Session 7:
Pollution Source Identification, Tracking, and Sanitary Surveys

Florida Beach Geomorphology and Beach Management Practices Correlate with Enterococci Percent Exceedances

Helena Solo-Gabriele
University of Miami

Abstract

Fecal indicator bacteria (FIB) data for Florida's 316 beaches that are monitored through the Florida Healthy Beaches Program were evaluated to identify possible correlations between FIB levels and beach physical characteristics and beach management practices. Beach characteristics were evaluated for coastal hydrodynamics using offshore bouy information and for observable features using Google imagery. Management information was obtained through a survey that was sent to beach managers. Results show that beach geomorphology (open coast versus bay and beach width), wave energy, proximity of rivers/canals/marshes, presence of piers/causeways, and degree of surrounding urbanization was correlated with FIB levels. For open coast beaches there was a statistically higher level of enterococci percent exceedence for beaches that display higher levels of urbanization (p=0.03), contain major human constructions like piers and/or causeways (p=0.03), and were narrower in width. Beaches within bays or reside in close proximity to marshes were also characterized by higher enterococci percent exceedences (p<0.01). From the beach management survey we also found that beaches that charged fees had lower FIB levels (p<0.01). Beaches that allowed dogs had higher FIB levels (although borderline not statistically significant, p=0.07). Results suggest that the cumulative effects of beach management practices can play a role in whether beaches comply with EPA recreational water quality guidelines.

Biosketch

Dr. Helena Solo-Gabriele is a professor in the Department of Civil, Architectural, and Environmental Engineering at the University of Miami's College of Engineering as well as the college's associate dean for research. Dr. Solo-Gabriele's research has focused on evaluating the relationship between the environment and human health and has spanned diverse areas that include evaluating the human health impacts of metals/pesticides in the environment and of microbial contaminants in coastal zones. Her microbial contaminants research focuses on evaluating the water-sand interface and the microbial contaminants in that area as well as the conditions under which microbes are released from that zone. Most recently, Dr. Solo-Gabriele has focused her efforts on evaluating data for many beaches; her presentation will summarize her work on beaches throughout the State of Florida.

Slide 1

Florida Beach Geomorphology and Beach Management Practices Correlate with Enterococci Percent Exceedences

Helena Solo-Gabriele, Ph.D.
Professor and Assoc. Dean for Research
University of Miami, College of Engineering

EPA National Beaches Conference
April 14, 2016

Slide 2 — Acknowledgments

Elizabeth Kelly, Allison Donahue, Ryan Mustacato, Sabina Rokhimbekova, Sathvika Ramaji

Zhixuan Feng, Laura Vogel

David Polk & Samir Elmir

Missing: Henry Barreras, Nicole Johnson, Hannah Lockwood

Ad Renters

Florida HEALTH

National Institute of Environmental Health Sciences

National Science Foundation

OCEANS AND HUMAN HEALTH CENTER

OHH

Slide 3 — Outline/Methods

- Florida's Beach Monitoring Program
- Florida's Beach Geomorphology
  - Wind Data and Beach Slopes (Feng et al.)
  - Visual from Google Earth (In progress)
- Beach Management
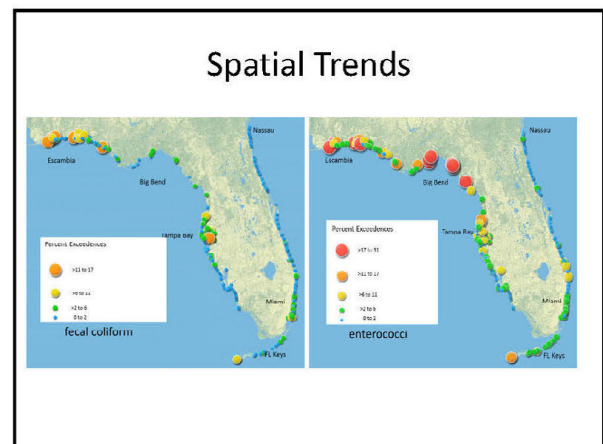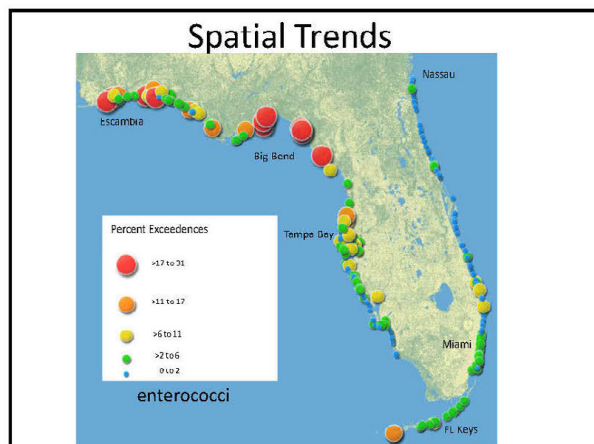 - Survey. Sewage, stormwater, solid waste, human and animal densities, seaweed and grooming, fees (In progress)
- Conclusions & Recommendations
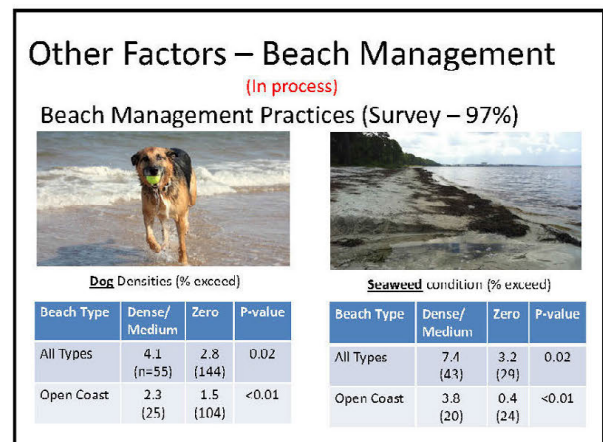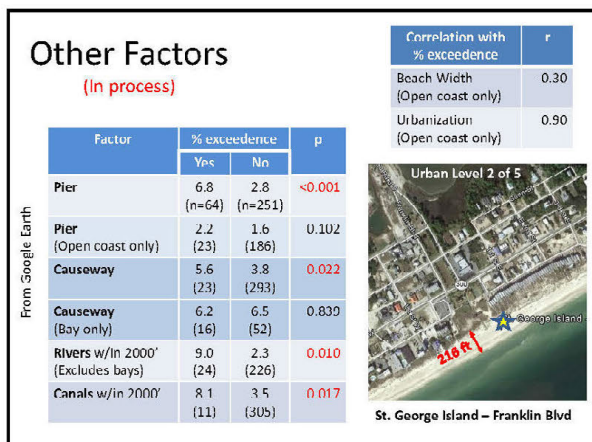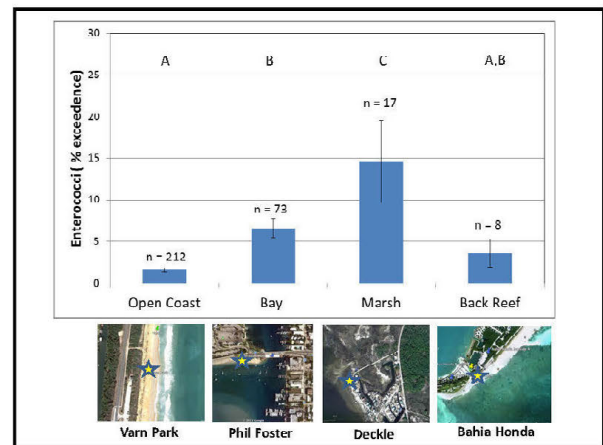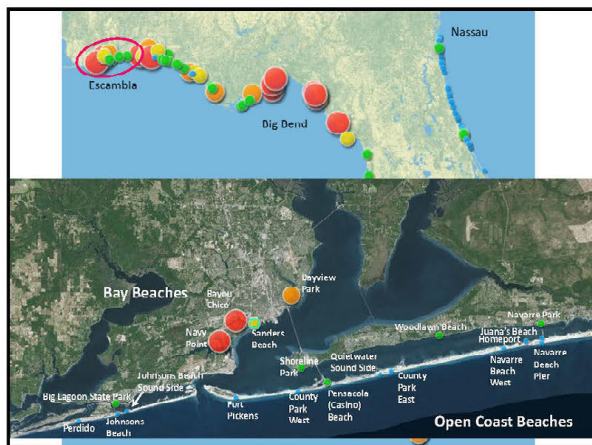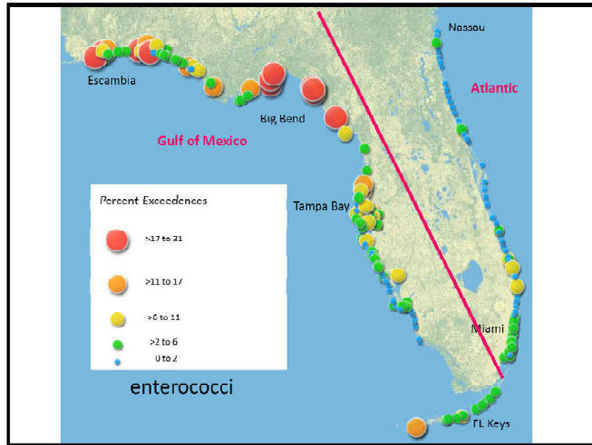
Association with Enterococci

