

US EPA ARCHIVE DOCUMENT

Thursday, April 14
1:30 p.m.–3:00 p.m.

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 buoy 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 exceedance 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 exceedances ($p<0.01$). 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.



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

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

EPA National Beaches Conference
April 14, 2016

Acknowledgments

Elizabeth Kelly, Allison Donahue, Ryan Mustacatu, Sabina Rakhimbekova, Sathvika Ramaji

Zhixuan Feng, Laura Vogel

David Polk & Samir Elmir

Missing: Henry Barreras, Nicole Johnson, Hannah Lockwood

Ad Reniers

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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

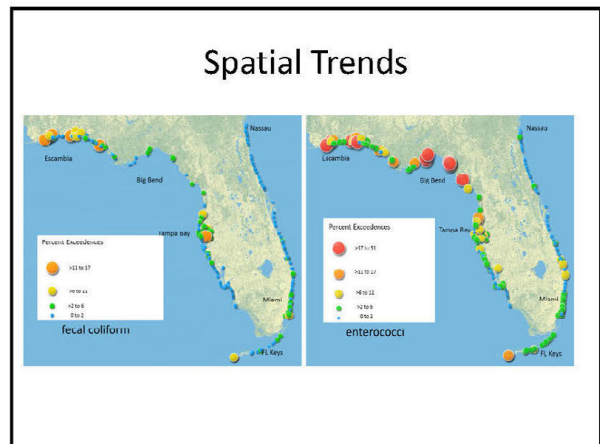
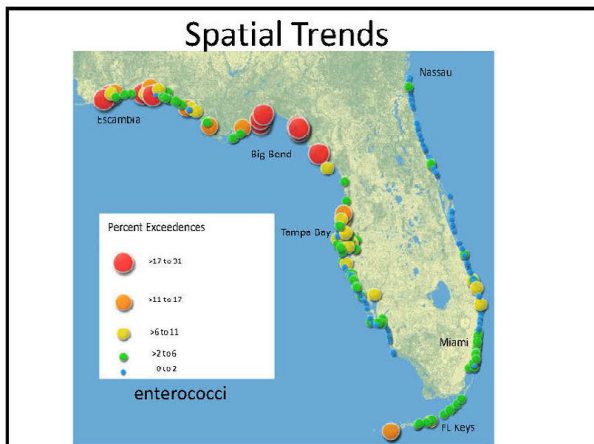
} Association with Enterococci

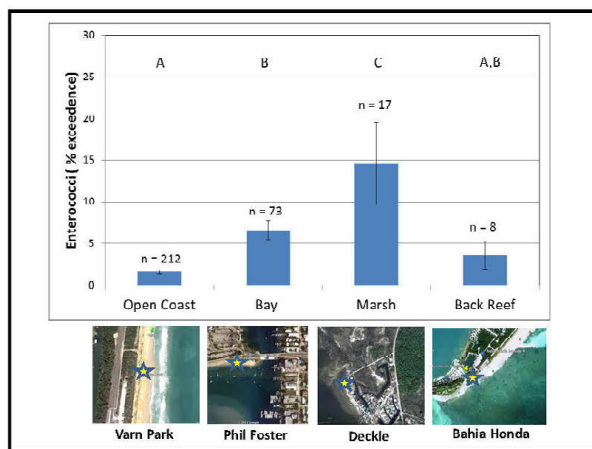
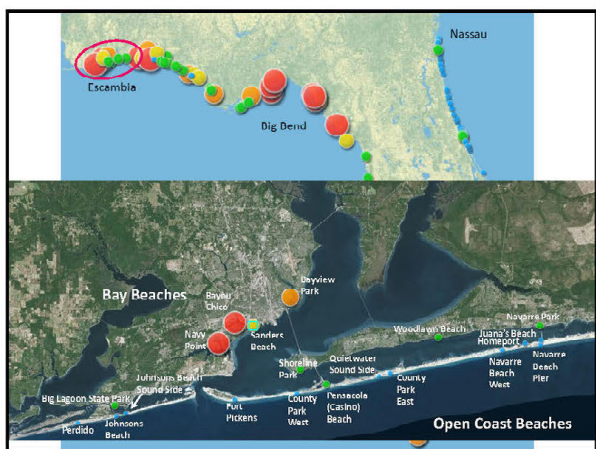
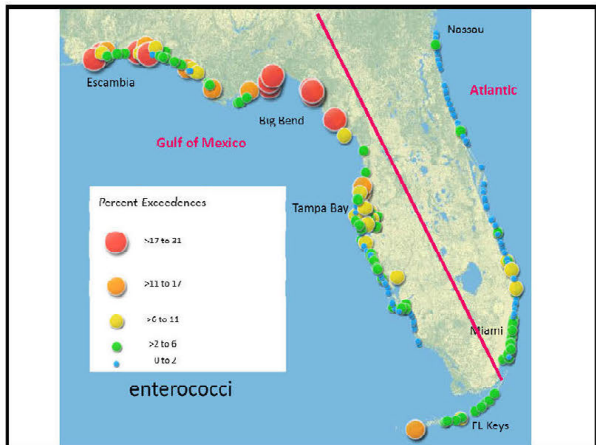
Florida Healthy Beaches Data

