

Thursday, October 14
8:00 a.m. – 9:40 a.m.

Concurrent Track I:
Identifying and Solving Beach Water Quality Problems
Session Five:
Source Identification



EPA Guidance Manual on Source Identification

Gerald Stelma, Jr.

U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory

Biosketch

Dr. Gerard N. Stelma Jr. received a Bachelor's degree from the University of Michigan in 1965 and a PhD in microbiology from Michigan State University in 1974, specializing in bacterial physiology. He performed postdoctoral research at Purdue University from 1974 until 1976, where he studied spore coat synthesis in *Bacillus cereus*. He did additional postdoctoral work at the University of Wisconsin from 1976 until 1978, performing research on structure/activity relationships of *Staphylococcus enterotoxins*. He was a Research Microbiologist for the US Food and Drug Administration from 1978 until 1987. During his tenure there, he worked on the development of methods to detect pathogens and toxins in foods and on methods to distinguish between virulent and avirulent strains of bacterial pathogens. He joined the US Environmental Protection Agency's research staff in 1987 as a Research Microbiologist. From 1988 until 2002, he supervised a branch of EPA microbiologists and immunologists in the development of methods to detect hazardous microorganisms drinking water, recreational water and indoor air. He is currently a science advisor to the Director of the Microbiological and Chemical Exposure Assessment Research Division of EPA's National Exposure Research Laboratory.

Abstract

Beach closures or violations of total maximum daily loads of fecal organisms in watersheds frequently generate a need to identify the major sources of contamination or, at least, determine whether the source is human or animal. A few years ago *E. coli* ribotyping was the only method available for microbial source tracking (MST). Recently, however, a number of diverse methods are reported to be effective for MST; and it has become difficult for beach managers and other local officials to choose the method that is best for their specific needs. The USEPA is writing a guidance document to assist the users of MST methods in choosing the most appropriate method for their individual beaches or watersheds. The MST guide document contains descriptions of each published method, including references; the assumptions on which the methods are based; the limitations of each method; data collection and analyses and method performance. The final chapter provides decision criteria and includes a decision tree which guides the reader through the various scenarios in which MST may be useful. Each decision point in the tree contains a menu of the most appropriate methods for the user's needs. The document is comprehensive, including both library-dependent and library-independent molecular methods, as well as library-dependent phenotypic methods.



The EPA MST Guidance Document

Gerard N. Stelma Jr.
Jorge SantoDomingo

Although this work was reviewed by EPA and approved for presentation, it may not necessarily reflect official Agency policy.

When are MST Methods Useful

- To supplement sanitary surveys
 - Identify sources of beach contaminants
 - Identify sources of TMDL violations
- For risk analyses
 - Human vs animal
 - Domestic animal vs wildlife

Why is a Guidance Document Needed?

- Recent proliferation of new methods
 - Genomic
 - Phenotypic
 - Cultural
 - Non-cultural
 - Different levels of discrimination
- Most useful method depends on circumstances

The Theory Behind MST

- Genomic Methods
 - Parallel evolution of mammalian host and intestinal flora
- Phenotypic Methods
 - Uses of different antibiotics for different animal species
 - Parallel evolution of mammalian host and intestinal flora

Levels of Discrimination Available

- Human vs animal
- Human vs domestic animal vs wildlife
- Human vs species of domestic animal
- Specific population of human or animal
 - Specific community of humans
 - Specific herd or flock of animal

Content of MST Guidance Document

I Introduction

- What is MST?
- Definitions of terms

II Decision Criteria

- When MST methods should be used
- Importance of sanitary surveys
- Decision tree



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Decision Tree

- **Questions;**
 - Is the problem adequately defined?
 - Has an adequate sanitary survey been conducted?
 - How many sources were identified?
 - Is the study area of manageable size?
 - What is the desired level of discrimination?

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Content of MST Guidance Document

III MST Approaches

- Summary descriptions of all current methods
 - Explanations of how they work
 - Summary tables with advantages and disadvantages
 - References

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Content of MST Guidance Document

IV Data Collection and Analysis

- Design sampling around study objectives
- General principles for sampling
- Library construction and validation
- Spatial and temporal variability
- Similarity measurement methods

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V Performance Standards

- Universal quality measures
- Method-specific controls
- Method-specific performance criteria

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VI Assumptions and Limitations


- Characteristics of source identifiers
 - Specificity
 - Distribution in host
 - Geographic range
 - Temporal stability
 - Survival in water

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Content of MST Guidance Document


VII Applications of MST Approaches

- Eight case studies are presented
- A glossary of MST terms is presented



SUMMARY

- **Content of The EPA Guidance Document**
 - Decision criteria for choosing the appropriate method
 - Detailed method descriptions
 - Data collection and analysis
 - Performance standards
 - Assumptions and limitations
 - Case studies



The Future of MST

- **Technology is changing rapidly**
 - New methods will arise
 - Some methods will become obsolete
- **Library-independent methods will probably be the trend**



Questions and Answers

Q: Is this on the Web site?

Gerard N. Stelma, Jr.

Not yet—it is still being reviewed. Everything has to be peer-reviewed before we can make it public. But I expect it to be available by the end of the calendar year.

Q: Will any of these methods become part of the regulations?

Gerard N. Stelma, Jr.

Because there are so many different needs and so many different levels of specificity and so on that are available, I don't see us ever becoming prescriptive. I don't think there will ever be a regulation. I think it will always be up to the user to choose the most appropriate method.

Q: Can you describe the methods that will be available in the future?

Gerard N. Stelma, Jr.

I can give you some examples. Some specific species of bacteroides are carried only by one particular type of animal. Betty Olsen, from the University of California, Irvine, has found some toxin genes that are carried only by *E. coli* of human origin and some other ones that are only carried by *E. coli* of porcine origin and some of bovine origin. So, you don't need a library—you just look for that specific gene.

Q: What do you mean by a library?

All of us carry a number of *E. coli* in our intestines, and if you look at a community or at sewage, there are even more out there. And so, there are so many types of *E. coli* that you can find in a contaminated environment, and if the theory is correct, there are some of these strains of *E. coli* that are common in the community and you've got to just go through and do ribotyping on a number of *E. coli* from, say, a particular sewage plant. The patterns that you get from ribotyping a large number of strains become your library. Then, when you go out to the contaminated water, you look at the ribotypes of the various organisms you isolated from the water and try to match those patterns to your human library, or whatever other species you are looking for. There are several PCR methods that are out there, too, that are library dependent, that you get different patterns on the gel from different strains of *E. coli*, and because there are so many possibilities, you have to isolate a large number of *E. coli* from each possible species that contaminated the water and you have to make a library of those various patterns.



Tiered Approach for Identification of a Human Fecal Pollution Source at a Recreational Beach: Case Study at Avalon Bay, Catalina Island, California

Alexandria Boehm

Stanford University, Department of Civil and Environmental Engineering

Biosketch

Dr. Boehm is the Clare Boothe Luce assistant professor of environmental engineering and science at Stanford University. Dr. Boehm received a B.S. with honors from California Institute of Technology in Pasadena, CA and her M.S. and Ph.D. in Environmental Engineering from the University of California Irvine. She has been at Stanford for two years and prior to that was a faculty fellow at University of California Irvine. Her research interests include coastal water quality, coastal transport processes and their influence on pollution, water borne pathogens, microbial pollution, water quality indicators, and particle fate in water.

Abstract

Recreational marine beaches in California are posted as unfit for swimming when the concentration of fecal indicator bacteria (FIB) exceeds any of seven concentration standards. Finding and mitigating sources of shoreline FIB is complicated by the many potential human and non-human

sources of these organisms and the complex fate and transport processes that control their concentrations. In this study, a three-tiered approach is used to identify human and non-human sources of FIB in Avalon Bay, a popular resort community on Catalina Island in southern California. The first and second tiers utilize standard FIB tests to spatially isolate the FIB signal, to characterize the variability of FIB over a range of temporal scales, and to measure FIB concentrations in potential sources of these organisms. In the third tier, water samples from FIB hot spots and sources are tested for human-specific bacteria *Bacteroides/Prevotella* and enterovirus to determine whether the FIB are from human sewage or from non-human sources such as bird feces. FIB in Avalon Bay appear to be from multiple, primarily land-based, sources including bird droppings, contaminated subsurface water, leaking drains, and runoff from street wash-down activities. Multiple shoreline samples and two subsurface water samples tested positive for human-specific bacteria and enterovirus, suggesting that at least a portion of the FIB contamination is from human sewage.



A Tiered Approach for the Identification of a Human Fecal Pollution Source at a Recreational Beach: Case Study at Avalon Bay, Catalina Island, California, USA



Alexandria Boehm, Jed Fuhrman, Robert Mrse, and Stanley Grant

Image from www.catalina.com

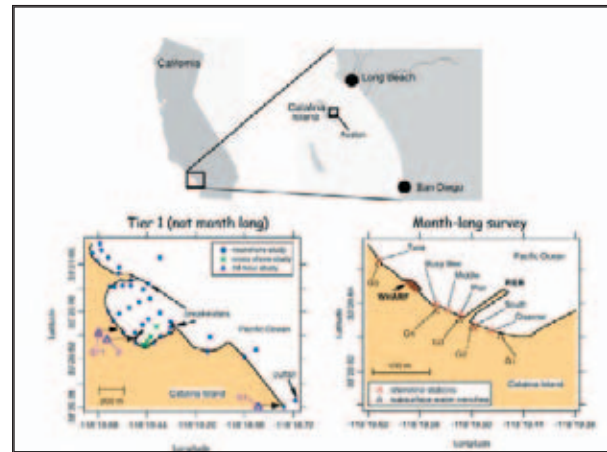
The Problem

- Avalon Beach had high levels of fecal indicator bacteria (FIB), especially enterococci (ENT)
- City officials could not readily identify and remediate the problem.
- Possible sources
 - treated sewage effluent
 - nuisance runoff
 - bird and wild animal feces,
 - sewage-contaminated groundwater
 - boat sewage collection tanks

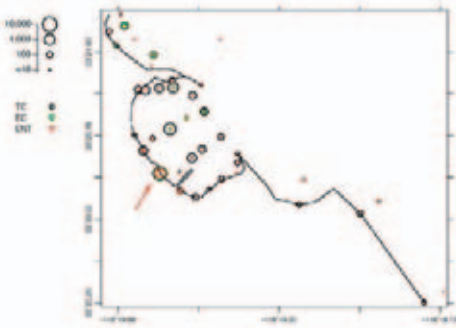


Our Approach

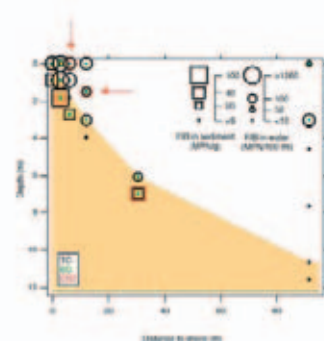
- **Tier 1: Use FIB analyses to document spatiotemporal variability and identify hot spots**
 - Nearshore bay survey: conducted during a flood tide, at night
 - Cross-shore transect: sediment and water tested
 - 24 hour study: document sunlight and tide effects
 - Month long, twice daily shoreline survey
- **Tier 2: Use FIB analyses to document potential sources**
 - Grab samples of runoff, feces, subsurface waters, cooling water discharge, leaking pipes under pier and wharf
 - Intensive sampling of subsurface waters
- **Tier 3: Use novel, human-specific indicators to determine if human sewage contributes to FIB.**
 - Hot spots and sources assayed for *Bacteroides/Prevotella* DNA (Bernhard and Fields) and enterovirus genomic RNA (Monpoeho et al.)