Slide 4 — Florida Healthy Beaches Data

- Period of Record: 2000 to 2014 (2015)
- Sampling Frequency: Monthly → Weekly → Variable
- Converted FIB measures to "percent exceedences"

FIB	n	Threshold for % exceed
Enterococci	182,000	104 CFU/100 ml
Fecal Coliform	154,000	400 CFU/100 ml

- No. Points per Beach: 120 to 690 (enterococci)
- No. Beaches: 316

Slide 5 — Spatial Trends

Percent Exceedences
- >17 to 31
- >11 to 17
- >6 to 11
- >2 to 6
- 0 to 2

enterococci

Slide 6 — Spatial Trends

fecal coliform

enterococci

Slide 1 (top-left map):

Nassau

Escambia

Atlantic

Big Bend

Gulf of Mexico

Tampa Bay

Percent Exceedences

>17 to 31
>11 to 17
>6 to 11
>2 to 6
0 to 2

Miami

FL Keys

enterococci

Slide 2 (top-right map):

Marsh Beaches

Shell Point

Mash's Sands

Open Coast

Alligator Point

Bay Beach

Carabelle Beach

St. George Island – State Park

St. George Island, 11th Street East
St. George Island, Franklin Street

Open Coast Beaches

Slide 3 (middle-left map):

Nassau

Escambia

Big Bend

Bay Beaches

Bayou Chico

Bayview Park

Navy Point

Sanders Beach

Woodlawn Beach

Juana's Beach Homeport

Navarre Park

Shoreline Park

Quietwater Sound Side

Navarre Beach West

Navarre Beach Pier

Johnson Beach Sound Side

County Park West

Pensacola (Casino) Beach

County Park East

Big Lagoon State Park

Perdido

Johnsons Beach

Fort Pickens

Open Coast Beaches

Slide 4 (middle-right chart):

A   B   C   A,B

Enterococci (% exceedence)

n = 17

n = 73

n = 212

n = 8

Open Coast   Bay   Marsh   Back Reef

Varn Park   Phil Foster   Deckle   Bahia Honda

Slide 5 (bottom-left):

Other Factors
(In process)

	Factor	% exceedence		p
		Yes	No	
From Google Earth	Pier	6.8 (n=64)	2.8 (n=251)	<0.001
	Pier (Open coast only)	2.2 (23)	1.6 (186)	0.102
	Causeway	5.6 (23)	3.8 (293)	0.022
	Causeway (Bay only)	6.2 (16)	6.5 (52)	0.839
	Rivers w/in 2000' (Excludes bays)	9.0 (24)	2.3 (226)	0.010
	Canals w/in 2000'	8.1 (11)	3.5 (305)	0.017

Correlation with % exceedence	r
Beach Width (Open coast only)	0.30
Urbanization (Open coast only)	0.90

Urban Level 2 of 5

St. George Island

218 R?

St. George Island – Franklin Blvd

Slide 6 (bottom-right):

Other Factors – Beach Management
(In process)
Beach Management Practices (Survey – 97%)

Dog Densities (% exceed)

Beach Type	Dense/ Medium	Zero	P-value
All Types	4.1 (n=55)	2.8 (144)	0.02
Open Coast	2.3 (25)	1.5 (104)	<0.01

Seaweed condition (% exceed)

Beach Type	Dense/ Medium	Zero	P-value
All Types	7.4 (43)	3.2 (79)	0.02
Open Coast	3.8 (20)	0.4 (24)	<0.01

Conclusions:
(Factors Associated With Low Bacteria)

Hydrodynamics (Feng et al. 2016)
- High Wave Energy, Steeper Slopes

Physical Features
- Open Coast < Bay < Marsh
- No Piers, No Causeways, Low Urban
- Wide Beaches
- Rivers/Canals Far Away

Beach Management
- No Dogs
- No Seaweed
- Charge Fees
- Covered Trashcans

Recommendations
1) Evaluate high enterococci relative to fecal coliform
2) Evaluate sand quality.
3) Evaluate impact of water chemistry at marsh beaches.