- 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





Other Factors (In process)

Factor	% exceedence		p
	Yes	No	
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)	0.830
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 – Franklin Blvd

Other Factors – Beach Management (In process)

Beach Management Practices (Survey – 97%)

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

Beach Type	Seaweed condition (% exceed)		P-value
	Dense/ Medium	Zero	
All Types	7.4 (43)	3.2 (29)	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 post-doctoral 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
 GEORGIA SOUTHERN UNIVERSITY
 aaslan@georgiasouthern.edu

Disclaimer

This presentation was prepared by Dr. Aslan under grant award #NA14NO54190117 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.

Study Site



Sample Collection

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

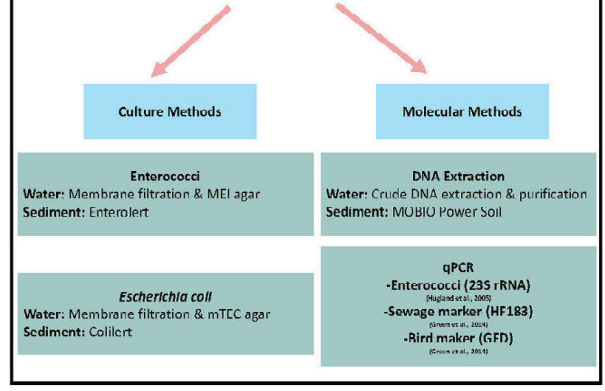
Sediment samples: 6 sites monthly, 14 sites one in winter, one in the summer

Beach sanitary surveys

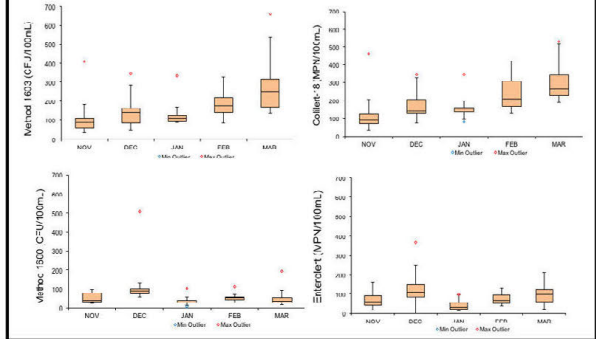
Temperature, salinity, dissolved oxygen, pH, turbidity