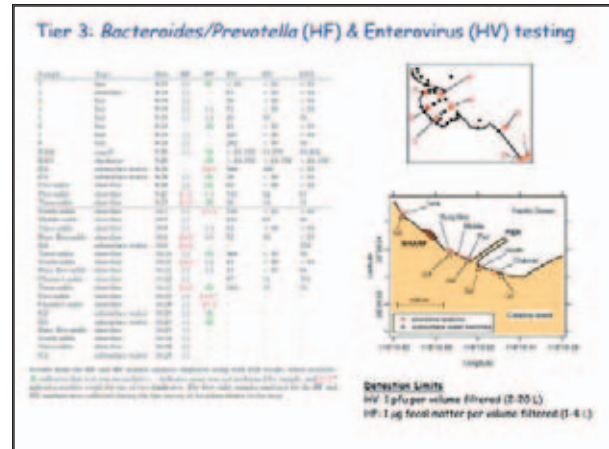
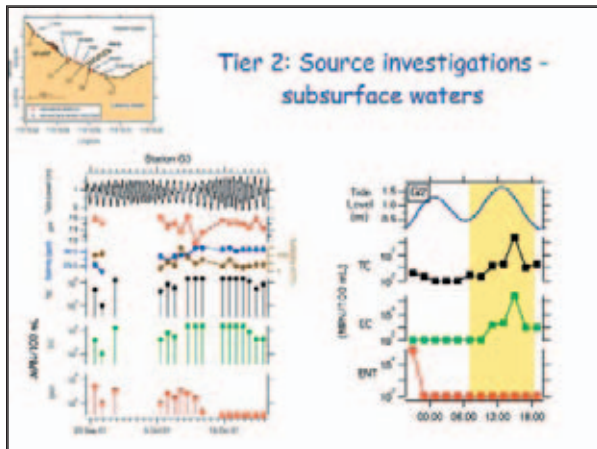
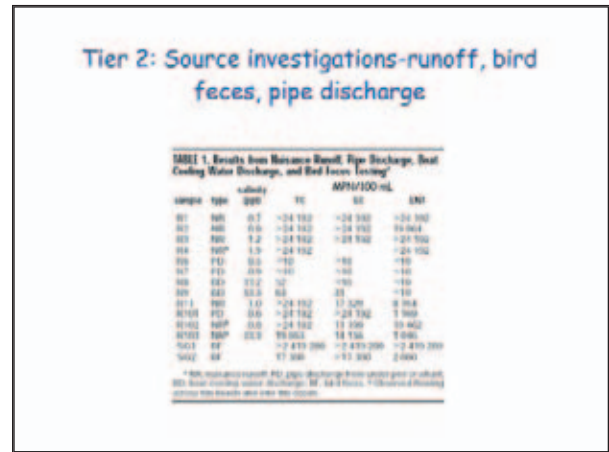
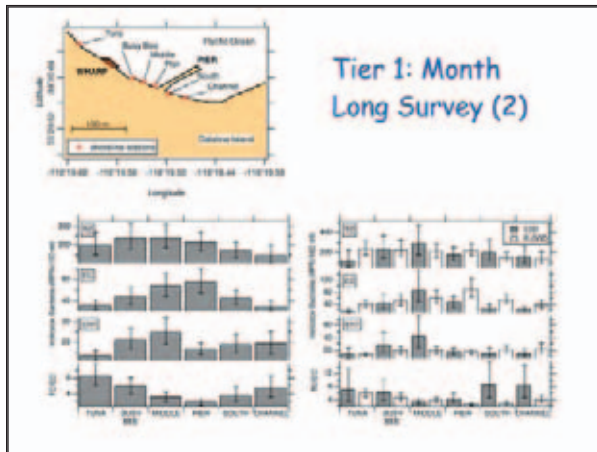
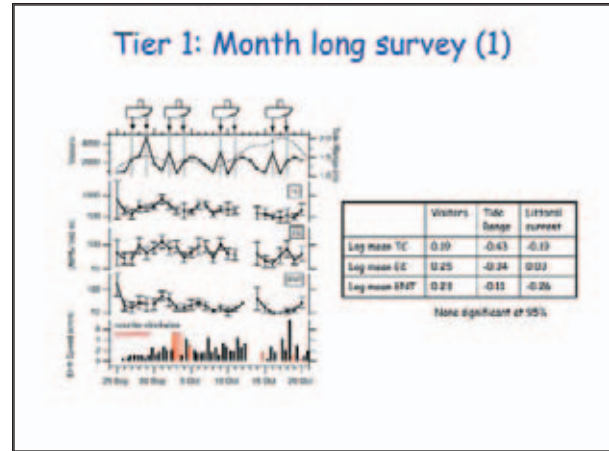
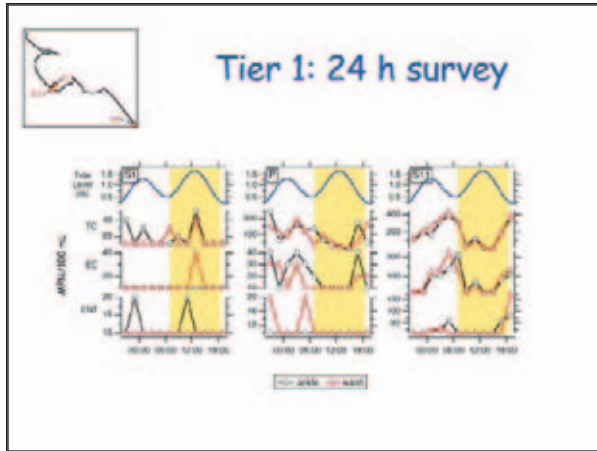


Tier 1: Nearshore bay survey



Tier 1: Cross shore survey



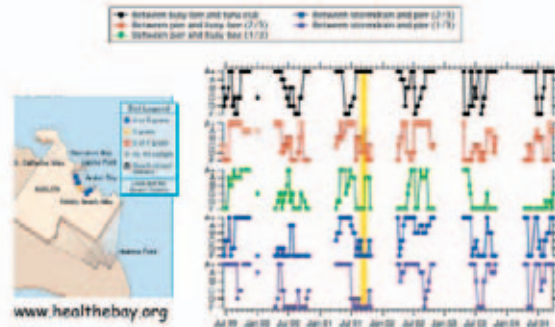




Implications

- FIB at Avalon Beach are from sources inside the bay, from the land side of the beach.
 - Nearshore bay survey, cross shore survey, ebb tide signal (24 hour study, month long survey)
 - At least a portion of the pollution is from human sewage
 - Extraordinarily high levels of FIB in groundwater
 - HF and HV markers found at every shoreline site (except middle) and in a groundwater sample
- City of Avalon slip-lined their sewer lines adjacent to the beach in the winter of 2001/2002

Has water quality improved?



Acknowledgements

- Funding from
 - The City of Avalon
 - Los Angeles Regional Water Quality Control Board
 - State of California Clean Beaches Initiative Prop 13
- Assistance
 - Ryan Reeves, Cynthia Jensen-McMullin, Mark Bachman, Lisa Gilbane, Burt Jones



Questions and Answers

Q (Donna Francy, USGS): I really like your tiered approach, and I think it's a really good way to go about it, instead of just going out there and ribotyping everything. So you found that it's partially human, at least, but then they took these remediation steps and that didn't help. So what do you think you should do next? Are there any other potential sources? Do you think it might be a nonhuman source also, like wildlife?

Alexandria Boehm

I haven't kept up with all the maintenance activities in the city of Avalon, but my first guess would be that the slip lining did not work. Also, the city is so densely populated and I'm not sure how the sewerage infrastructure is set up there and I think it might be possible that there are leaking sewer lines in other places where they did not slip line. If they wanted to do another study, then I would see if there is the same problem there, and if it is, then I would say the sewer lines are leaking somewhere and they need to do something about it.

Q: Can you define "nuisance runoff"? Is that from rain or dry weather flows? Also, how did you eliminate urban runoff? Did you do a loading estimate?

Alexandria Boehm

It may only occur in California, but "nuisance runoff" is the water that we see in the gutter when it hasn't been raining. In Avalon, they hose down the streets at night and the streets lie right next to the beach so that water from the hosing down we would call "nuisance runoff," or any water just trickling along when it hasn't been raining.

Q: How did you eliminate the urban runoff, the surface water, and the nuisance flows? Did you do a loading estimate?

Alexandria Boehm

No, we didn't say that it couldn't be nuisance runoff. We didn't eliminate that, but none of the nuisance runoff came back positive that we tested for the HF or the HV marker. Surely they are contributing a fraction of the pollution to the beach, so we did not eliminate it.

Q: Was it just one field event for the Bacteroides?

Alexandria Boehm

The design of our project was to first identify locations, and then sample those locations maybe a couple times, but we found the Bacteroides multiple times at multiple stations. So it was not just one sample.



Fecal Source Identification with Bacteroidetes Molecular Markers

Katharine Field

Oregon State University, Department of Microbiology

Biosketch

Dr. Kate Field is an Associate Professor in the Department of Microbiology at Oregon State University, where she also co-directs the Biore-source Research Interdisciplinary Program. Her research concerns new and rapid biotechnical methods of detecting and identifying bacterial pollution and pathogens in the environment, the study of microbes in natural populations, and the spread of antibiotic resistance in the environment. She has degrees from Yale University, Boston University, and University of Oregon. She is the author of two lab texts on molecular biology, and is on the editorial board of the journal *Applied and Environmental Microbiology*. Her research has been widely published and she has been an invited speaker for the World Health Organization, Food Safety Research Consortium, American Academy of Microbiology, American Society for Limnology and Oceanography, Environment Canada, British Department of the Environment and European Union, among others.

Abstract

Fecal contamination of seawater is widespread in the coastal ocean of the United States, causing illness and beach closures, impacting shellfish harvest, and degrading habitat. Human

and animal feces pose different threats to human health, but epidemiological data that link human health outcomes to exposure in water do not distinguish human from animal feces. Current methods of measuring fecal contamination with public health indicator bacteria do not identify its source. Often fecal pollution cannot be corrected, because the source is not known. We have developed a rapid and accurate method of identifying the source of certain kinds of feces in water, utilizing a PCR assay that targets host-specific groups of Bacteroidetes fecal bacteria. The method differs from existing methods of detecting fecal pollution in that it detects genetic marker sequences that identify bacterial groups specific to the host species that produced the feces, allowing discrimination among different potential sources. This method performed well in a comparative study of fecal source tracking methods. Field studies in Tillamook Bay, Oregon, and Mission Bay, California, demonstrate this approach. The method has been tested throughout the U.S., in Canada, Ireland, and New Zealand. Utilizing the same technology, we also developed a quantitative (Q-PCR) assay for Bacteroidetes bacteria that is being tested as a rapid method of detecting fecal pollution. Both of these methods use small water samples, do not require isolating and growing the bacteria, do not require a library, and are rapid and accurate.



Fecal Source Identification with Bacteroidetes Molecular Markers

Katharine G. Field
Oregon State University

Some of the following information represents unpublished research results. If you wish to use or reproduce any of this information, please contact the author directly:
kate.field@orst.edu, (541)737-1837





References:
 Dik, L. B. and K. G. Field 2004. Rapid estimation of numbers of fecal Bacteroidetes by use of a quantitative PCR assay for 16S rDNA genes. *Appl. Environ. Microbiol.* 70: 5465-5467.
 Field, K. G., E. C. Chen, L. E. Dink, J. A. Peltzman, J. P. Griffiths, P. A. Hilliers, M. C. LaMontagne, J. La B. H. Olson, M. T. Simonich. 2002. A comparative study of culture-independent, library-independent genotypic methods of fecal source tracking. *J. Wat. Health* 1 (4): 385-394.
 Beuchard, A. E., T. Ouyard, M. T. Simonich, and K. G. Field. 2009. Application of a rapid method for identifying fecal pollution sources in a multi-use estuary. *Water Research* 39: 909-923.
 Beuchard, A. E., and K. G. Field. 2000. A PCR assay to discriminate human and ruminant feces based on host differences in *Bacteroides-Plesiomoda* 16S ribosomal DNA. *Appl. Environ. Microbiol.* 66: 4371-4374.
 Beuchard, A. E., and K. G. Field. 2000. Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal excretion. *Appl. Environ. Microbiol.* 66: 1507-1514.
 Field, K. G. 2004. Fecal Source Identification. In "Waterborne Zoonoses: Identification, Causes and Control", J. A. Colwell, A. Dubost, G. Ross, J. Bahram, E. Cam, D. G. Clive, G. P. Cross, S. Pappas and V. P. G. Gibson, eds. World Health Organization, WHO Publishing Alliance Notes, London: review article.

- Rationale
- Identifying markers: T-RFLP, clone library analysis, and subtractive hybridization
- A quantitative assay for rapid detection of fecal pollution
- Sensitivity and specificity
- Field studies
- New research directions

Fecal Pollution

- a continuing problem in coastal and fresh waters
- causes economic losses, human health risks, and environmental damage
- standard methods of measuring fecal pollution are slow, and do not distinguish its source

Potential Sources of Fecal Pollution:

 Enteric viruses...	Runoff from sewage, septic tanks, and agriculture	 E. coli O157, Salmonella, Cryptosporidium...
 Salmonella, Giardia, Cryptosporidium...	Wildlife	

CHEMICAL METHODS

- caffeine, fecal sterols and stanols, laundry brighteners, surfactants, and fragrances are used to diagnose human fecal contamination
- Their spread, persistence, and transport in water is not well correlated with microbial pathogens



- Two "DNA fingerprinting" methods, **Ribotyping** and **PFGE**, can be effective methods of fecal source identification
- Based on culturing and analyzing fecal isolates
- Slow and expensive
- Geographic variation: need a new "library" (set of bacterial isolates from feces) for each new area
- Potential problem: both *E. coli* and Enterococci grow in the environment, and may have environmentally-adapted types

Our Goal:

- A method of source detection that
 - is fast (less than 24 hours) and inexpensive
 - doesn't require culturing
 - doesn't require a site-specific library
- based on extracting DNA from water samples and amplifying diagnostic gene sequences from **uncultured Bacteroidetes** rDNA genes

Why *Bacteroidetes*?