Conduct human health study in Big Bend

Questions? hmsolo@miami.edu

Source Tracking of Ankle-Depth Water Quality Impairment at Freshwater Beaches in Ontario, Canada

Thomas Edge, PhD
Environment Canada

Abstract

Ankle-depth *E. coli* concentrations were investigated at 15 freshwater beaches in southern Ontario, Canada. Across about 1,300 sampling events, ankle-depth *E. coli* concentrations were found to exceed chest-depth *E. coli* concentrations most often at sheltered beaches (91%), followed by exposed beaches (84%) and river beaches (68%). Ankle-depth *E. coli* concentrations could exceed 10,000 colony-forming units (CFUs) / 100 milliliters (mL) at times at 10 of the 15 beaches, and two sheltered beaches had ankle-depth *E. coli* concentrations that could exceed 100,000 CFU / 100 mL. Pair-wise analyses found that ankle-depth *E. coli* concentrations were significantly higher than adjacent chest-depth concentrations at each of the 15 beaches investigated ($p < 0.05$). Microbial and chemical source tracking techniques are being applied to assess the potential significance of this water quality impairment. Application of a suite of microbial assays (e.g., endpoint and qPCR fecal marker assays for human, gull) and chemical assays (e.g., caffeine, carbamazepine) have indicated that bird fecal contamination rather than human fecal contamination is the primary fecal pollution source at a number of beaches. Additional research is required to better understand the potential for exposure in ankle-depth waters at freshwater beaches and the potential for health risks.

Biosketch

Dr. Tom Edge is a research scientist at Environment and Climate Change Canada's National Water Research Institute in Burlington, Ontario. He received his doctorate in environmental microbiology from Carleton University in Ottawa, his master of science degree from the University of Ottawa, and his bachelor science degree in marine biology from the University of Guelph. Dr. Edge conducts research on microbial source tracking and waterborne pathogens in source waters used for drinking, recreation, and shellfish production across Canada. He has served as president and a board member of the Great Lakes Beach Association and has been a scientific advisor to the International Joint Commission and Health Canada on beach issues and Canadian recreational water quality guidelines.

Sources of Enterococci and their Relation with Environmental Factors at an Inland Beach

Asli Aslan, PhD
Georgia Southern University

Abstract

Nonpoint sources of pollution have been reported as an alternative source of high enterococci levels in beaches. In this study, persistent high levels of enterococci were investigated at an inland beach in Georgia that has been under permanent advisory since 2005. On a monthly basis for 1 year, data were collected on Enterococci (using method 1600 for water, Enterolert for sediment, and method 1611 for water and sediment), source tracking markers (HF183 and GFD), temperature, salinity, pH, dissolved oxygen, and turbidity. Microbial source tracking analyses showed high HF183 concentrations around the beach at the beginning of the study (405 calibrator cell equivalents/100 milliliters [ml]) and further investigations proved an illicit sewage leak close to the beach. Culturable enterococci in water significantly decreased below 70 CFU/100 ml criteria value and HF183 was below detection limits after controlling the sewage intrusion to the beach. In addition, wildlife (particularly avian species) was a major source of enterococci in this area. GFD marker ranged from 152–1077 gene copies/100 ml in HF183 negative water samples. Sediment also harbored culturable enterococci and concentrations ranged from 29–24196 MPN/100 ml throughout the study, showing significant relation to temperature ($p < 0.001$). Tidal movements and precipitation were the major factors ($p < 0.001$) for elevated concentrations of enterococci after controlling the point source. Further studies are needed to investigate the relation between culturable enterococci and pathogens such as enteric viruses to assess health risks upon exposure to contaminated sediments on inland beaches.

Biosketch

Dr. Asli Aslan is a water microbiologist whose primary research areas include microbial source tracking, health risk assessment of water resources, and ecology of pathogens in the aquatic environment. Her research program bridges ecosystems and public health. Dr. Aslan is currently an assistant professor at the Department of Environmental Health Sciences at Georgia Southern University. She holds doctoral and master of science degrees from Istanbul University, Institute of Marine Sciences and Management, and has completed her postdoctoral studies at Michigan State University, College of Natural Resources and Agriculture. Dr. Aslan has conducted and participated in several health-related water microbiology projects in Turkey, Malawi, Ghana, the United States, and other countries; authored various manuscripts; and presented at numerous conferences. She has been serving in various state and federal agencies and organizations as an advisor, reviewer, scientific committee member, and/or affiliated faculty member.

Sources of enterococci and their relation with environmental factors at an inland beach

ASLI ASLAN
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Disclaimer

This presentation was prepared by Dr. Aslan under grant award #NA14NOS4190117 to the Department of Natural resources from the Office of Ocean and Coastal Management, National Oceanic and Atmospheric Administration. The statements, findings, conclusion and recommendations are those the author(s) and do not necessarily reflect the views of DNR, OCRM or NOAA.

Coastal Georgia

- 3,400 miles of tidal shoreline
- 100 miles of sand beach
- 300 square miles of open Atlantic Ocean
- 9 major estuaries
- 14 barrier islands

Courtesy of Ms. Elizabeth Cheney

Georgia Beaches
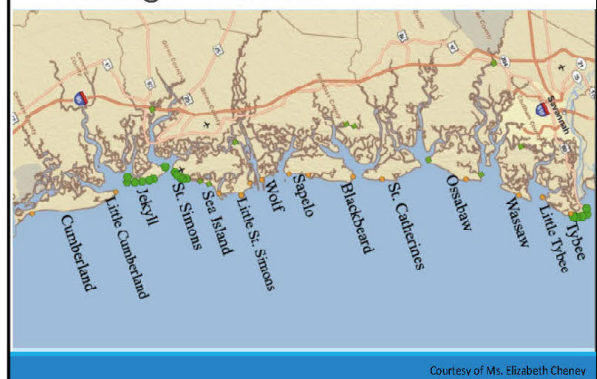
Courtesy of Ms. Elizabeth Cheney