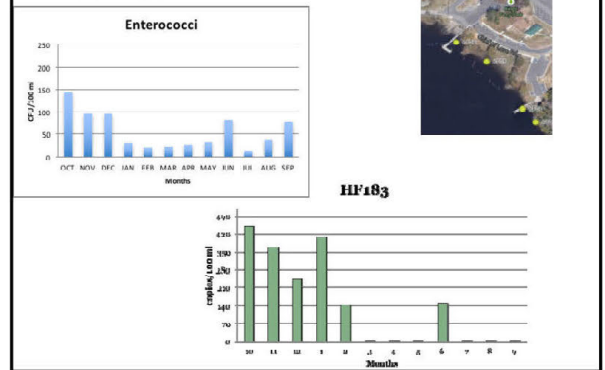
Laboratory Analysis



Culture methods comparison



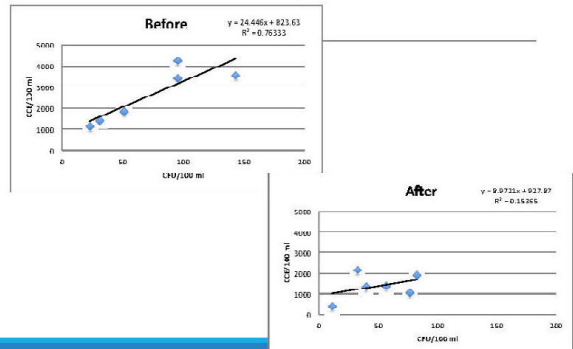
Beach



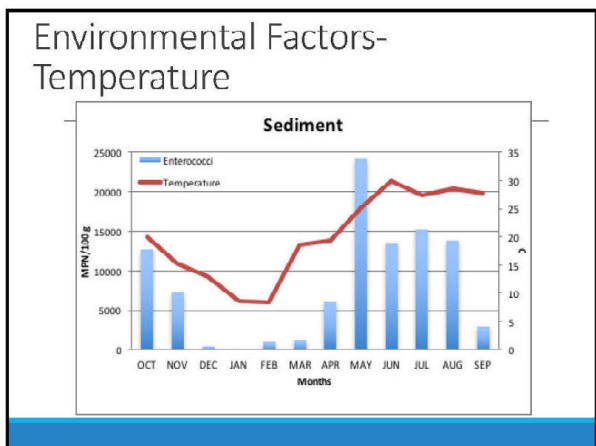
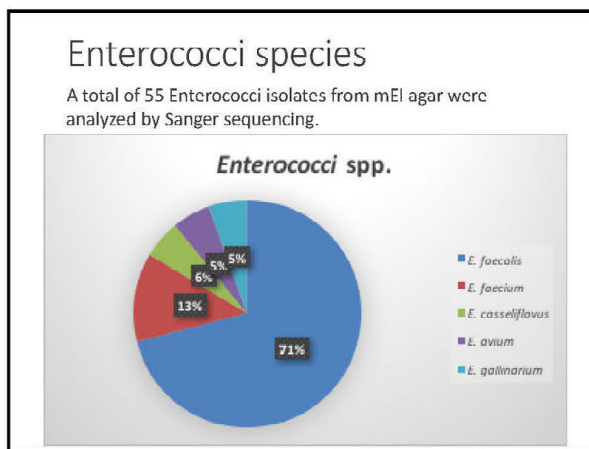
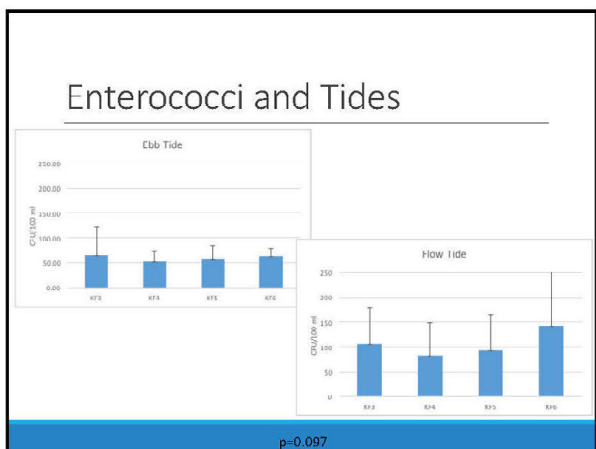
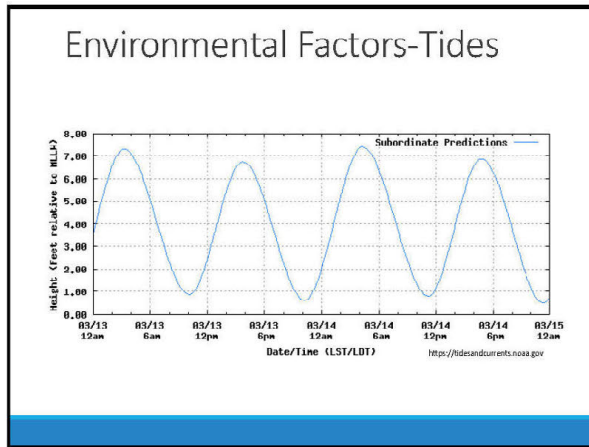
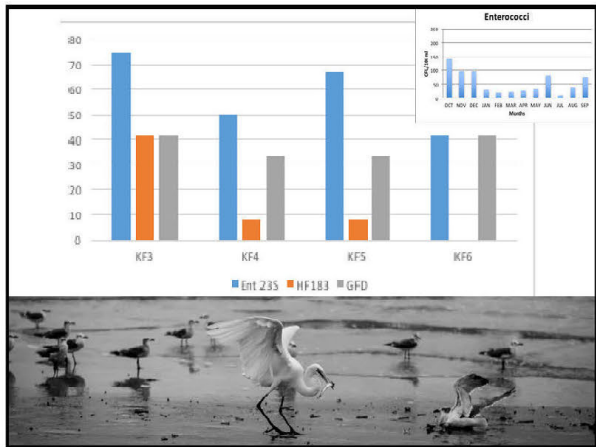
	Enterococci (MPN/100 ml)	Enterococci 23S (CCE/100 ml)	HF183 (copies/100 ml)
KF18	4,543	22,235	1,566,373
KF20	91	950	300
KF3	49	1,350	142



Culture versus qPCR



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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.



