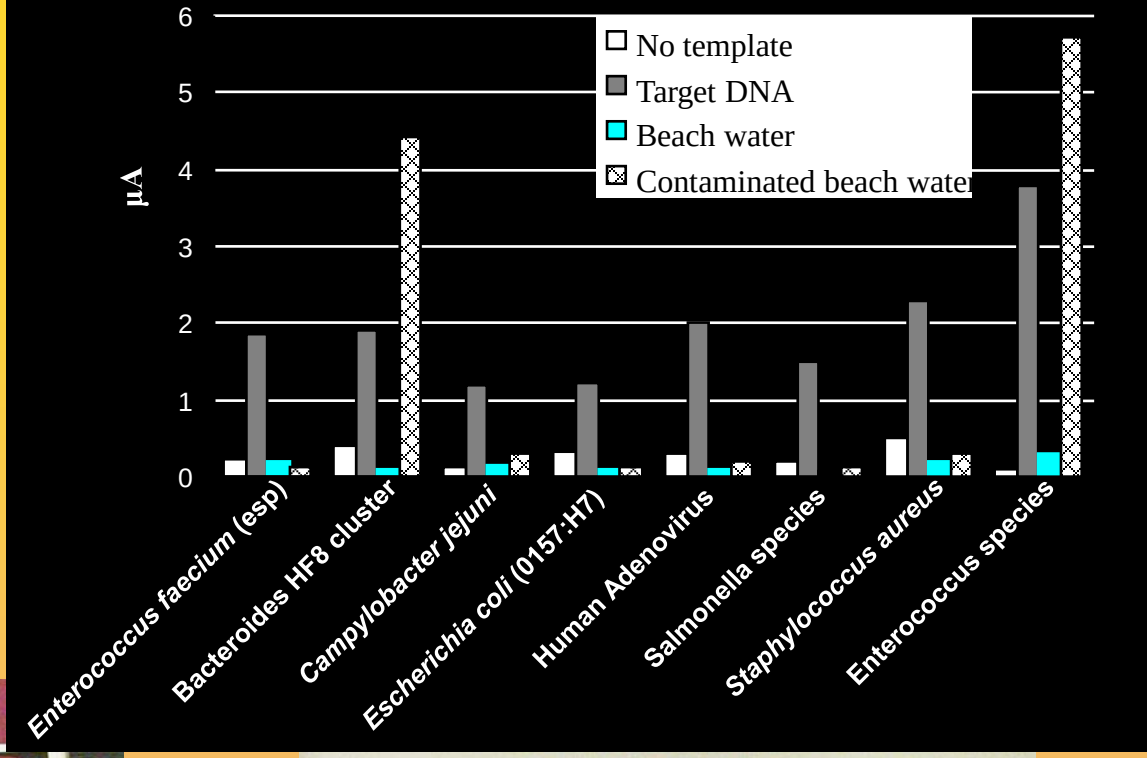
Residential Yard
25 Mar 2006

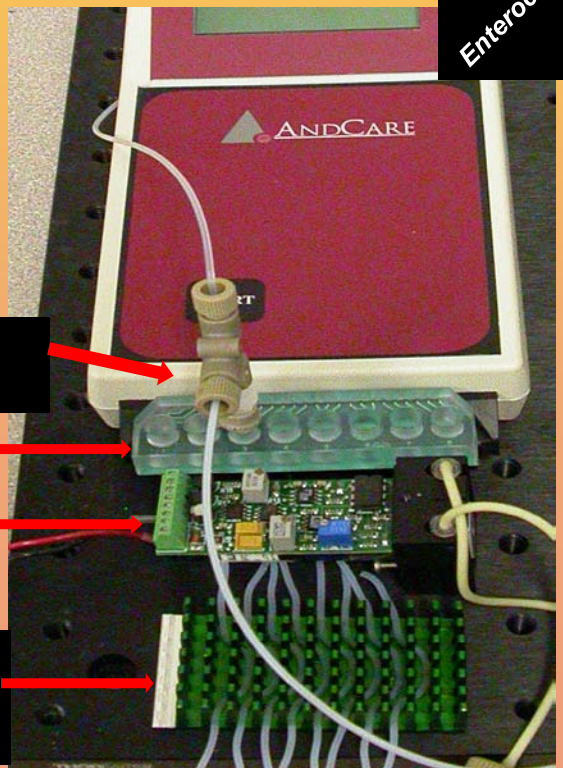
- Reported in: Sinigalliano et al., 2007, PNAS 104:9029-9034
- Reviewed in: Dobbs, 2007, PNAS 104:9103-9104