- found in both human and animal feces
- significant portion of the flora (more than *E. coli*)
- only in animal body cavities, not in water
- anaerobic: limited survival and reproduction upon release
- strain differences among different animal hosts

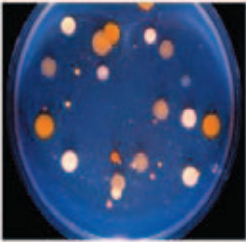
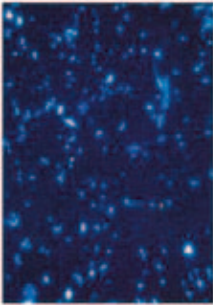
Why not *E. coli* or enterococci?

- Both the freshwater indicator *E. coli* and the saltwater indicator enterococci spp. can survive and proliferate in the environment.
- Furthermore, evidence suggests environmentally-adapted strains of indicators.
 - Fujioka et al. *J. Appl. Microbiol. Symp. Suppl.* Vol. 85
 - Desmarais et al. *Appl. Environ. Micro.* Vol. 68(3), 2002
 - Alm et al. *Water Research.* Vol. 37, 2003
 - Byappanahalli et al. *Appl. Environ. Micro.* Vol. 69(8), 2003
 - Whitman et al. *Appl. Environ. Micro.* Vol. 69(8), 200

Why use molecular methods, that don't require culturing?

Culture bias: most microbes in natural populations can't be cultured, and the ones that grow are not necessarily representative of what's in the natural population

"The Great Plate Count Anomaly": only 1 to 0.001% of microbes in the environment can be cultured.

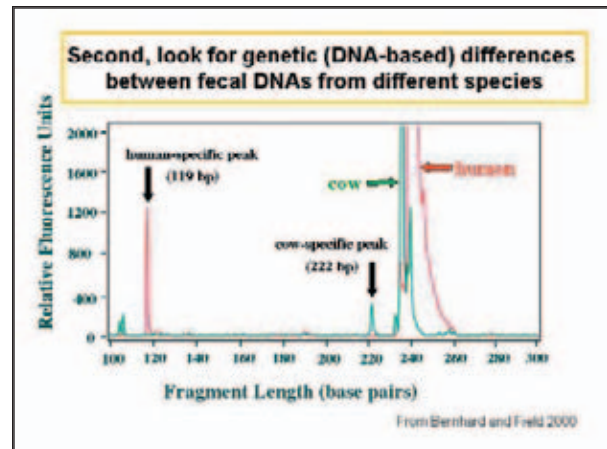
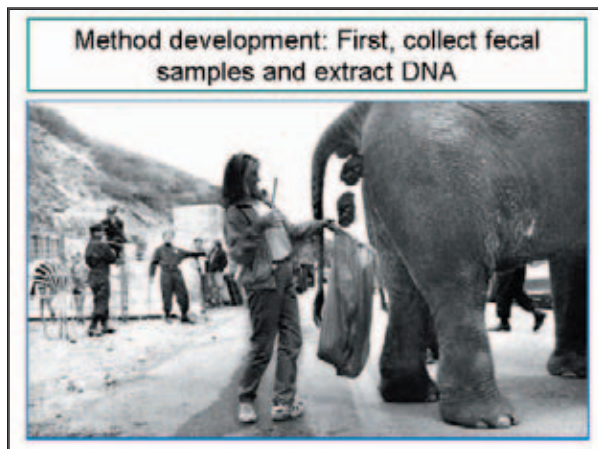
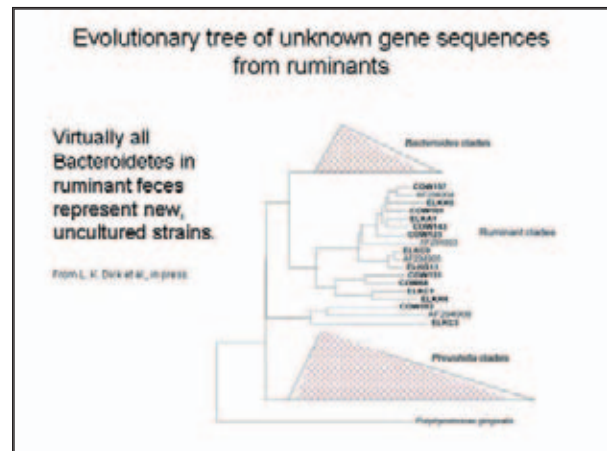
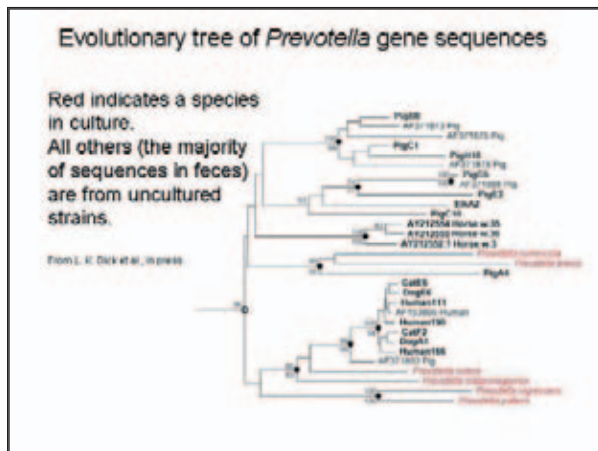
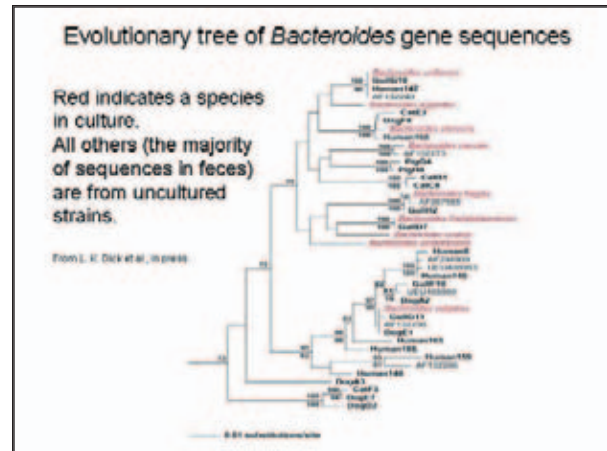
Plate Counts	Direct Counts
	

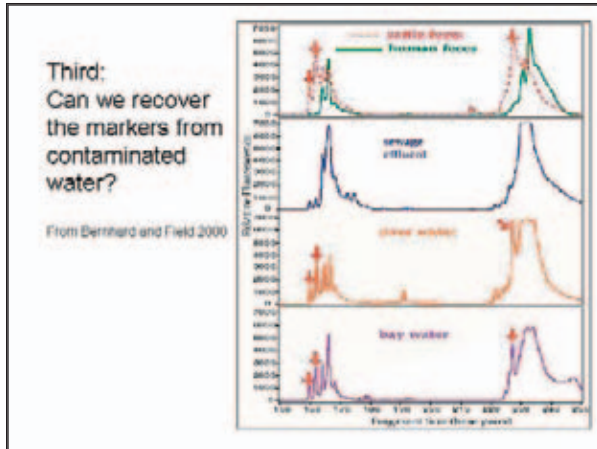
Courtesy Mike Rappo, HIMB



Why use uncultured Bacteroidetes as markers?

The most common fecal Bacteroidetes remain uncultured, and cultured strains do not adequately represent fecal diversity. Using molecular methods to detect allows us to access the uncultured majority.

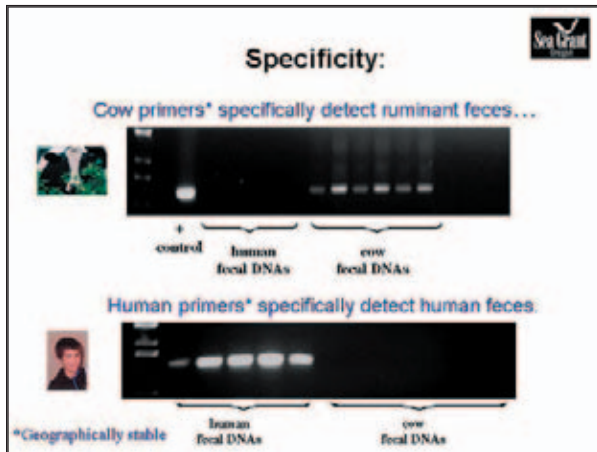




Fourth, sequence the unique types and
design host-species-specific "primers"

What is a primer?

- a short piece of artificial DNA that will interact only with Bacteroidetes DNA from a specific type of feces
- PCR only works (synthesizes DNA) if this interaction takes place
- so if we put a cow primer into the PCR and it works, the sample contains cow feces.



Clone library analysis yielded more host-specific primers: PIG and HORSE

FECAL DNAs AMPLIFIED with PIG PRIMER

FECAL DNAs AMPLIFIED with HORSE PRIMER

But this approach doesn't work well for closely-related hosts and host species that share fecal bacteria...

Many animal species have unique clusters of Bacteroidetes sequences, suggesting coevolution: e.g. cow, pig, horse...

However, humans and their pets share Bacteroidetes sequence types, suggesting horizontal transfer of fecal bacteria between humans and pets





SOLUTION: SUBTRACTIVE HYBRIDIZATION

- "Target" DNA is hybridized to "subtractor" DNAs fixed in microtiter wells.
- Unique target sequences, left in solution, are recovered, cloned and sequenced, and used to design new primers
- Results: new primers for elk, dog





From L. K. Dick et al., in press




Results of subtractive hybridization

From L. K. Dick et al., in press

Dog primer highly specific
DF475F/Bac708R




Elk primers distinguished elk from cow feces, but also amplified sheep fecal DNA
EF447F/EF990R



Primer Sensitivity


Examples of sensitivity assays: (primers paired with Bac708R; target = plasmid)

Target copy number
 10⁷
10⁶
10⁵
10⁴
10³
10²
10¹



GenBac CF128 CF193

Effect of Matrix:
DF475F
Sensitivity
62%, 2 mM Mg²⁺,
30 cycles



10⁸ 10⁷ 10⁶ 10⁵ 10⁴ 10³ 10² 10¹
plasmid alone plasmid + seawater extract

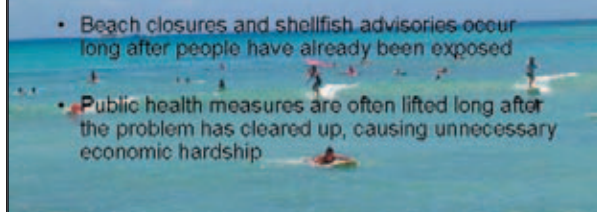
From L. K. Dick et al., in press

Sensitivity: Summary

MARKER (paired with 708R)	TARGET	SENSITIVITY (gene copies detected)
GenBac	All fecal Bacteroidetes	10
HF134F	human	100
HF183F	human	100
CF128F	ruminant	100
CF193F	ruminant	100
PF163F	pig	100
HoF597F	horse	100
DF475F	dog	100

NEEDED: RAPID, QUANTITATIVE ASSAY


- Problem: Traditional assays for fecal pollution take a minimum of 24 hours
- Beach closures and shellfish advisories occur long after people have already been exposed
- Public health measures are often lifted long after the problem has cleared up, causing unnecessary economic hardship



Rapid detection of fecal contamination based on Q-PCR of Bacteroidetes genetic markers

- "TaqMan" primers allow us to use Real-Time Quantitative PCR to measure the amount of these genes in water samples. **TNA= tag Nuclease Assay** (form of Q-PCR)
- Q-PCR of Bacteroidetes is correlated to sewage dilutions and to *E. coli* and enterococci MPNs.

From Dick and Frazar 2004



• A rapid, sensitive assay for fecal pollution.



Questions and Answers

Q: I don't think right now there are any truly quantitative methods that will allow us to say that Tellhook Bay is contaminated with 60 percent cow fecal matter and 40 percent human or anything like that, but do you think you can get at least an estimate of the prevalent sources? It looks to me like your method could be at least semiquantitative.

Katharine Field

It's easy to count the number of genes in a sample, so we can be quantitative in that sense, but the problem is that you don't know whether or not those markers have survived. If it's 2 weeks after the pollution event, is it the same proportion of survival as it was at the moment that it dropped into the water? So, what we are working on right now is looking at the survival profiles and correlating them with the survival of specific pathogens. We've got the 0157 strain of *E. coli* and we have some viral pathogens.

Q (Stephan Wuertz, CCD): My question goes in the same direction. Your last comment indicated that you may have evidence of bacteroidetes that have been released from different species that may have different survival properties. Do you have any indication that that is really the case? That would have implications for quantitative microbial source tracking.

Katharine Field

We don't have too much evidence except for some anecdotal evidence that we've seen with our field samples. I have a grad student right now that is growing the markers and labeling them with bromidioxuridine so that she can look at survival versus growth over time, and her experiments are working really well right now. We are hoping that within a year we'll have more specific information. But I would say that Ali Boehm's data were very nice. To me, it looked like her human fecal and human viral markers were not correlated.