Kings Ferry Beach

Under permanent advisory since 2005.

http://www.paddling.net/
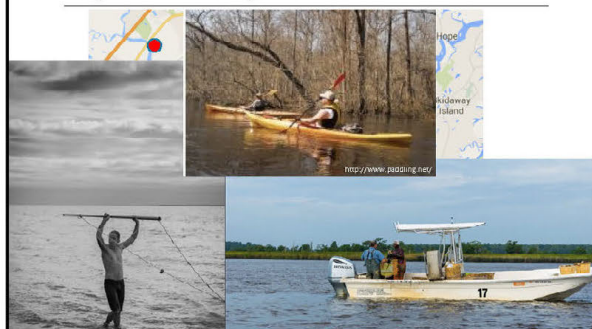
Study Site

Sample Collection

Water samples: 14 sites monthly in winter, twice a day (low-high tide) during the summer

Sediment samples: 6 sites monthly, 14 sites one in winter, one in the summer

Beach sanitary surveys

Temperature, salinity, dissolved oxygen, pH, turbidity

Laboratory Analysis

Culture Methods	Molecular Methods
Enterococci **Water:** Membrane filtration & MEI agar **Sediment:** Enterolert	**DNA Extraction** **Water:** Crude DNA extraction & purification **Sediment:** MOBIO Power Soil
Escherichia coli **Water:** Membrane filtration & mTEC agar **Sediment:** Colilert	**qPCR** ·Enterococci (23S rRNA) (Haigland et al., 2005) ·Sewage marker (HF183) (Green et al., 2014) ·Bird marker (GFD) (Green et al., 2014)

Culture methods comparison
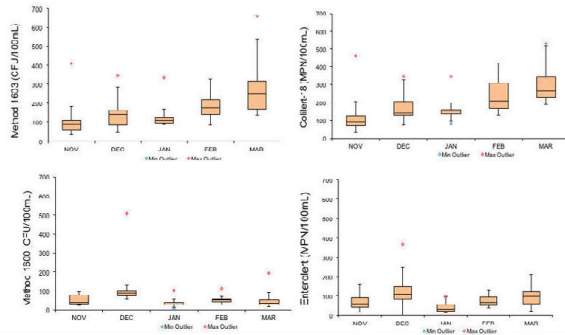
Beach
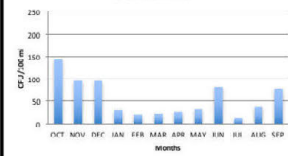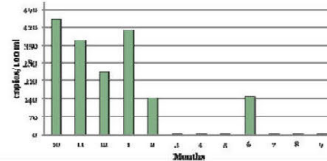
	Enterococci (MPN/100 ml)	Enterococci 23S (CCE/100 ml)	HF183 (copies/100 ml)
KF18	4,543	22,235	1,566,373
KF20	91	950	300
KF3	49	1,350	142

Culture versus qPCR

Environmental Factors-Tides

Enterococci and Tides

p=0.097

Enterococci species

A total of 55 Enterococci isolates from mEI agar were analyzed by Sanger sequencing.

Enterococci spp.

- *E. faecalis* — 71%
- *E. faecium* — 13%
- *E. casseliflavus* — 6%
- *E. avium* — 5%
- *E. gallinarum* — 5%

Environmental Factors-Temperature
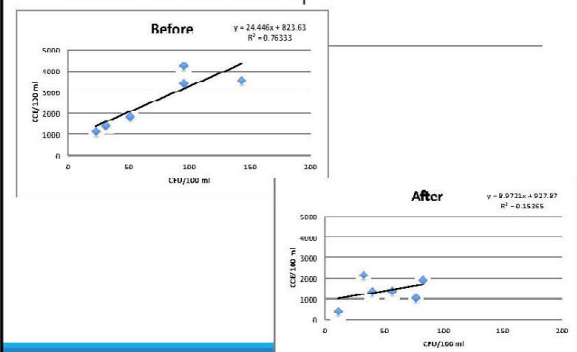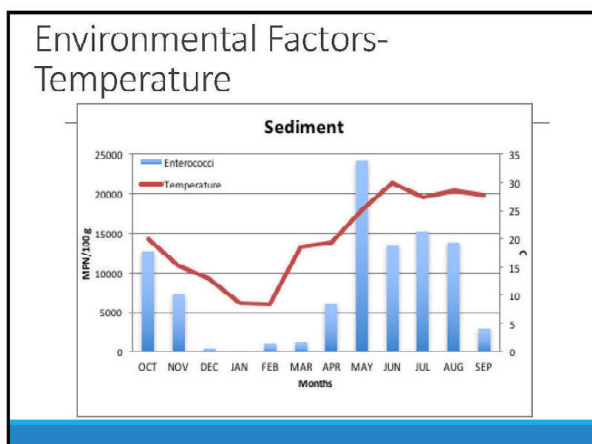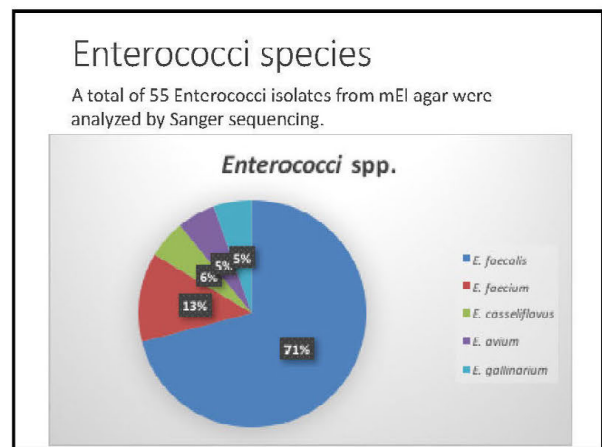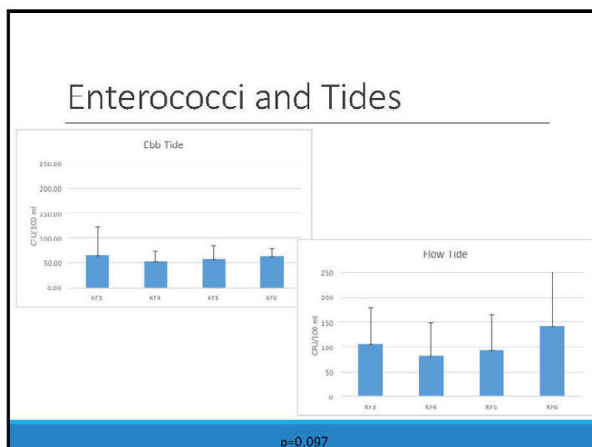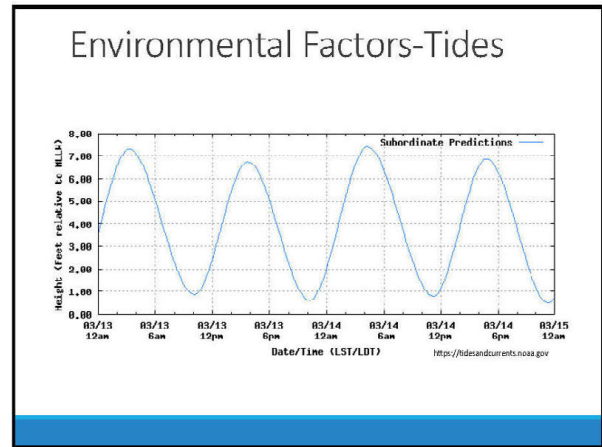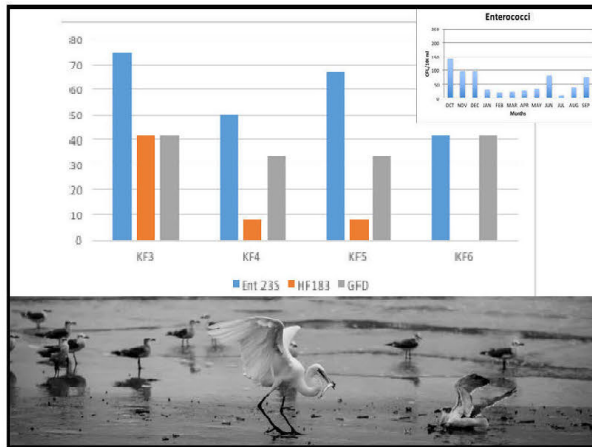
Conclusions