Acknowledgements

Elizabeth Cheney

Jill Andrews

Stefanie Nagid

Emily Markesteyn

William Pitts

Clark Alexander

Aslan Lab @GeorgiaSouthern:

Asheley Poole

Kendall Anderson

Spencer Barron

Jessica Sexton





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



Fecal Pollution contains a complex mixture of organisms



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

Fecal anaerobes
95% of microbiota
***Bacteroides* spp.**
Lachnospiraceae

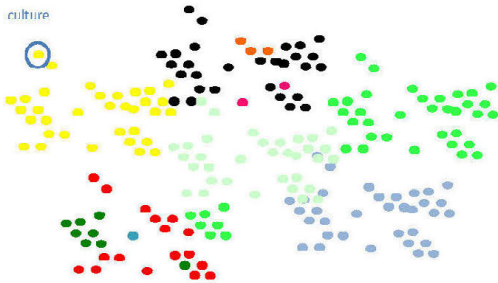


Pathogens
intermittent
Salmonella
Cryptosporidium
viruses

Klebsiella pneumoniae
Enterobacter cloacacae

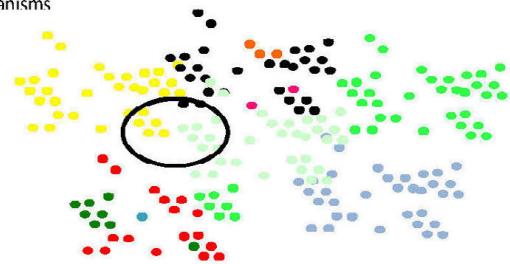
Bifidobacteria
Prevotella spp.

Characterizing sewage Sewage organisms 10⁹ cells per ml



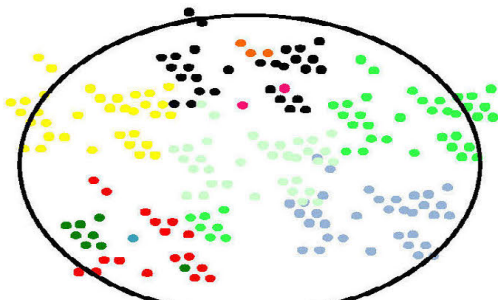
Sewage organisms 10⁹ cells per ml

Cloning and sequencing the 16S rRNA gene- "who's there"
1000-2000 sequences is a good effort- only see the most abundant organisms



Sewage organisms 10⁹ cells per ml

Illumina MiSeq or HiSeq sequencing
On average 100,000 sequences per sample



Sequencing Sewage V6 rRNA gene

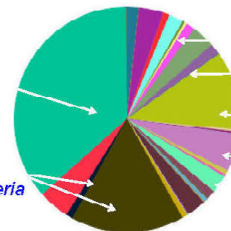
>100,000 reads per sample
800 different taxa
20,000 different sequences

80% environmental origin

20% faecal origin

Acinetobacter
Aeromonas
Pseudomonas

Arcobacter
Betaproteobacteria



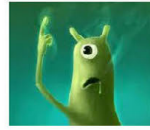
Actinobacteria
Bacteroides
Trichococcus

Lachnospiraceae
Ruminococcaceae

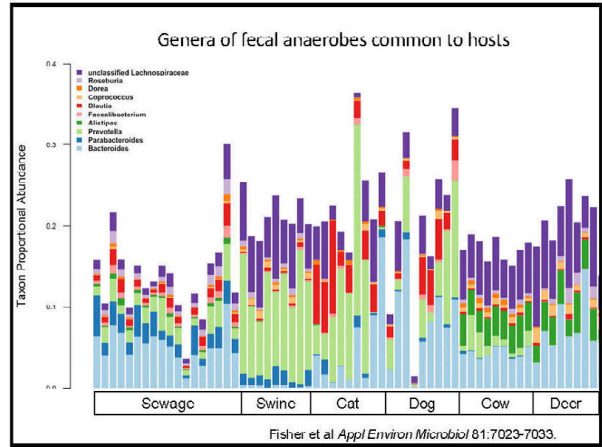
McEllan et al. (2010) *Environmental Microbiology* 12(2), 378-397
VandeValle et al. (2013) *Environmental Microbiology* 14(9), 2538-2552