Q (Kelly Goodwin, NOAA, Atlantic Oceanographic and Meteorological Lab): Do you have a gull-specific marker? And, have you or anyone looked at fish or marine mammals?

Katharine Field

All of those are things that we are working on. The gull is particularly refractory and we think we have figured out why that is, and that we are getting somewhere with it right now. I hope that we'll soon have some information. I also have some marine samples sitting in our freezer and I need more students and more money to do those.

Q: Are there ways for other labs to use your primers or do they have to start at point zero and develop their own primers as well? And, can you talk a little about cost for people who don't have their own lab?

Katharine Field

Some primers are not yet published but are in press. Many have been published already. The quantitative assay just came out last month. For research purposes, anyone can use them. For commercial purposes, my university is trying to get some sort of patent, but they have been trying to do this for 6 years and they are not having a lot of success. So, I'm not holding my breath on this, but that is the way my university is trying to play it, in terms of commercial application. We ourselves analyze samples for people all the time. People call us up and say they have certain questions or certain studies and ask if we can do it and we do, and the cost is about \$50 a sample. We are also starting a collaboration with Mohsen Orodpour in Seattle because we see how our two different approaches of methods really get at different aspects of the same thing and can work very nicely together.



Using Microbial Source Tracking in New Hampshire: Applications, Results and Challenges

Stephen Jones

University of New Hampshire

Biosketch

Dr. Stephen Jones is a research associate professor of marine science and natural resources at the University of New Hampshire. Dr. Jones received his B.S. in Soil Science from the University of Maine in Orono, his M.S. in Soil Science at the University of Wisconsin in Madison and his Ph.D. in bacteriology from the University of Wisconsin in Madison. He conducted research on biodegradation of organic chemicals as a postdoctoral fellow in the Institute of Comparative and Environmental Toxicology at Cornell University from 1983-86, then became a research fellow and adjunct professor studying anaerobic digestion of municipal sludge in the Department of Civil Engineering at Syracuse University until 1987. Since 1987, he has been conducting research on a variety of environmental microbiological and toxicological issues at the University of New Hampshire's Jackson Estuarine Laboratory. He currently serves as the Director of the UNH Center for Marine Biology.

Abstract

Traditional investigatory methods are used by state agencies to track sources of fecal-borne microbial contamination that are causing pollution problems for recreational and shellfish growing

waters. While methods such as bracketing streams using microbial indicator organisms and shoreline surveys have been successful in identifying various pollution sources in coastal New Hampshire, estuarine and coastal waters still have elevated bacteria levels in some areas. Since 1999, the New Hampshire Department of Environmental Services has worked with University of New Hampshire researchers to identify specific source species using a microbial source tracking technique called Ribotyping. NHDES and UNH have applied this MST technique while investigating sources of bacterial contamination at recreational beaches, shellfish growing waters, freshwater streams, and tidal rivers. The results, which show the relative contribution of specific source species, have been used in a Total Maximum Daily Load study and to guide remedial actions in both estuarine and fresh waters. In some cases the results were as expected, in others the results indicated unexpected sources, which were eventually verified. Research is continually refining the methodology including a move from manual to automated ribotyping using a RiboPrinter. The cost for ribotyping is an issue that has led to several studies exploring the potential for using small source species databases that reflect local source species during the time of the study. Other ongoing research and experimental designs seek to expand possible applications of ribotyping for source tracking.



Using Microbial Source Tracking in New Hampshire: Applications, Results and Challenges

Stephen H. Jones, Ph.D.
Dept of Natural Resources
Center for Marine Biology
Jackson Estuarine Laboratory
University of New Hampshire
Durham, NH
s.h.jones@unh.edu

Natalie Landry & Sara Sumner
NH Department of Environmental Services
Concord, NH
landry.natalie@des.nh.gov
sumner.sara@des.nh.gov

NH-MST Partners & Organization

- Beach, Watershed Restoration & Shellfish programs at NHDES.
- Lab analysis & field research at UNH- Jackson Estuarine Laboratory
- Cooperative strategy for MST

Water Quality Issues NH estuarine & coastal waters

- Water quality concerns at ocean beaches.
- Shellfish harvesting is limited.
- Pollution problems associated with storm events.
- Remedial actions in some problem areas have not been successful in improving water quality.
- TMDL process necessitates pollution source identification.

Bacteria and Viruses



Septic Systems, Illegal Sewer Connections, Boat Sewage, Pets, Livestock, Wildlife

Water Quality Issues Pollution source investigations

- Traditional approaches have had some success:
 - Shoreline surveys, catch basins & storm pipes, septic system study
- Management need: better method for routine pollution source ID
- Decision 1999: adopt most promising research-level MST approach for development & application in NH → *E. coli*/RIBOTYPING
- Cooperative State and UNH investment in ribotyping
 - source species library (~1000 isolates/35 species) & RiboPrinter

Water Quality Issues Pollution source investigations-MST

- Mutual support and feedback:
 - State conducts surveys to meet management needs
 - UNH conducts supporting research to refine survey approach & enhance results
 - Different State programs share limited resources



Today's Topics

- Management applications
- Evolution of study design
- Gains in understanding limitations and uses

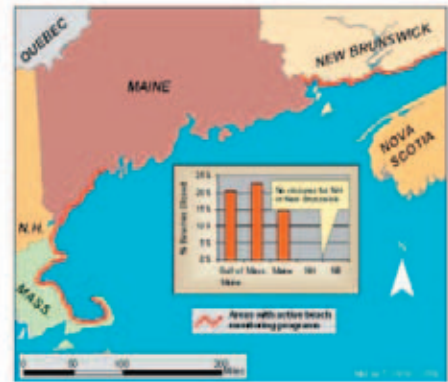
Escherichia coli Ribotyping

- **Genotypic:**
 - Distinguish among bacterial strains based on their genetic makeup
 - Target ribosomal RNA-DNA highly conserved, include some variable regions
- **Source identification:**
 - *E. coli* strains yielding identical or highly similar DNA banding patterns assumed to be from the same source species
 - Source species database required
 - *E. coli* used as indicator of freshwater sources to beaches/surface waters
- **UNH Methodology:**
 - Based on-Parveen et al. (1998) & Hartel et al. (2002)

Using microbial source tracking: Bathing Beaches



Monitored Beaches & Beach Closures in the Gulf of Maine (2002)

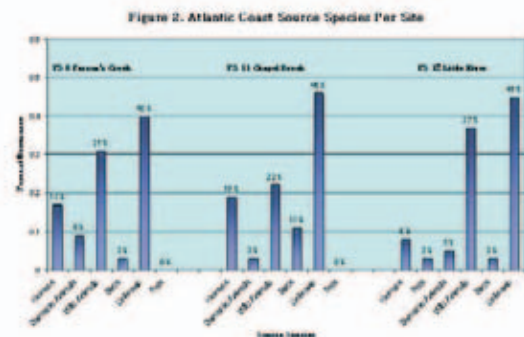


Using microbial source tracking: New Hampshire Coastal Beaches

- No advisories or closures through 2002
- 2003: 1 advisory
- 2004: 3 advisories

Trend is function of:

- Increased monitoring effort
- Previous 5 years of data show significantly increasing trend in bacteria at 5 of 8 beaches
- Weather influence not determined



-OTTERS, common wild animal source species, CONFIRMED at some sites



Using microbial source tracking: Bacterial TMDL

Hampton/Seabrook Harbor TMDL

- 3 designated uses relevant to bacterial pollution (shellfish harvesting, swimming, boating).
- Used ribotyping data to illustrate relative strengths of the different bacteria sources (modeling stormwater loads).

Using microbial source tracking: Stormdrain Discharges

E. coli concentrations > 100,000 cfu/100 ml

Source Species Identified in Stormwater Pipe Effluent

Sampling throughout 10/16/02 storm at end of pipe

E. coli concentrations: 120,000-1,120,000 cfu/100 ml

Source Species	Percentage
Human	26%
Birds	29%
Wild animals	17%
Pets	9%
Unidentified	20%

Source Species Identified in Stormwater Pipe Effluent

Composite samples from storm event above and below treatment system

E. coli concentrations (cfu/100 ml): ~9000 on 10/25 & 1800 on 10/29

Source Species	Percentage
Goose	10%
Human	2%
Dog	3%
Unidentified	22%
Cat	72%

*Follow-up: local sources found to be changing plastic bags of pet feces in storm drains

Source species for identified profiles --Hampton/Seabrook Estuary--

Source species type	% in study area
Humans/wastewater	26%
Pets	4%
Birds	7%
Livestock	8%
Wild animals	15%
Unidentified	40%

Using microbial source tracking: Target Remedial Efforts

- Prioritize efforts in areas where "human" sources had highest prevalence.
- Place more resources into boat pump outs, sewer/stormwater infrastructure, and septic system maintenance education.
- Shift focus from suspected source to confirmed source (Hampton Harbor, e.g., birds to human)



Using microbial source tracking: Recent Projects in NH

Approach	Design	Cost*
Stormdrain discharges	2 pipes, 5 samples throughout one storm at each pipe	\$5,000
Pollution Source sites from shoreline survey	13 sites, 6 samples/site, pre-scheduled sampling; add strains to library	\$79,000
Tidal creeks during wet weather (beach)	3 sites, 5 samples throughout two storms at each site	\$35,000
Entire Harbor (TMDL)	10 sites, monthly for 15 months; add strains to library	\$90,000

Using microbial source tracking: Challenges

- What is the management question?
- What is being impacted (e.g., shellfish growing waters, swimming beach, river)?
- SCALE OF STUDY-
specific source > problem area > watershed
\$ \$\$ \$\$\$
- Define conditions to enable narrowing of efforts that require ribotyping

Using microbial source tracking: Challenges

Study design

- Driven by management question & knowledge of area of concern
- Sample site location (e.g., bracket suspected sources & beach; spatial representation)
- Build local source species database
- Limited resources: exploit information from indicator analysis before ribotyping
- Over-sample then choose most critical (bacteria level, rainfall amount) samples to ribotype

Using microbial source tracking: Present Focus

SURVEY:

Defined study area:

Spatially intensive sampling includes & brackets suspected sources:

Wet/dry weather;

Source faces sampling for local library

RESEARCH:

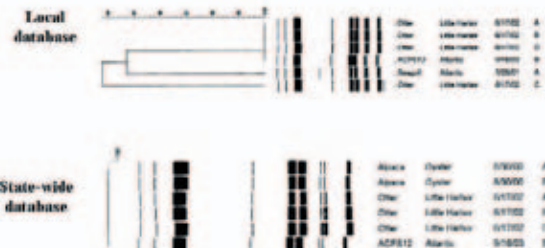
Ground-truth non-human sources in storm water runoff from defined catchments

Thank you!

"SOURCES" of support



Ribotyping results for one isolate (ACPS12) using local & large source species databases





Questions and Answers

No questions.



Replication of *E. coli* in Sand at a Temperate Freshwater Beach

Elizabeth Alm

Central Michigan University

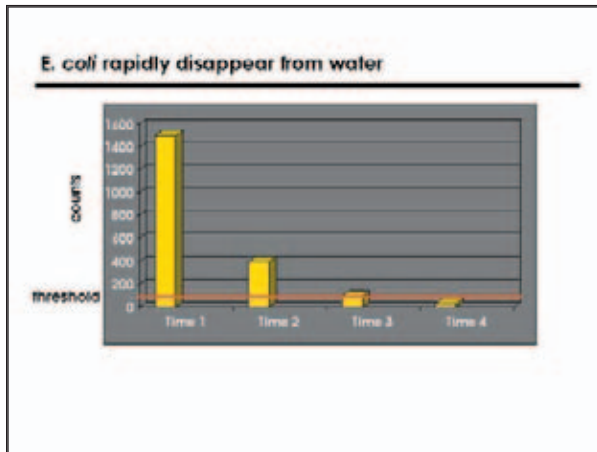
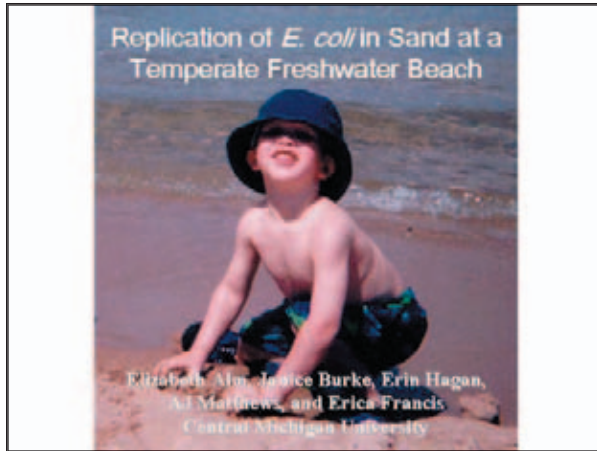
Biosketch

Dr. Elizabeth Alm is a professor of microbiology in the Biology Department at Central Michigan University. Dr. Alm received an A.B. in Biology from Randolph-Macon Woman's College in Virginia, a M.S. from Ball State University in Indiana, and a Ph.D. from the University of Illinois at Urbana-Champaign. She has been on the faculty at Central Michigan University since 1996. Dr. Alm has been studying microbial community structure in aquatic environments for over 12 years. For the past 4 years she has been focusing on the sources and fates of enteric bacteria at Great Lakes beaches. She is a participating faculty in the Michigan Water Research Center and in the Institute for Molecular Epidemiology.