- Method 1600 and Method 1611 results were significantly related in the presence of fresh sewage (p<0.001).
- Microbial source tracking (HF183 and GFD) is a powerful tool to identify the sources of high levels of Enterococci in rural environments.
  - Inform local agencies for the advantageous of new technology
- Environmental factors such as temperature and tidal movements are drivers for high levels of Enterococci.
  - Each beach is unique, ebb or flow tide can transport Enterococci depending on the location of the potential sources.
- Sediment harbors Enterococci and potentially acts as a reservoir in warmer temperatures.
- Future studies will involve detecting changes in the bacterial community structure and Enterococci diversity before and after the sewage leak.

Temporal Stability of Fecal Bacteria and Microbial Source Tracking Markers in Water: Implications for Source Allocation Using MST Markers

Mia Mattioli, PhD
Emory University

Abstract

A coupled field and laboratory study of the temporal stability of fecal bacteria was assessed to inform the use of microbial source tracking (MST) markers for inferring sources of fecal contamination in natural waters. The temporal stability of enterococci, *E. coli*, and human-associated markers of fecal pollution was assessed in seawater. Seawater from Half Moon Bay, California, was seeded with raw sewage and placed into 6–8-kilodalton dialysis bags. Approximately 30 dialysis bags were deployed at both the surface of the water column and at 1-meter depth and destructively sampled over 10 days during the summer and winter. A distinct difference in decay at surface and at depth was observed, presumably due to decreasing UVA and UVB wavelength intensity at depth. The absorbance of the ambient water illustrates that the water column is not colored and relatively clear. A model was developed for the depth-dependent inactivation rate of the fecal indicator bacteria (FIB) and human markers, and it shows how a depth averaged inactivation rate varies with depth of the water column. The decay of MST markers and FIB was the same in dark water and diverged in shallow waters. This finding suggests that the ratio method for source allocation using MST markers can be applied only in dark water.

Biosketch

Dr. Mia Catharine Mattioli is a joint post-doctoral scholar at Emory University and the Waterborne Disease Prevention Branch of the Centers for Disease Control and Prevention (CDC). She has a bachelor of science degree in biological engineering from the University of Georgia (UGA) and masters and doctoral degrees in environmental engineering from Stanford University. Dr. Mattioli's research is focused on the intersection between the environment and human health with a specific interest in the relationship between fecal indicators and enteric pathogens in various environmental matrices. During a postdoctoral fellowship at Stanford University, she examined the decay characteristics of fecal indicators and sewage-related bacterial communities in environmental waters. Dr. Mattioli is currently working on a collaborative project between the CDC, UGA, and Emory studying the use of ultrafiltration as a means for monitoring microbial contamination of irrigation waters.

Exploring the Microbiome for New Approaches to Microbial Source Tracking

Sandra McLellan
University of Wisconsin—Milwaukee

Abstract

Fecal microbiome studies offer new opportunities to identify alternative indicators for microbial source tracking that are host-associated and more informative than general indicators that are ubiquitously present in humans and other animals. Microbiome next generation sequencing technologies provide hundreds of thousands to millions of DNA sequences per sample. While mining the data can be challenging, it is clear that host-associated patterns in fecal microbiomes have important implications for water quality testing. Bacteroidales and Clostridiales are two bacterial groups that are particularly rich in potential host-associated indicators, including the organisms within the family Lachnospiraceae, which has not been explored in depth previously. We report the development of Lachnospiraceae qPCR assays that can be used in conjunction with established Bacteroidales methods to quantify sewage contamination in the Great Lakes, particularly in respect to characterizing recreational beach impairment. In addition to qPCR applications, next generation sequencing strategies can make use of distinctive host community trends within the broad groups of Bacteroidales and Clostridiales for the identification of fecal pollution sources. As microbiome studies increase the number of potential candidate host-associated indicator strategies, future laboratory validation and field studies will be critical to identify the most applicable methods for recreational water quality monitoring.

Biosketch

Dr. Sandra McLellan is a professor in the School of Freshwater Sciences at the University of Wisconsin-Milwaukee. Her research focuses on studying microbial communities in the urban environment and the implications for human and ecosystem health. Studies include determining the fate of pathogens in the Great Lakes and determining the causes of beach closings. Her laboratory employs next generation sequencing approaches to identify new indicators of pollution and pollution impacts. Dr. McLellan's group has identified several promising alternative indicators within diverse families of fecal bacteria and has developed microbial signatures for humans, cattle, and urban wildlife. These signatures are being tracked in urban waters to delineate the extent of sewage contamination and the drivers of pollution. Dr. McLellan interacts with a wide range of stakeholders, including public health officials and water resource managers to translate basic research into practical applications and sound policy. She graduated from the University of Wisconsin-Milwaukee in health sciences in 1990 and worked at Miller Brewing Company before entering graduate school. Dr. McLellan earned her graduate degree in environmental health at the University of Cincinnati College of Medicine in 1998.