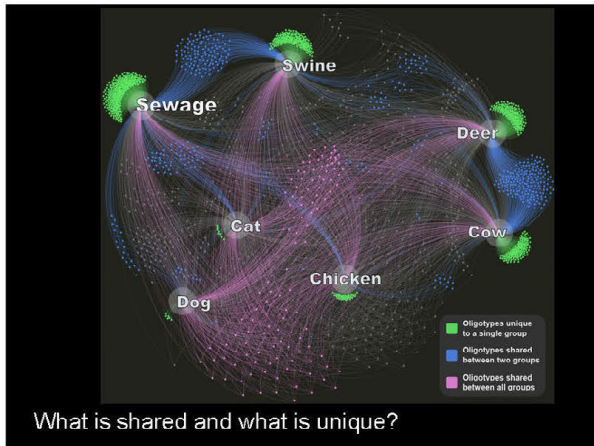
How do we make sense of millions of data points?



- | | |
|--------------|---------------------------------------|
| Sequence | Identify the members |
| | <i>Community analysis</i> |
| Raw reads | Assign taxonomy |
| Demultiplex | Cluster into OTUs (oligotyping) |
| Merge | Network analysis to compare sequences |
| Quality trim | LefSe analysis to identify biomarkers |
| Chimeras | |



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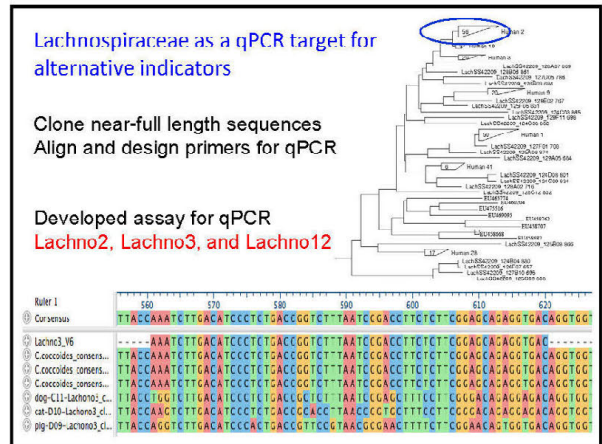
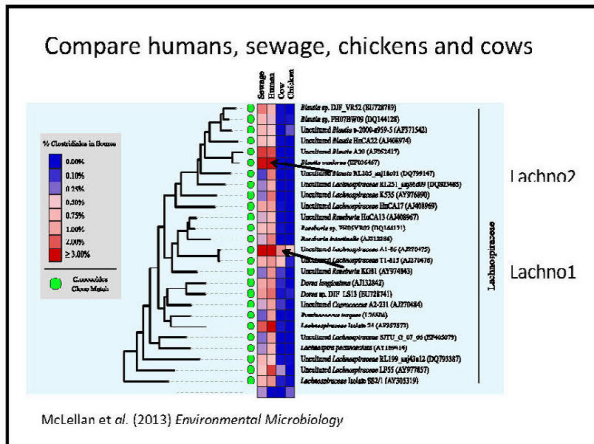