Abstract

Escherichia coli have been used as indicators of recent fecal contamination in beach monitoring and source-tracking programs. Recent investigations have demonstrated high abundances of *E. coli* in sand at temperate freshwater beaches. This study was initiated to test the hypothesis that high

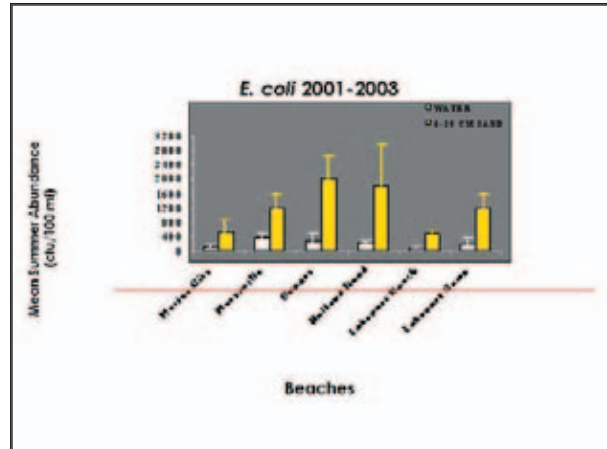

abundances of *E. coli* can be explained, at least in part, by the ability of *E. coli* to live and replicate in beach sand. In laboratory microcosm studies, *E. coli* densities increased from 1.9×10^2 to more than 2×10^7 CFU/100 g sand after 2 days of incubation at 19°C, and remained above 2×10^7 for at least 35 days. In field replication studies, performed in diffusion chambers incubated in Lake Huron foreshore sand, *E. coli* were able to multiply rapidly at the beach, reach high densities in the sand (approximately 7.5×10^7 CFU/100g), and to persist in a cultivable state at high density for at least 48 days. In another field study, *E. coli* O157:H7 was observed in sand biofilm communities, suggesting in situ replication of this *E. coli* pathotype. Beach monitoring programs operate under the assumption that *E. coli* in water originates from a recent fecal contamination event. This study supports suggestions from recent monitoring studies: Some *E. coli* populations may be indigenous to beach sand and may be a source to swimming water. The potential for indigenous sand populations of *E. coli* to re-enter swimming water at some later time would frustrate *E. coli*-based monitoring and source tracking studies.





Characteristics of the swash zone

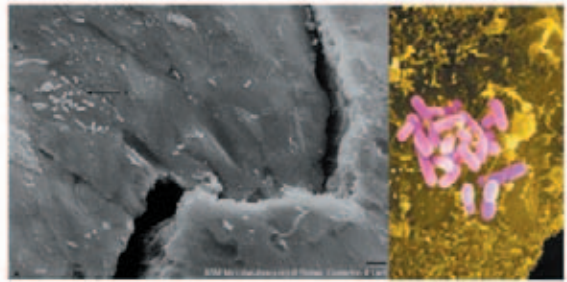
- particle size - >0.5 mm in diameter
- temperatures - 14 to 24.5°C (mean 19.6°C)
- moisture content - 12±3%
- nutrients - TP = 38ug/g;
TIN = 1ug/g



What is responsible for abundance of E. coli in sand?

1. filtration and concentration of E. coli by the sand
2. periodic direct fecal loading of E. coli to sand followed by persistence
3. deposition followed by replication of E. coli in the sand environment

E. coli attached in the intestine

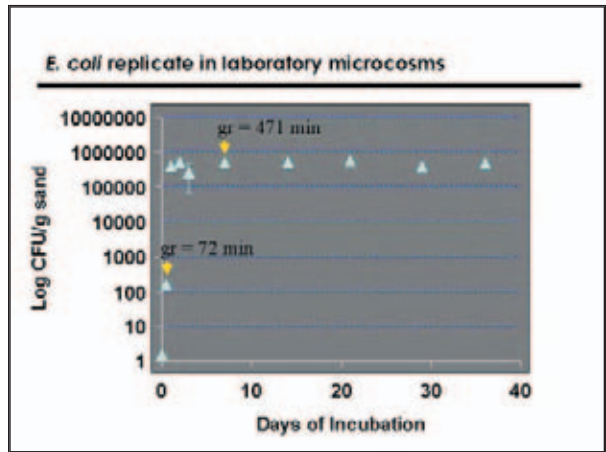
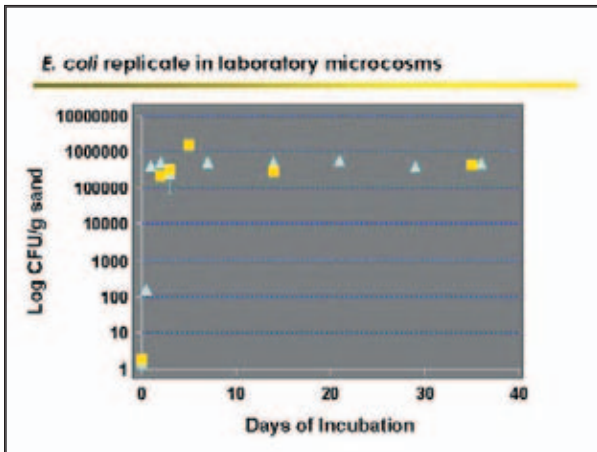
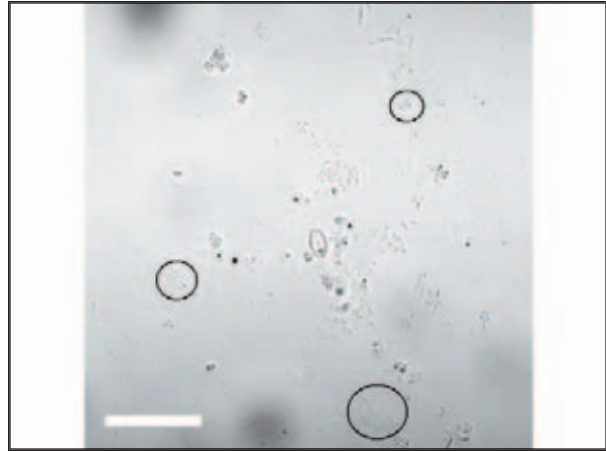
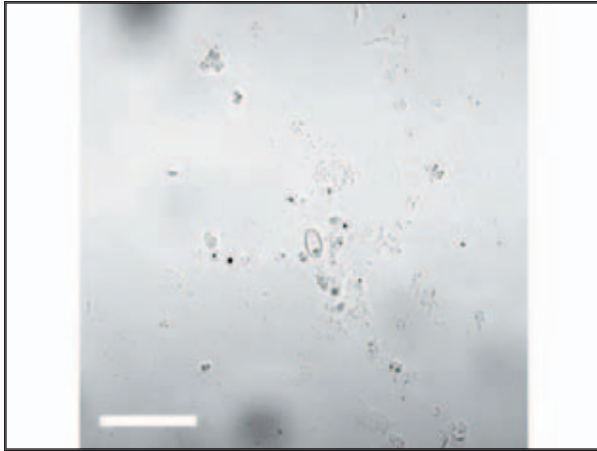


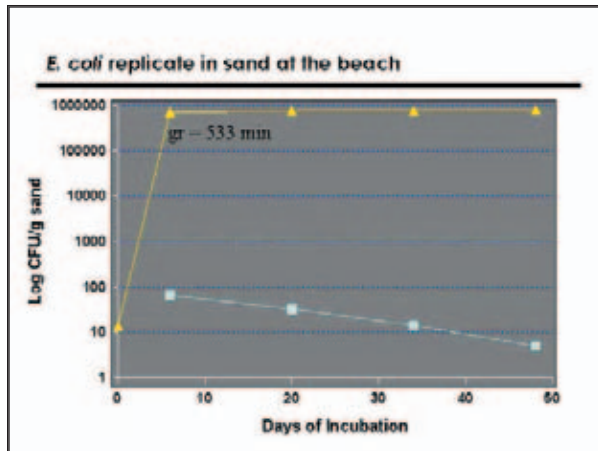
Sand grain habitat



Glass slides used as surrogate for sand grains

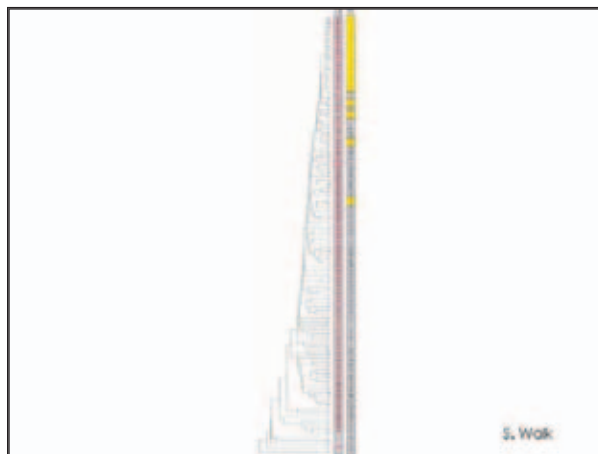






Might some *E. coli* be adapted to the beach?

with Seth Walk
and Dr. Tom Whittam
MSU



Summary

1. Dense populations of *E. coli* persist in sand
2. Some *E. coli* are indigenous to the beach

Significance

- Indigenous sand populations may be a source to water
? Indicator of recent contamination?
? Useful for source tracking?

Significance

- Indigenous sand populations may participate in lateral gene transfer and creation of new pathotypes.

Strain	lac	Kan	H ₂ r
Donor	-	-	-
Reclonants	-	R	R
Transconjugates	-	-	-

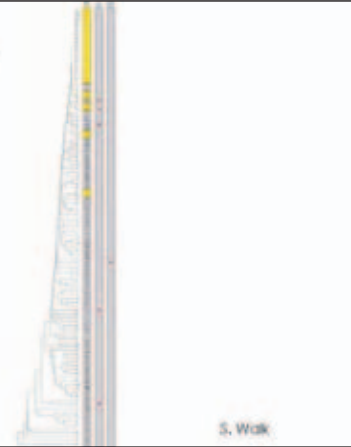


Significance

- Pathogens of fecal origin may persist in sand
- Susceptible persons may contact pathogens directly

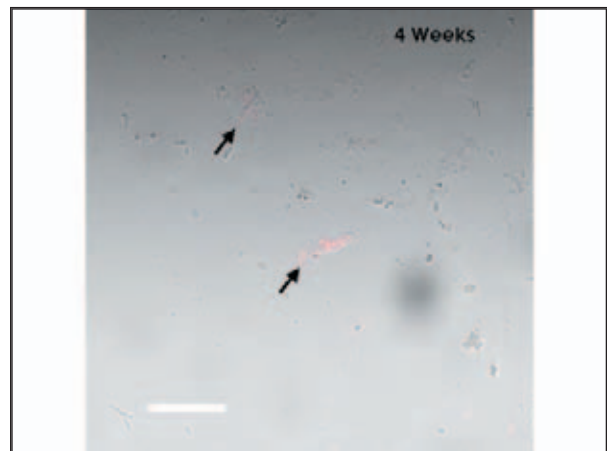
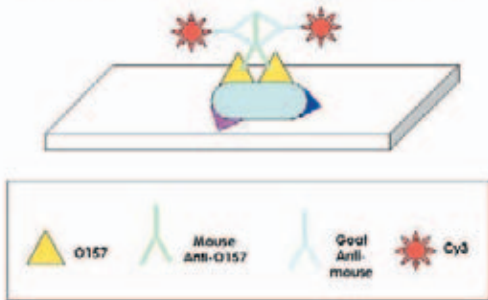