Exploring the microbiome for new approaches for microbial source tracking

Sandra L. McLellan, Ryan J. Newton, Jen C. Fisher, and Debra K. Dila
School of Freshwater Sciences, University of Wisconsin Milwaukee

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Fecal Pollution contains a complex mixture of organisms

Fecal indicator bacteria
Rare, <5% in mammals,
Up to 50% in birds
E. coli
enterococci

Klebsiella pneumoniae
Enterobacter cloaccae

Fecal anaerobes
95% of microbiota
Bacteroides spp.
Lachnospiraceae

Bifidobacteria
Prevotella spp.

Pathogens
intermittent
Salmonella
Cryptosporidium
viruses
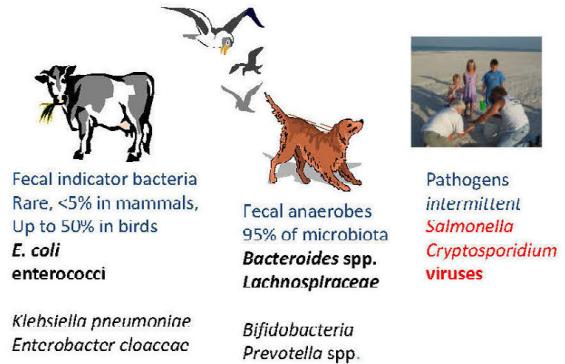
Characterizing sewage
Sewage organisms 10^9 cells per ml

culture
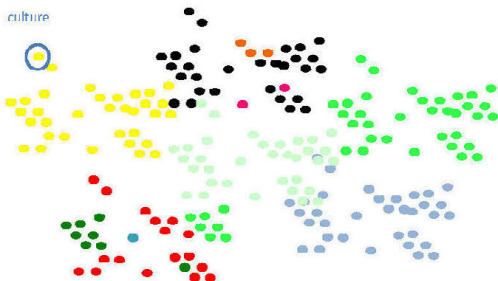
Sewage organisms 10^9 cells per ml

Cloning and sequencing the 16S rRNA gene- "who's there"

1000-2000 sequences is a good effort- only see the most abundant organisms

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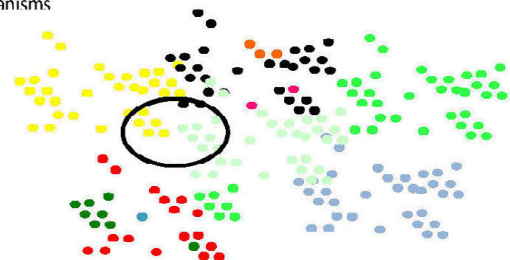
Sewage organisms 10^9 cells per ml

Illumia MiSeq or Hiseq sequencing
On average 100,000 sequences per sample
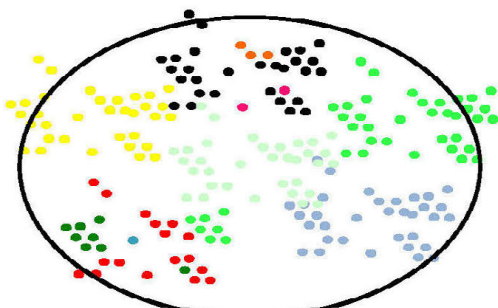
Sequencing Sewage
V6 rRNA gene

>100,000 reads per sample
800 different taxa
20,000 different sequences

80% environmental origin

Acinetobacter
Aeromonas
Pseudomonas

Arcobacter
Betaproteobacteria

20% faecal origin
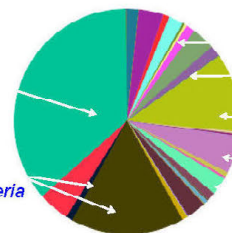
Actinobacteria
Bacteroides

Trichococcus

Lachnospiraceae
Ruminococcaceae

McLellan et al. (2010) *Environmental Microbiology* 12(2), 378–392
VandeWalle et al. (2013) *Environmental Microbiology* 14(9). 2538–2552

How do we make sense of millions of data points?

Sequence — **Identify the members**
Community analysis

Raw reads
Demultiplex — Assign taxonomy
Merge — Cluster into OTUs (oligotyping)
Quality trim — Network analysis to compare sequences
Chimeras — LefSe analysis to identify biomakers

Genera of fecal anaerobes common to hosts

Fisher et al *Appl Environ Microbiol* 81:7023-7033.

What is shared and what is unique?
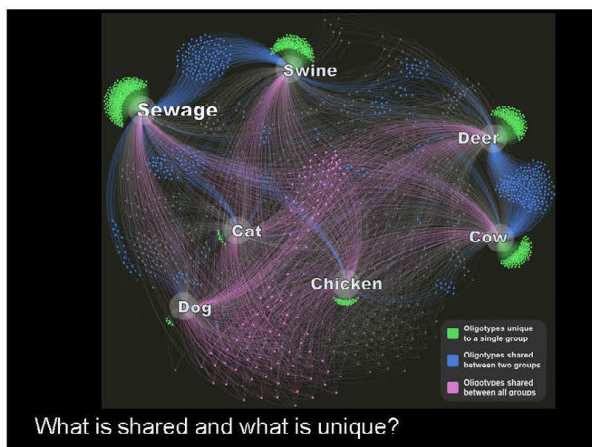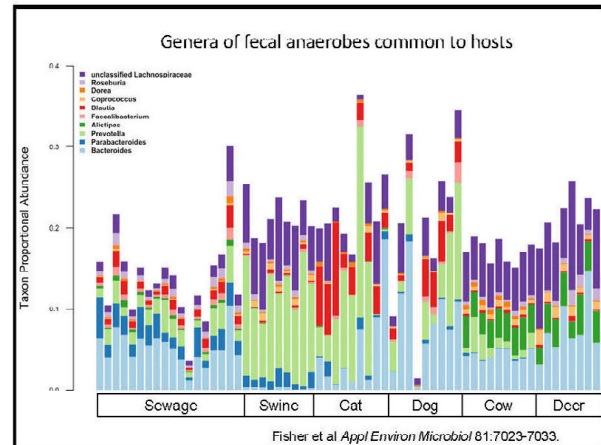
What taxa contain preferred and unique sequences?