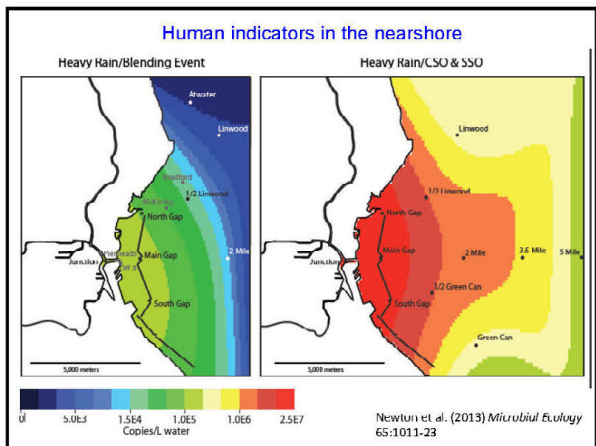
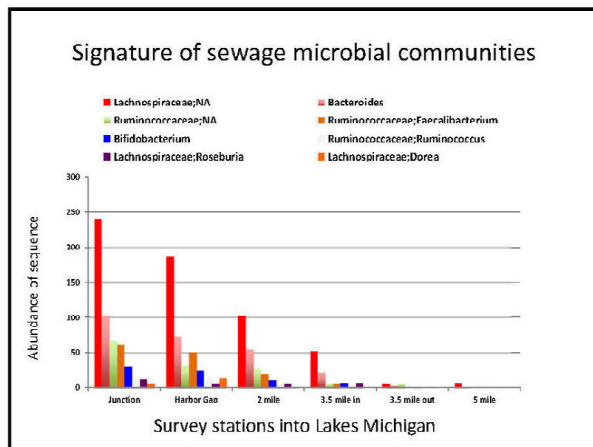
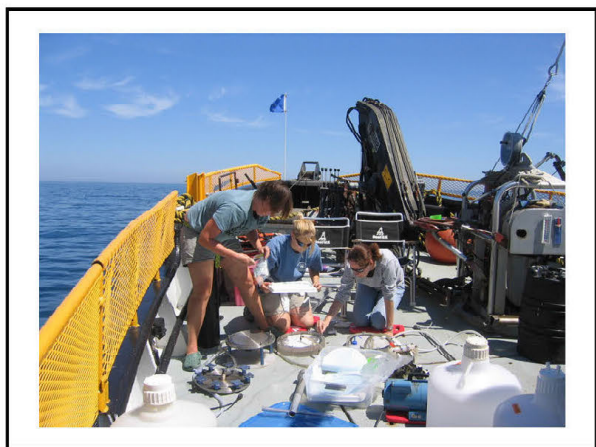
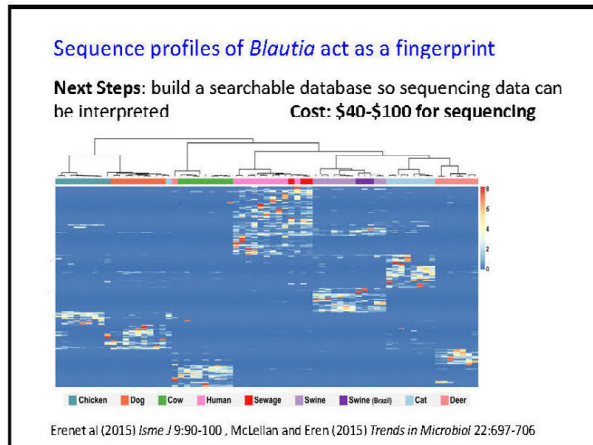
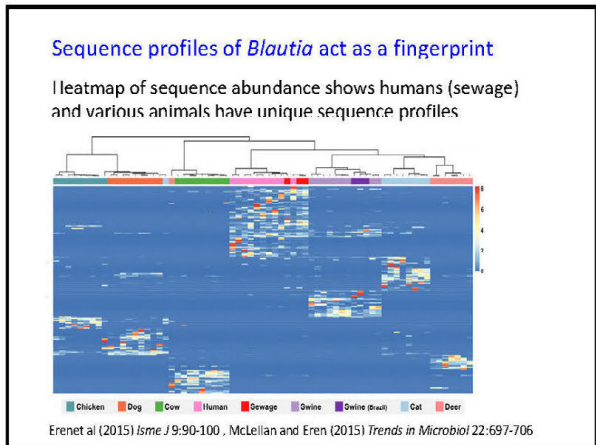
What taxa contain preferred and unique sequences?

Taxa	Sequence	Rank in Sewage
Bacteroides	CGAGCAGAAI IACGSCCGIAACCCAI I G	1
Bacteroides	CGGGCAACATGATGCATGTACCCAGTG	4
Roseburia	AGCTTCCTCCCTCCACTCTCAGT	6
Bacteroides	CGAGCACTATGATCGTTCACGAGCAG	7
Bacteroides	CGAGCAACATATGATGATATCTCTGTG	9
Blautia	AATCTCACCGCTTACCTCTTGACGT	13
Alistipes	GCATCTAGGCTCTCGG	16
Blautia	AGTGCCACCCTCTCATCTTACGCA	17
Lachnospiraceae	TGCTTCCCTACCGCAATGTCTCTTGACAGT	18
Bacteroides	CGAGCAACATATGCGCCATACGAGCGT	20
Bacteroides	CGAGCAAAATATGCCCATACCCCATTTG	21
Blautia	AGTCTCACCGCTCTGCTCTTACAGA	22
Dorea	GCACGCCCTAAGTACT	23
Roseburia	AGCTTCCTCCCTCCACTCTCAGT	24
Faecalibacterium	TCIALGALGSLALAI I LG	25
Bacteroides	CGAGCAACATATGCGCCATACGAGCGT	26
Blautia	AATCTCACCGCTCTCTTACGGA	27
Bacteroides	CGAGCAACATATGATGATATCTCTGTG	28
Dorea	GCACGCCCTAAGTACT	29
Alistipes	CTGATCGAGGTCTCAG	31