Virulence genes are present in beach isolates

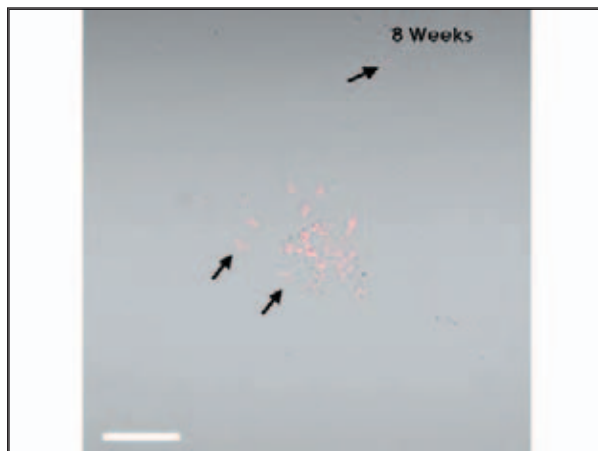


S. Wain

Antibody staining for O157

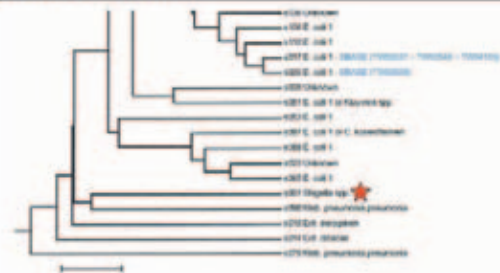


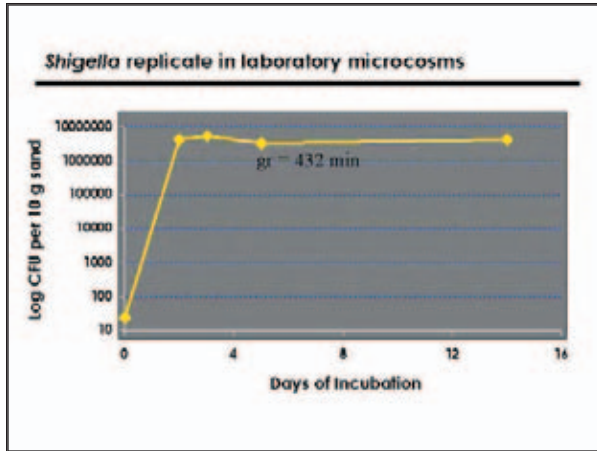
4 Weeks



8 Weeks

Shigella is present







Questions and Answers

Q: In your microcosm experiment where you spiked with the isolate that you've grown in the lab, you showed it could reproduce in your microcosm in the absence of competition. Have you done another experiment where there was competition to see whether that is still occurring?

Elizabeth Alm

In other experiments that we've tried to set up, for instance when we were trying to set up the assays to look at the exchange in antibiotic resistance, finding a pair of *E. coli* that we could maintain in our columns at the ratio that we wanted was challenging. Very often one strain would push the other one out and take over. So I think that we have a lot of evidence that competition is occurring and is probably a very important mechanism for regulating these populations.

*Q: Can you justify your choice to compare directly *E. coli* in cfu/100 grams of sand to *E. coli* in cfu/100 milliliters?*

Elizabeth Alm

Not very well because they are very different matrixes, and for the volume of sand there are a lot more attachment sites, so it is a bit like comparing apples and oranges. So, doing it on a per volume basis was the best that we could come up with, but I wouldn't say that a direct comparison like that is a fair comparison.

Q: That information you provided on Shigella and 0157 growing in the sand is pretty frightening. You started your talk out with a picture of a kid wearing a swim diaper, and you are talking about control at the source. Do you think that kids in swim diapers may be something we need to control at beaches?

Elizabeth Alm

Yes, definitely. I think that is a real problem and that a lot of studies have shown that bathers can carry—not just children but adults too—fecal organisms microorganisms on their skin that comes right off when they get into the water. So, I think that a lot more public awareness of the contributions they make is definitely important. I don't think the swim diapers do too much to keep the organisms out. It may remove the visible floaters, but not the bacteria and viruses.

Thursday, October 14
10:20 a.m. – 12:00 p.m.

Concurrent Track I:
Identifying and Solving Beach Water Quality Problems
Session Six:
TMDLs



A Watershed Scale Approach for Developing a Bacterial TMDL in an Urbanizing Puget Sound Embayment

Christopher May

Battelle Marine Science Laboratory

Biosketch

Dr. Christopher W. May, senior research scientist and engineer at the Battelle Marine Sciences Laboratory (MSL), is a freshwater ecologist and environmental engineer with expertise in urban watershed assessment and management. His areas of interest include stormwater management, watershed analysis using geographic information systems (GIS), salmonid habitat assessment, urban stream rehabilitation, water quality monitoring, stream biological assessment, and watershed restoration. His current research at Battelle focuses on the linkage between upland watersheds and nearshore-marine ecosystems, including natural processes and land-use impacts. Prior to joining the MSL team Dr. May was a research engineer at the University of Washington Applied Physics Laboratory (UW-APL). His research there centered on the cumulative impacts of urbanization on native salmonids in small streams in the Puget Sound lowland eco-region. Dr. May is an adjunct faculty member of Western Washington University, Huxley School of Environmental Studies, University of Washington, Tacoma Environmental Science Program, and the University of Washington, Professional Engineering Program.

Abstract

Shellfish are icons of the Pacific Northwest, associated with many recreational, cultural and economic values. Clean water is essential for shellfish harvesting. However, an increase in human population and development within nearshore environments and adjacent watersheds has degraded water quality by increasing the incidence of

bacterial pollution, resulting in increased closures for shellfish harvesting, as well as restrictions on fishing and contact recreational activities such as boating and swimming. While research has long demonstrated that urbanization alters water quality in upland streams and rivers, primarily through the loss of native vegetative cover, increased impervious surfaces, altered hydrology and other impacts, the relationships between patterns of landscape alteration and the health of shellfish growing areas are generally not well understood.

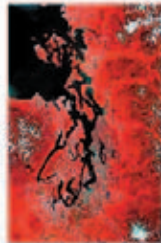
This research project explored the relationships between urbanization and nearshore water quality using a landscape scale analysis of the Sinclair-Dyes Inlet watershed. A landscape-scale empirical analysis of urbanizing sub-basins was conducted. Using bacterial contamination as the indicator of nearshore water quality conditions, we identified the landscape factors that best explained water quality conditions in nearshore shellfish growing areas. Across all sub-basins, we found that the loss of native forest cover, impervious surface area, and road density are the best predictors of nearshore water quality conditions. Within the more urbanized areas, the amount and connectivity of impervious surface areas explained most of the variance in bacterial pollution. In addition, the type and extent of the stormwater conveyance and treatment network significantly influenced bacterial contamination levels in the nearshore environment. The Sinclair-Dyes Inlet study was used to develop a TMDL implementation plan. A dynamic model was also developed as part of this project. The findings of this study also have broad implications for land-use and stormwater management policies in other coastal areas of the country.



A Watershed Scale Approach for Developing a Bacterial TMDL in an Urbanizing Puget Sound Embayment

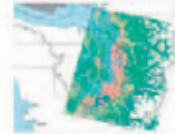


Battelle
The Business of Innovation
Pacific Northwest
National Laboratory
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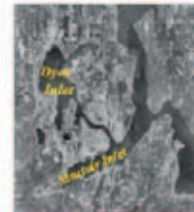


Chris May Battelle-PMML
Jerry Sherrell PMMS
Bob Johnston UQH-GP/NAWA
PF Wang USM-SP/NAWA
Brian Skahill AD0641ES

Project Location



Sinclair-Dyes Inlet
Project ENVVEST



Beneficial Uses

- **Shellfish Harvest**
- **Fishing**
- **Contact Recreation**
 - Swimming
 - Boating
- **Fish & Wildlife Habitat**
- **Shoreline Development**
- **Commercial & Industrial Activity**
 - Shipping
 - Marinas
 - Shipyards

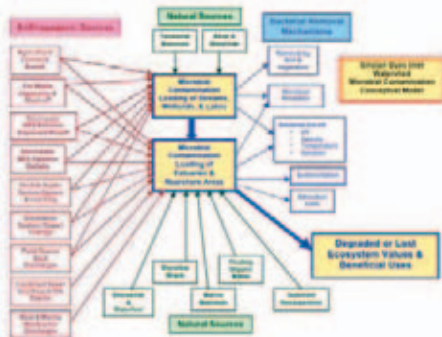


Pollution Sources

- Wildlife
- Livestock Manure
- Pet Waste
- On-Site Sewage Systems
- WWTP
- Combined Sewer Overflows
- Stormwater Runoff
- Marinas and Boats



Microbial Pollution Conceptual Model



Background

How does nearshore WQ change as shoreline and upland watershed land-use and land-cover changes during the development process?



Less Suitable ← → More Suitable

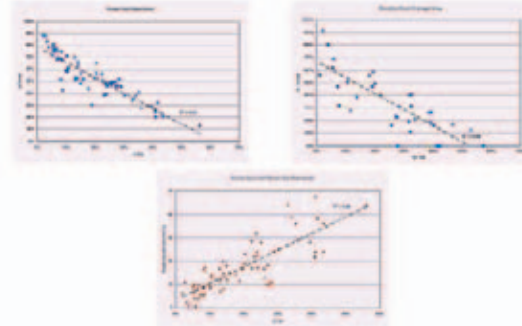




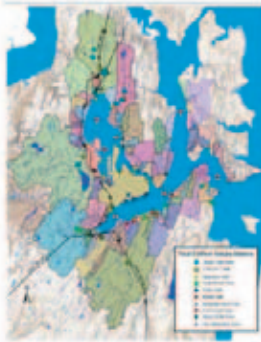
Landscape Metrics

- % Forest Cover (Coniferous/Deciduous/Mixed)
- Road Density
- % Total Impervious Area
- % Rural Residential
- % Suburban Residential
- % Urban Residential
- % Commercial
- % Industrial

LULC Analysis



Project ENVVEST



Watershed-based TMDL

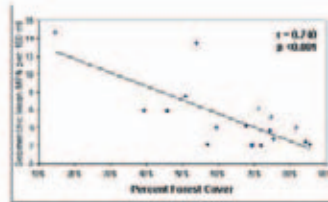
FC data sources:

- WA-DOH (Marine)
- KCHD (Streams & Marine)
- ENVVEST (Marine, Streams & Stormwater Outfalls)

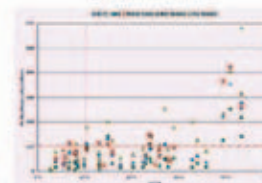
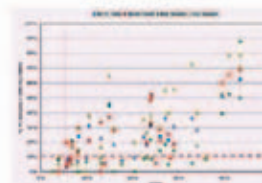
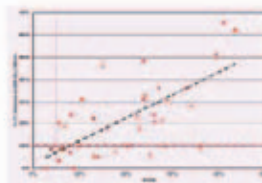
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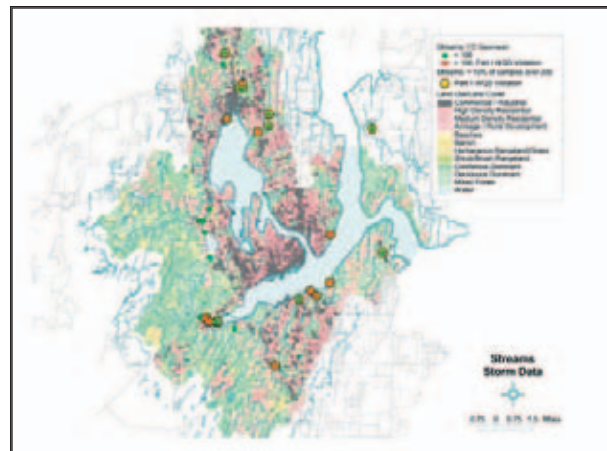
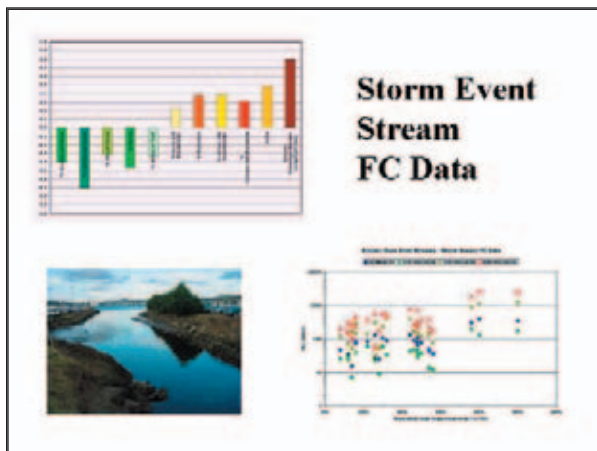
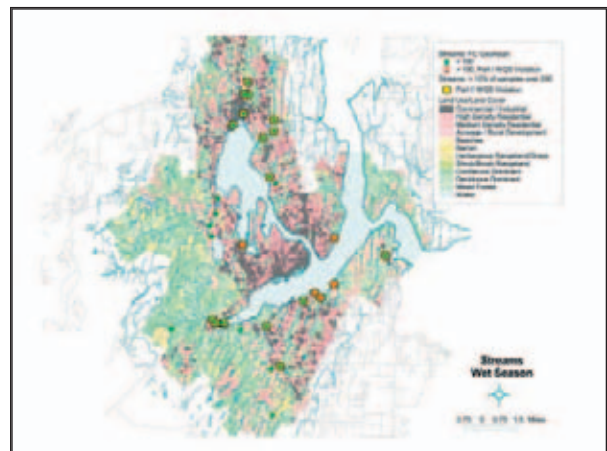
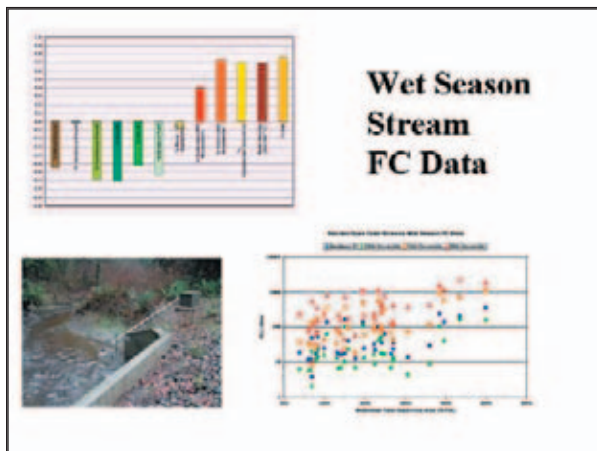
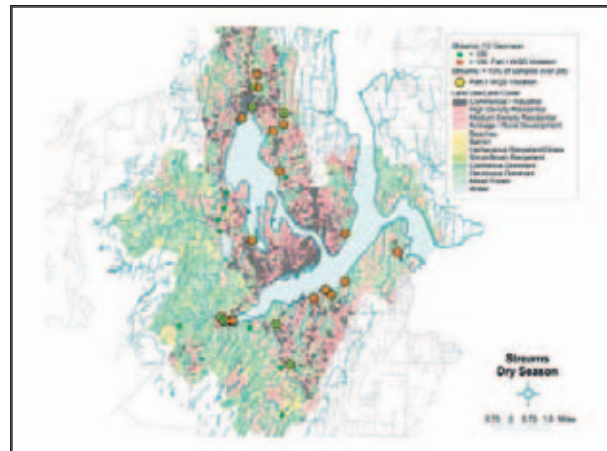
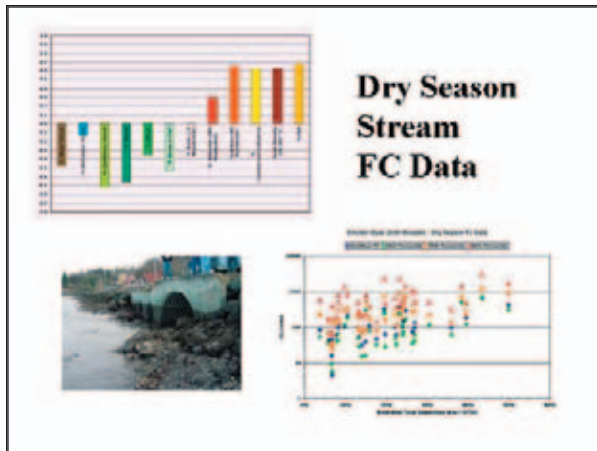
- Dry Season
- Wet Season
- Storm Event