Fisher et al 2015 *Appl Environ Microbiol* 81:7023-7033

Lachnospiraceae very promising

Compare humans, sewage, chickens and cows

McLellan et al. (2013) *Environmental Microbiology*
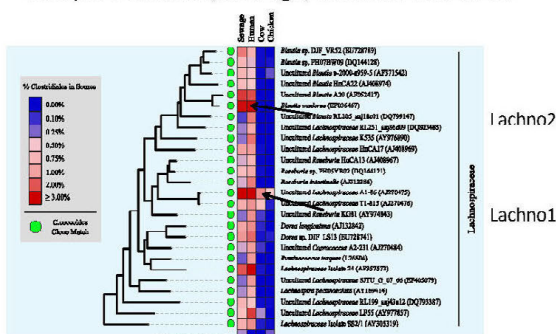
Lachno2

Lachno1

Lachnospiraceae as a qPCR target for alternative indicators

Clone near-full length sequences
Align and design primers for qPCR

Developed assay for qPCR
Lachno2, Lachno3, and Lachno12
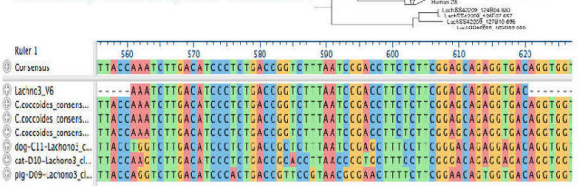
Sequence profiles of *Blautia* act as a fingerprint

Heatmap of sequence abundance shows humans (sewage) and various animals have unique sequence profiles

Chicken Dog Cow Human Sewage Swine Swine (Brazil) Cat Deer
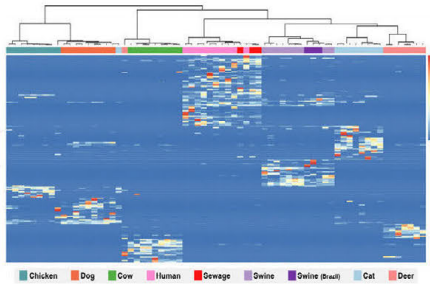
Erenet al (2015) *Isme J* 9:90-100 , McLellan and Eren (2015) *Trends in Microbiol* 22:697-706

Sequence profiles of *Blautia* act as a fingerprint

Next Steps: build a searchable database so sequencing data can be interpreted   **Cost: $40-$100 for sequencing**

Chicken Dog Cow Human Sewage Swine Swine (Brazil) Cat Deer
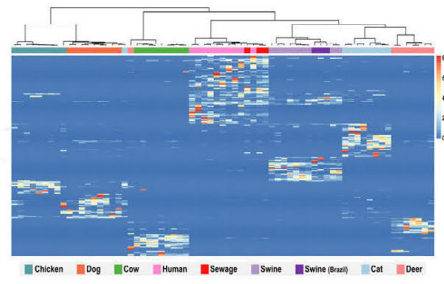
Erenet al (2015) *Isme J* 9:90-100 , McLellan and Eren (2015) *Trends in Microbiol* 22:697-706
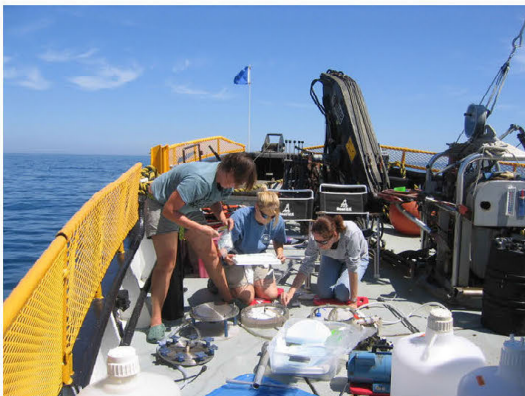
Signature of sewage microbial communities

Lachnospiraceae;NA   Bacteroides
Ruminococcaceae;NA   Ruminococcaceae;Faecalibacterium
Bifidobacterium   Ruminococcaceae;Ruminococcus
Lachnospiraceae;Roseburia   Lachnospiraceae;Dorea

Survey stations into Lakes Michigan
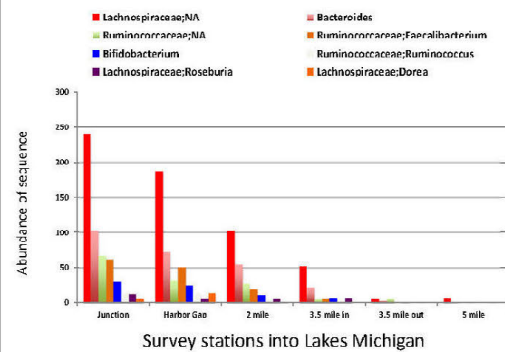
Human indicators in the nearshore

Heavy Rain/Blending Event   Heavy Rain/CSO & SSO

0   5.0E3   1.5E4   1.0E5   1.0E6   2.5E7
Copies/L water
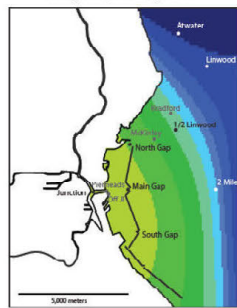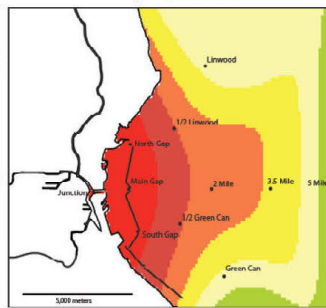
Newton et al. (2013) *Microbial Ecology* 65:1011-23

Characterizing the gull microbiome

- Gulls can be major sources of fecal contamination
- Gulls have been shown to harbor high abundances of FIB, *E. coli* and enterococci, which can be detected readily as part of routine beach monitoring

Despite the ubiquitous presence of gull fecal contamination at beaches, the microbial community is relatively poorly characterized