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

Lachnospiraceae very promising





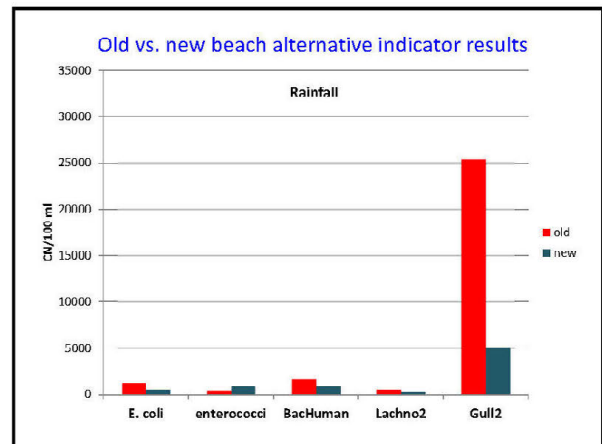
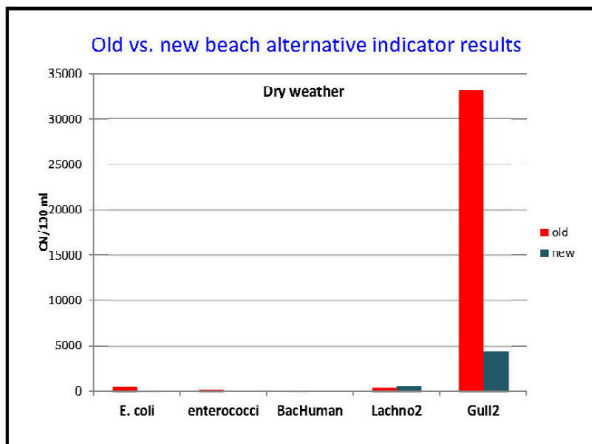
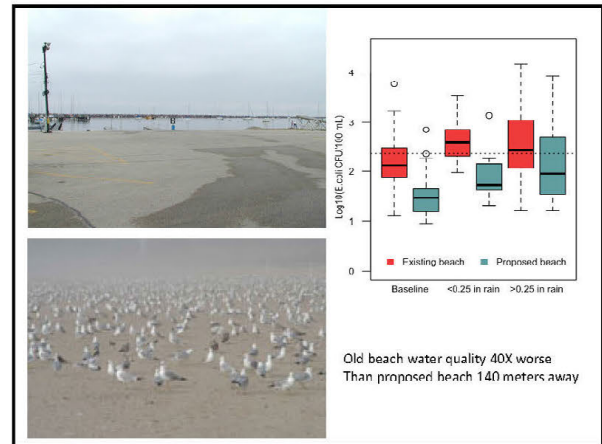
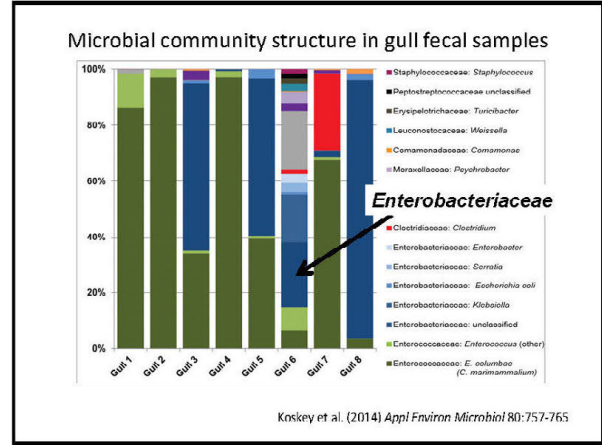
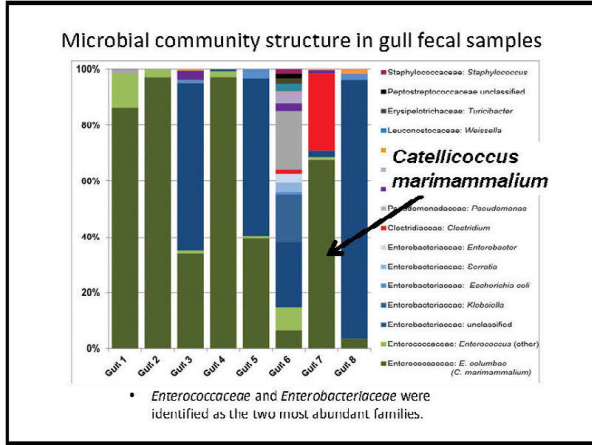
Characterizing the gull microbiome