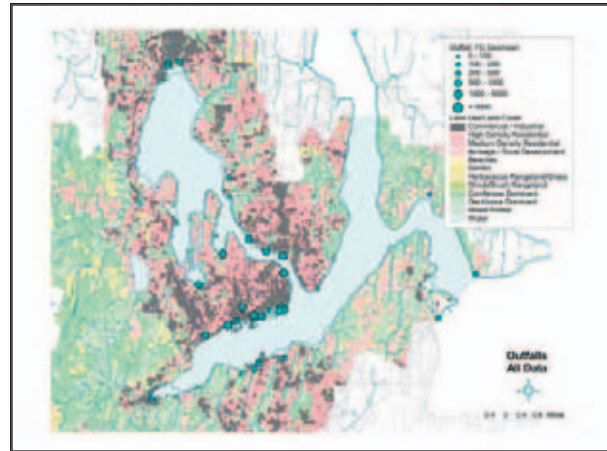
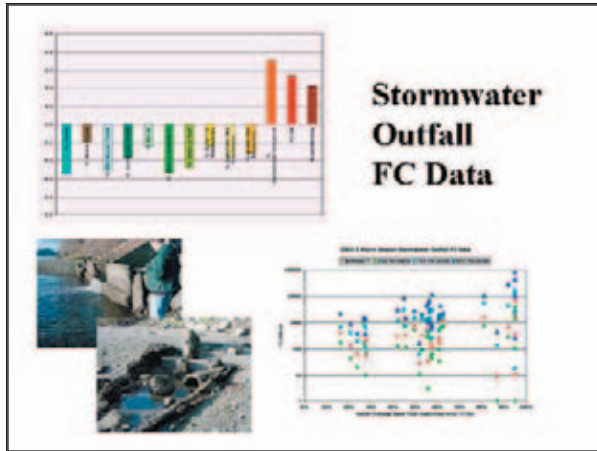
Nearshore & Marine FC Data



Stream FC Data





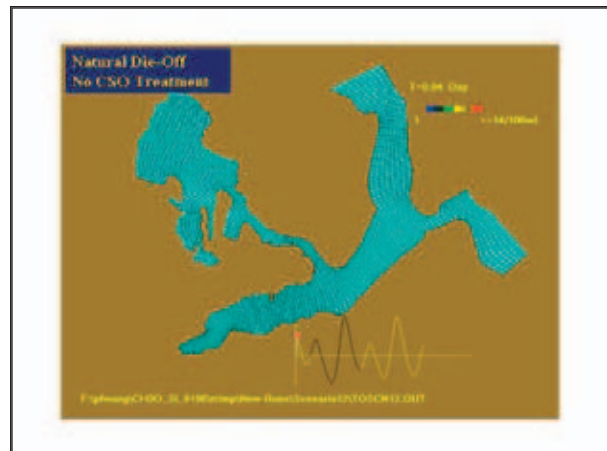
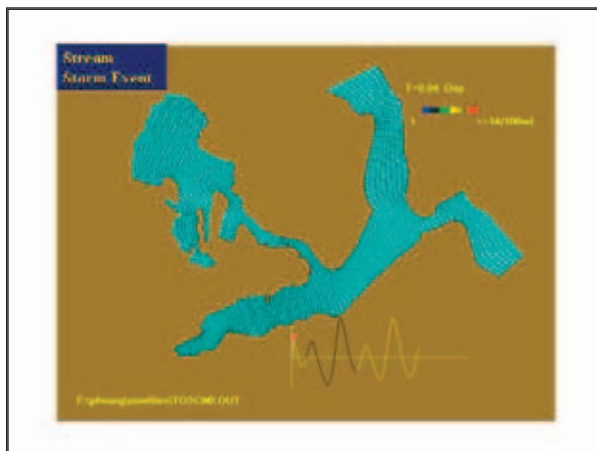


Summary Conclusions

- Highly developed shorelines, streams draining urbanized sub-basins, and stormwater outfalls can be significant sources of bacterial contamination to the nearshore environment.
- Most developing watersheds have numerous sources of bacterial contamination (illicit connections, CSO events, failing septic systems, leaking sewers, livestock manure, pet waste, etc.) making treatment very difficult.
- In developing watersheds, stormwater is a major transport mechanism for bacterial pollution, especially where "hard" conveyance systems (curb & gutter, drain-inlets collection, and piped conveyance) are in place.
- Violations of WQS are very common during storm events, but appear to be transient. Dry season problems appear to be the most chronic.
- Pollution Identification and Correction (PIC) programs have been very effective in reversing bacterial pollution trends, as have infrastructure improvement projects (CSO & sewer upgrades).

Management Implications

- Must consider all potential sources of bacterial pollution, including wet season sources such as stormwater runoff and dry season sources such as on-site septic system & sewer leakage.
- Source control (PIC) programs can be very effective if implemented on a watershed scale with active stakeholder involvement (KCHD & KCD). Microbial Source Tracking (MST) is also an option.
- In general, watershed and nearshore development, under current standards, may be inconsistent with some other beneficial uses, but better source control, low impact development practices, innovative stormwater treatment, & watershed management have the potential to reduce or eliminate this conflict.
- Modeling can be a useful tool for watershed management.





Questions and Answers

Q: How much money did it take to create your model?

Christopher May

I can't really tell you, as we probably have to talk man-hours and things like that.

Q: Too many zeros?

Christopher May

Not really. We have the technique down. For the upland part, we use an HSPF-based model, which is fairly simple. Then, the CH3D model was the dynamic model used for the water column, and that takes a little doing, but I think we've worked the bugs out so it's not that difficult anymore. So, I can talk to you about how many man-hours it took.

Q: Did you verify the model?

Christopher May

Yes, we have done synoptic surveys, and all the sample points at the same time, plugged it into the model and its pretty close.



Improving Beach Water Quality through TMDLs: A Case Study of Santa Monica Bay Beaches

Renee DeShazo

Los Angeles Regional Water Quality Board

Biosketch

Renee DeShazo is the Basin Planning Coordinator for the Los Angeles Regional Water Quality Control Board. In this role, she oversees development of all regional basin plan amendments that incorporate or revise water quality objectives, beneficial uses and implementation policies for water quality standards. Ms. DeShazo also initiates early review of basin planning issues related to TMDL development, and works closely with the multidisciplinary TMDL Units on the basin planning components of TMDL development. She was the lead staff person in the development of the Santa Monica Bay Beaches Bacterial TMDLs and continues to work closely with stakeholders in the development of monitoring and implementation plans for those TMDLs. Prior to her position with the Regional Board, Ms. DeShazo worked for the Santa Monica Bay Restoration Project, and prior to that she was employed by the Massachusetts Department of Environmental Protection. Her educational background includes a Bachelor of Science degree from the College of William and Mary and a Master's degree from the University of North Carolina at Chapel Hill.

Abstract

Santa Monica Bay beaches are an icon and a major source of revenue to the Los Angeles Region, while Santa Monica Bay is the major receiving water for urban runoff and effluent from wastewater treatment plants for one of the largest population centers in the United States. As such, many of the beaches along Santa Monica Bay experience poor bacteriological water quality, particularly during wet weather when storm water runoff is conveyed through numerous storm drain outfalls to the beaches. Yet, beach usage remains significant during winter months given the mild climate of Southern California and the year-round popularity of surfing and other water-related recreational activities. To address bacterial contamination at these beaches, the State adopted Total Maximum Daily Loads (TMDLs). These TMDLs are based on the principles that bacteriological water quality must be at least as good as at a reference site and there shall be no degradation of existing shoreline water quality if historical water quality is better than the reference site. The TMDLs have a multi-part numeric target that includes four bacterial indicators. Using the principles above, a certain number of exceedances of the single sample limits for these indicators are allowed at the beaches. This approach is supported by a diverse group of stakeholders, including cities responsible for complying with the TMDLs as well as environmental organizations committed to ensuring the highest achievable level of public health protection for the local residents and visitors to the Bay's beaches.



**Making the Most
of a Difficult Situation:**
 The SMB Beaches Wet Weather Bacteria TMDL

 National Beaches Conference
 October 14, 2004

Background

- Dry-Weather TMDL
 - Easier problem to tackle
 - Much progress made already (i.e. LFDs)
 - 3 to 6 years for implementation
 - Approach same as Wet-Weather

Wet Weather...Should we care?

- 1½ million beach visitors/month during winter months (Nov. to Mar.)
- Winter users are likely frequent users (e.g. surfers)
 - Longer and more frequent exposure than the average population
 - Likely to place a higher value on beach water quality

Health Risks of Swimming in Water with Elevated Bacteria Densities

- Epidemiological studies show links between bacteriological water quality and health risks
- Health effects observed include:
 - vomiting, fever, stomach pain, diarrhea
 - eye, ear and skin infections
 - respiratory ailments

Health Risks (continued)

Health Risks at Proposed Numeric Targets
based on Santa Monica Bay Epidemiological Study

Bacterial Indicator	Health Risk	Number per 10,000
Enterococcus	Diarrhea with blood	27
	Gastroenteritis I	130
Total coliform	Skin rash	165
Fecal/total ratio	Nausea	230
	Diarrhea	281
	Gastroenteritis II	98
	Chills	117
Fecal coliform	Skin rash	74

Water Quality Objectives = TMDL Numeric Targets

Target	Single sample limit	Geometric mean limit
Total coliform	10,000	1,000
	1,000	N/A
	when F:T>0.1	
Fecal coliform	400	200
Enterococcus	104	35



Wet-Weather Problem Identification

- LA County DHS Data (1996-2000):
 - 60% of shoreline monitoring locations exceeded standards more than LCB
- Heal the Bay Annual Beach Report Card:
 - 60% of beach locations received a grade of C or lower
- SCB Shoreline Microbiology Survey (2000):
 - 58% of sites exceeded standards

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Wet Weather Source Characterization

- Storm-water runoff is primary source of elevated bacteria densities
- Natural runoff contributes to some exceedances
- Supported by historical shoreline data & SCB survey, which show much higher levels of exceedance at freshwater outlets

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Wet Weather...What can we do?