Portable & In-Situ Biosensors



Prototype handheld automated multiwell PCR analyzer and electrochemical detector, with PC interface



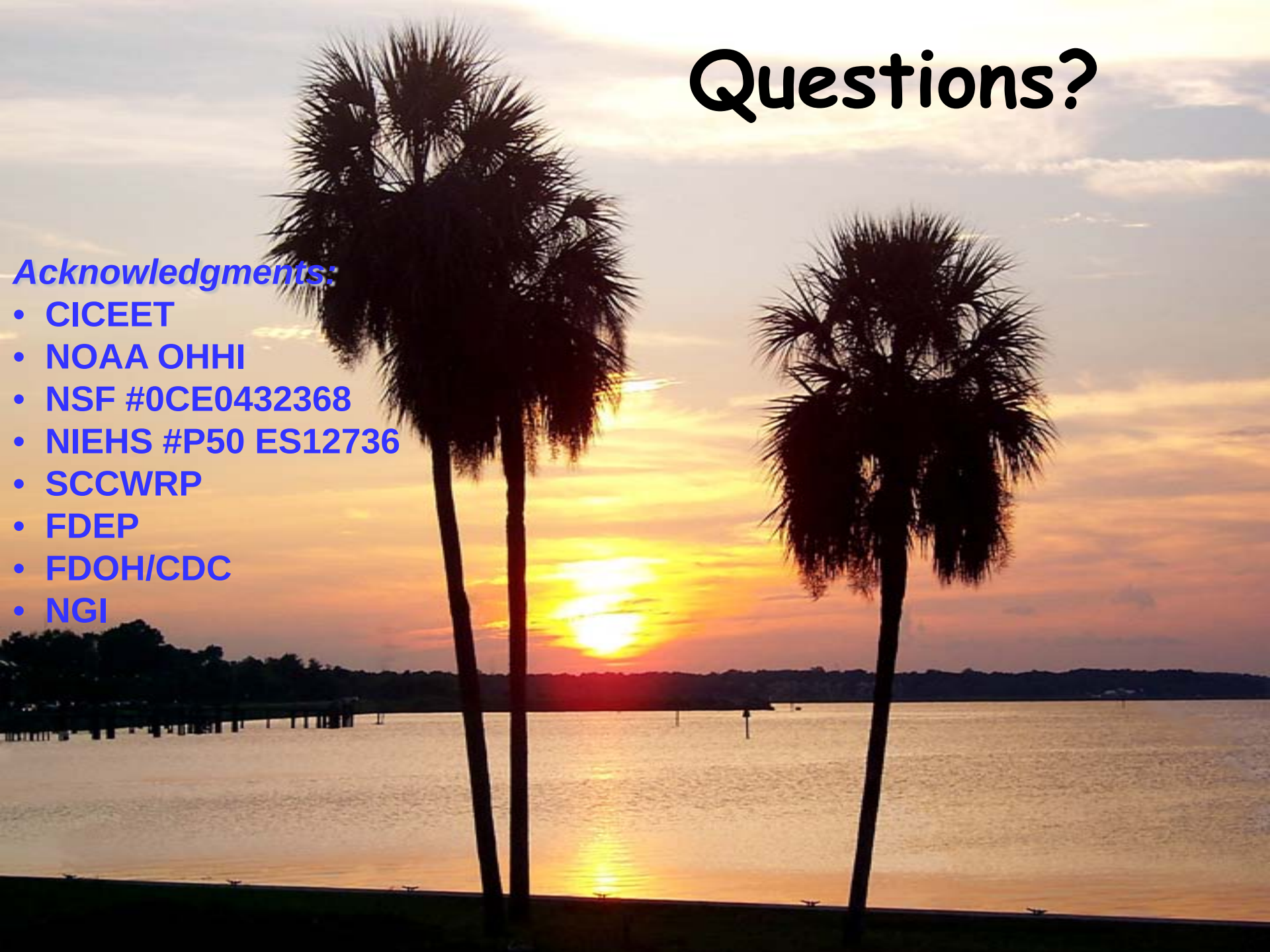


The Product:

biotechnological innovations to produce timely & accurate reporting of ecosystem health and environmental safety

apid, Reliable, Affordable Biosensing

Health risks?



Questions?

Acknowledgments:

- **CICEET**
- **NOAA OHHI**
- **NSF #0CE0432368**
- **NIEHS #P50 ES12736**
- **SCCWRP**
- **FDEP**
- **FDOH/CDC**
- **NGI**