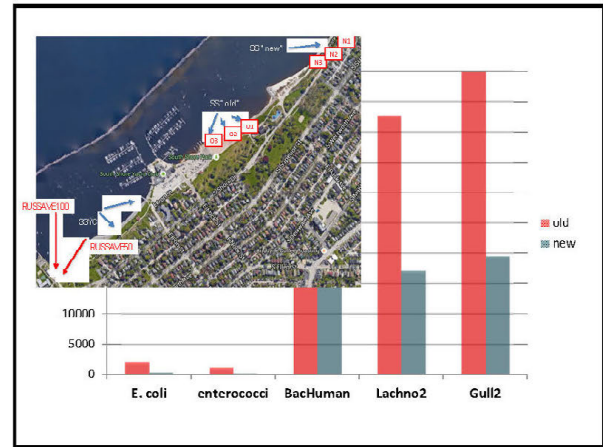
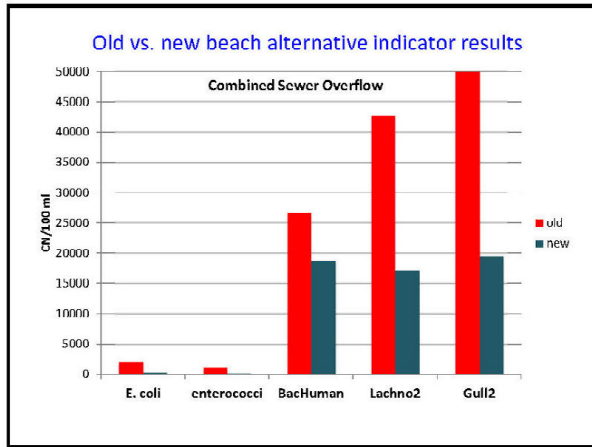
- Gulls can be major sources of fecal contamination
- Gulls have been shown to harbor high abundances of FIB, *E. coli* and enterococci, which can be detected readily as part of routine beach monitoring

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



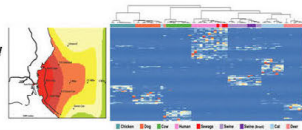
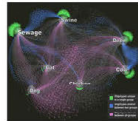
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Conclusions

- Next generation sequencing reveals *Bacteroides* and *Lachnospiraceae* are rich in host indicators
- Highly abundant in gulls *Catellibacterium 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





Question & Answer Session

Question 1

Ali Boehm: Why are we measuring Gen-Bac [general bacteroides marker]? They are not part of the criteria.

Answer 1

Sandra McLellan: Our lab stopped measuring Gen-Bac several years ago. We found it in several samples along Lake Michigan. In the last 10 years we have seen other organisms that can serve that same purpose. You still want some kind of qPCR for all bacteroides, though.

Answer 1 (follow-up)

Mia Mattioli: I agree. We didn't look for it. We did the decay rates though; they decay similarly to the human markers.

Answer 1 (follow-up)

Jodi Harwood: We use Gen-Bac as a control.

Question 2

Jodi Harwood: I have questions for Helena [Solo-Gabriele], do you analyze effects of temperature on indicator bacteria? Gulf waters are so different from East Coast waters.

Answer 2

Helena Solo-Gabriele: We put all the data into one number. There seems to be an optimal temperature that the microbes grow in. I think there is a temperature effect.

Question 2 (follow-up)

Jodi Harwood: We see high levels around vegetation. I am also wondering about the birds because they tend to have high levels of enterococcus compared to fecal coliforms. For Asli [Aslan]. Your signal persisted?

Answer 2 (follow-up)

Asli Aslan: We saw it right after a precipitation event.

Comment 2

Jodi Harwood: We have issues in Florida with deer feces; we can't use it because we have a lot of deer.

Answer 2 (follow-up)

Sandra McLellan: That's one thing that prompted us to look for another marker. HF183 is good, but we wanted a different one.

Question 3

(Unknown): Do you think the higher concentration in the sediment during the warmer season is related to decay?

Answer 3

Asli Aslan: It looks like it is related, but we need more research.

Question 4

Richard Whitman: For Helena [Solo-Gabriele]. The data seems to lend itself to regression tree/decision tree. Did you try that?

**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. It 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.