Microbial community structure in gull fecal samples

Enterococcaceae and *Enterobacteriaceae* were identified as the two most abundant families.
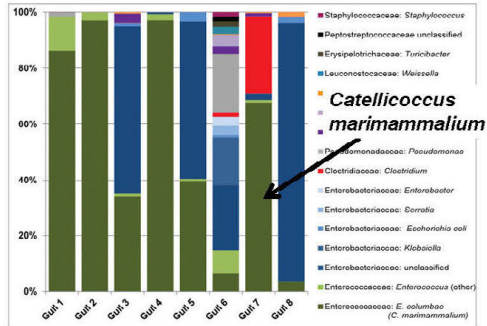
Microbial community structure in gull fecal samples
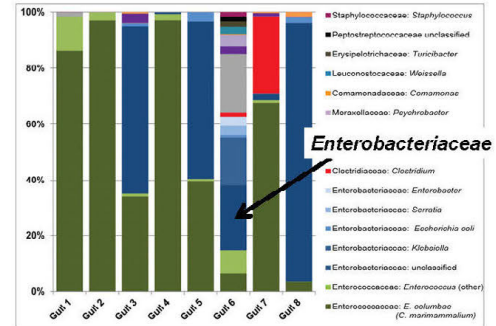
Koskey et al. (2014) *Appl Environ Microbiol* 80:757-765

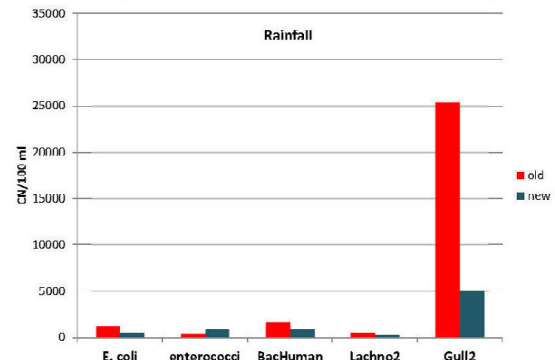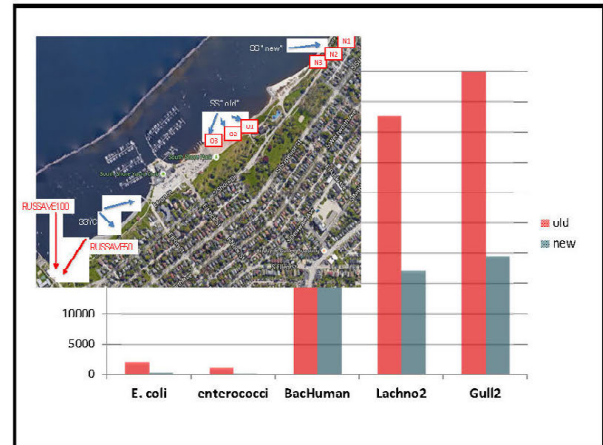South Shore Beach, Milwaukee WI worst water quality on Lake Michigan
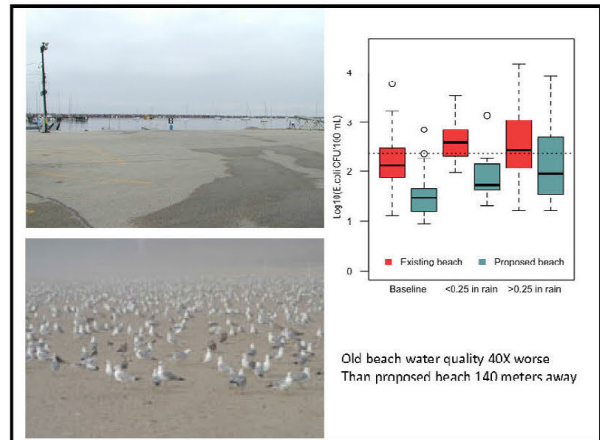
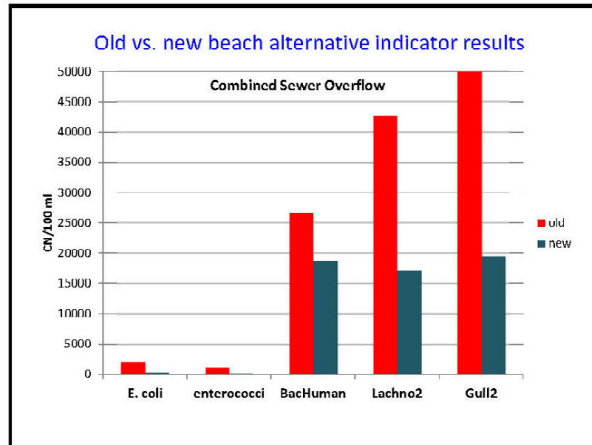Old beach water quality 40X worse Than proposed beach 140 meters away

Old vs. new beach alternative indicator results

Old vs. new beach alternative indicator results
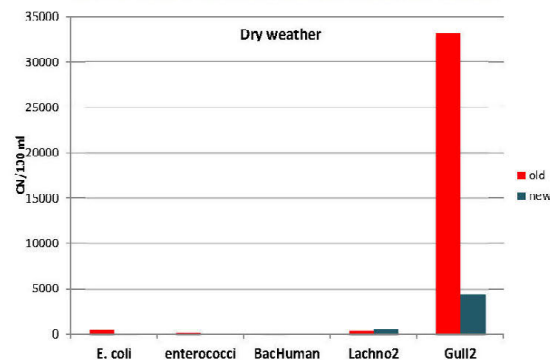
Old vs. new beach alternative indicator results

Combined Sewer Overflow

Conclusions

- Next generation sequencing reveals *Bacteroides* and *Lachnospiraceae* are rich in host indicators

- Highly abundant in gulls *Catellicoccus marimammalium* the most abundant taxon among all samples.

- Sequencing water samples takes advantage of complex profiles

- qPCR for new targets can show sources and quantify signal

Answer 4

Helena Solo-Gabriele: We first broke the beaches into six categories. We didn't do it methodologically, though.

Question 5

Richard Whitman: Your results have tremendous implication for site selection of beaches and site design. I have tried to get the Army Corps of Engineers to think about that and develop strategies for when and where to remediate.

Answer 5

Helena Solo-Gabriele: I have looked a lot at bays versus open beaches. There is a value to bay beaches, lots of characteristics that people like. But if you have it, you have to manage it to minimize other sources of contamination, like not allowing dogs. You don't have as much flushing there.

Question 6

Marirosa Molina: For Sandra [McLellan]. Can you comment on the aging of sewage? You have standing water in the pipe and other factors that could change things in the micro community.

Answer 6

Sandra McLellan: We have tried to look at that in several ways. That is why we wanted to develop electrophoresis markers: they are gram positive and might be hardier in the environment. Its acts a little like a stoichiometric clock. They disappear slightly differently. We are hoping to do some plume studies next year and sample day after day to help us answer those questions.