- Problem
 - Single sample "not-to-exceed" objectives
 - Bacteria is not solely a human-caused problem
 - Bacteria is ubiquitous in environment
 - Nature of So. Cal. storm events
 - short, intense
 - large peak flows & volume

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Implementation Procedures for Bacteria Objectives

- Two implementation procedures proposed for single sample objectives
 - Reference System/Antidegradation Approach
 - Used in this TMDL
 - Natural Sources Exclusion Approach
 - Use if an appropriate reference system cannot be identified
- Does not apply to geometric mean objectives
- May only be applied in context of a TMDL

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Why a Reference System/Antidegradation Approach?

- Not intent to require treatment or diversion of natural creeks that convey bacteria from natural sources
- Northern SMB sub-watersheds average 85% open space & associated beaches still exceed objectives occasionally

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Objectives of Wet-Weather TMDL: The "Reference System/Anti-Degradation Approach"

- Water quality is at least as good as that of a natural system
- No degradation of existing shoreline water quality where it is better than natural system

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Reference System Approach

- Three criteria for selecting local reference beach:
 - Watershed is predominately open space
 - Field observations indicate little human impact in watershed
 - Freshwater outlet onto beach
 - Adequate shoreline monitoring data

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Waste Load Allocations

- Expressed as "allowable exceedance days" for single sample targets
 - Bacterial density and frequency of single sample exceedances are most relevant to public health
 - 'Appropriate measure' consistent with the definition in 40 CFR 130.2(l)

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Waste Load Allocations

- Each of the 55 shoreline monitoring stations are assigned a final allowable number of exceedance days during wet weather
- All responsible jurisdictions and agencies within a sub-watershed are jointly responsible for complying with the WLA at the receiving shoreline monitoring location

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Criteria for Determining Allowable Wet-Weather Exceedance Days

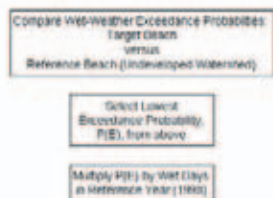
The Role of the Reference System and Anti-degradation

- Select smaller of two criteria based on historical data (1995-2000):
 - Wet-weather exceedance probability of the reference system
 - Wet-weather exceedance probability at a particular beach monitoring site

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Method for Setting Allowable Wet-Weather Exceedance Days

Decision-making Process for Determining Allowable Exceedance Days



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Critical Condition

- Wet Weather
 - Historical shoreline monitoring data and SCB survey show a higher level of exceedance during wet weather
- Selected 90th percentile storm year based on wet days as critical/reference year
 - 1993 had 75 wet days

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Wet-Weather Results for Leo Carrillo Beach Reference System

- Historical shoreline data for Leo Carrillo Beach
 - 22% wet weather samples exceeded standards
 - Also express as 0.22 probability of exceedance, given a wet day
 - Translates to 17 allowable exceedance days (0.22 x 75 wet days in "reference year" of 1993)*

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Sample of Wet-Weather Waste Load Allocations by Beach

Beach	Wet-Weather Exceedance Probability	Wet Days in Reference Year	Allowable Wet-Weather Exceedance Days
Leo Carrillo Beach (reference site)	0.22	* 75	= 17
Surfrider Beach	0.60	* 75	= 17
Santa Monica Canyon	0.33	* 75	= 17
Santa Monica Pier	0.16	* 75	= 17
Manhattan Beach Pier	0.06	* 75	= 5
Long Point	0.05	* 75	= 4

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Schedule based on Implementation Approach

- Two broad approaches
 - Integrated Water Resources (IWR) Approach
 - Single-purpose Approach
- Schedules tailored to approach
 - As short as possible given public health risks
 - No more than 18 years for IWR Approach
 - No more than 10 years for Single-purpose Approach

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What is an IWR Approach?

One that:

- Integrates planning for future wastewater, storm water, recycled water, and potable water needs
- Focuses on beneficially re-using or infiltrating storm water at multiple points throughout a watershed
- Addresses multiple pollutants
- Realizes water quality and other public goals
 - e.g. water supply, recreational opportunities, and open space

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Why different schedules?

- To realize multiple benefits, IWR Approach requires more complicated planning & implementation, such as
 - identifying and designing viable integrated projects;
 - designing for multiple pollutants;
 - siting transmission infrastructure, storage and recharge areas at multiple points in a watershed;
 - identifying markets for reclaimed stormwater; etc.

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Monitoring Program Objectives

- Re-evaluate possible reference system approaches, including the site(s) and years used
- Re-evaluate allowable exceedance days based on anti-degradation criterion and final compliance point (wave wash)
- Re-evaluate potential implementation scenarios based on refined source characterization
- Assess compliance with interim and final allowable exceedance days

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Refinements to Wet-Weather Allowable Exceedance Days

- Revise TMDL 4 years after effective date to re-evaluate wet-weather allowable exceedance days
 - Re-evaluate selection of reference system
 - Re-evaluate selection of reference year
 - Collect daily shoreline monitoring data from wave wash rather than 50 yards away

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Summary

- Wet Weather Only
- Significant water quality & public health issue
- ~ 40% reduction in wet-weather exceedance days *Bay-wide*
- Increased protection for 7.3 million visitors to beaches during winter wet season
- Money well spent, considering magnitude of direct spending by visitors & importance of beaches to the local economy

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Questions and Answers

No questions.



Delisting of Recreational Beaches on the 303(d) List for Exceedances of Bacterial Water Quality Standards

Lisa Kay

MEC-Weston Solutions, Inc.

Biosketch

Ms. Lisa Kay has over 19 years of experience in water quality assessments relating to the Clean Water Act, primarily involving project development, study design, project management, and quality assurance oversight. She assists her municipal clients in NPDES compliance; TMDL studies, watershed management planning, and the development of grant funded projects. She co-designed the NPDES storm water-monitoring program for the 22 municipal copermittees in San Diego County. She has been managing the implementation of this urban runoff program since the year 2000. Ms. Kay is the Water Resources Practice Leader for MEC-Weston Solutions, Inc.

Abstract

In southern California, there are numerous shoreline water quality monitoring sites located along coastal beaches, bays, and harbors that are monitored for bacterial indicators (total coliform, fecal coliform, and enterococcus). Due to exceedances of bacterial indicator standards, many of these sites are listed as impaired on the California

State Water Resources Control Board (SWRCB) 303(d) List. In December 2003, the SWRCB developed draft guidance criteria for removing sites from the 303(d) List (a process known as delisting). The primary consideration for removal of a water segment from the 303(d) List is an exceedance frequency of water quality standards of less than 10% of the analyses conducted (with at least 90% confidence). In this assessment, five years of bacterial data from all of the beach sites within the City of San Diego that are listed on the 2002 303(d) List were reviewed and compared to the draft guidance criteria. A total of 62 sites are identified on the List, including 45 that are located in Mission Bay, which is listed in its entirety. Of the 17 sites listed outside of Mission Bay, 11 were recommended for delisting. Within Mission Bay, nearly half the sites monitored were recommended for delisting. The SWRCB delisting guidance provides a meaningful, statistically based process for removal of sites from the 303(d) List. The results of the assessment using the process suggest that many of the sites that are currently on the 303(d) List within the City of San Diego should be considered for delisting.



MEC
MARINE ENVIRONMENTAL CONSULTANTS

Delisting of Recreational Beaches on the 303(d) List for Exceedances of Bacterial Water Quality Standards

*Lisa Marie Kay,
Stephen Gruber,
Susan D. Watts*

City of San Diego Beaches

Project Objective

- Compile and summarize the available bacterial data of all the shoreline sites monitored in the City of San Diego,
- Assess the data at sites on the 2002 303(d) List, and
- Identify those sites to be considered for removal (delisting) from the 303(d) List

Draft 303(d) Delisting Criteria CA State Water Resources Control Board

- *SWRCB's Draft Functional Equivalent Document (December 2003).*
- Removal criteria: exceedance of water quality standards of less than 10% of the analyses.
- The AB411 single sample limits were used in this document to determine the number of exceedances for a given sample size.

Applicable Water Quality Standards

	30-Day Limit ¹	Single Sample Limit
Total Coliform	1,000 MPN/100 ml ²	1,000 MPN/100 ml if Fecal > 10% of Total, or 10,000 MPN/100 ml ³
Fecal Coliform	200 MPN/100 ml	400 MPN/100 ml
Enterococcus	35 MPN/100 ml	104 MPN/100 ml

¹ 30-day limit is based on the geometric mean or on total fecal coliform.
² Method 1603 maximum number.
³ Total coliform single sample limit of 10,000 MPN/100 ml is used when the fecal coliforms are greater than 10% of total coliforms.

Information Assessed

- Five years of data were assessed: from January 1999 through October 2003.
- Data were compiled from raw data for all shoreline sites within the City of San Diego.
- Weekly monitoring by City of San Diego or the County Department of Environmental Health (DEH).
- Samples were analyzed for three bacterial indicators: total coliform, fecal coliform, and Enterococcus.



Draft Guidance

- For numeric water quality objectives for bacteria in water, SWRCB (2003) states that the primary consideration for removal of a water segment from the 303(d) List shall be the following criteria:
 - "Numeric water quality objectives or standards for bacteria are exceeded in fewer than 10 percent of the samples with a confidence level of 90 percent using a binomial distribution."

September 2004 Final Guidance

- Uses binomial distribution with <10% exceedance (n=26)
- Also applies site specific exceedance frequency IF used to place beach on list
- Proposes use of reference beach approach for comparison

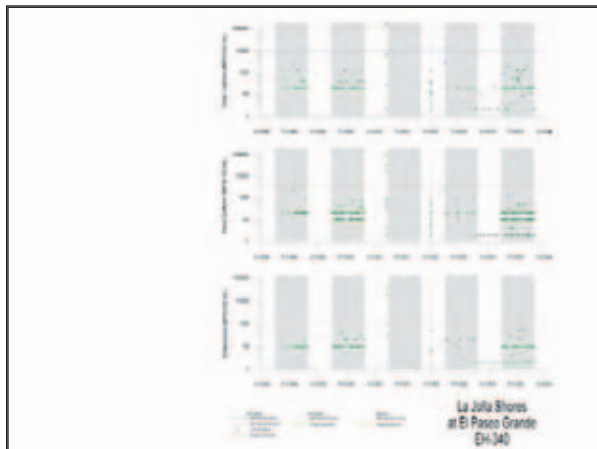
City of San Diego Beaches



Data Representation

303(d) List Site Name	Location	Station	Total Analyses	No	Yes	Allowable (Yes/Total)	Percent
Mission Bay	MDW04	MDW04	231	127	104	22	95.28

- Total number of analyses for all three indicators
- (No) = number that did not exceed criteria
- (Yes) = number that did exceed criteria
- (allowable) = The number allowed by SWRCB
- (percent) = percentage exceedances relative to the total number of analyses



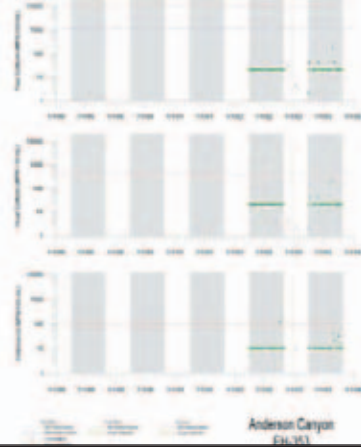
City of San Diego Beaches





Anderson Canyon

303(d) List Site Name	Location	Station	Total Analyzes	No	Yes	Allowable (Yes/Total)	Percent
Pacific Ocean Shoreline, Miramar Reservoir RA	Anderson Canyon	BH-222-0-M	171	170	1	11	0.00
		BH-253-75-L	9	9	0	na	0.00
		TOTAL	180	179	1	12	0.06



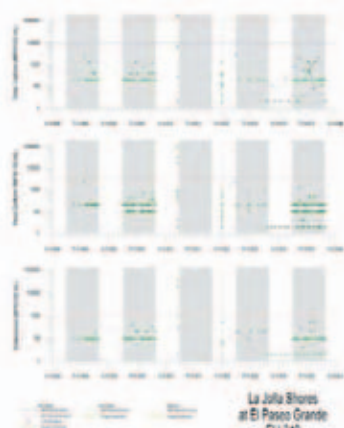
La Jolla Shores at Paseo Grande

The number of exceedances of water quality standards at this site are presented in Table 5. A total of 366 analyses were performed from 1999 through 2003. Of these, there were only six exceedances of the bacterial standards for all three indicators: one for total coliform, three for fecal coliform, and two for Enterococcus (Figure 3). Six exceedances out of 366 analyses is well below the number of exceedances allowed by the SWRCB guidance document (28). These data suggest that the El Paseo Grande site should be considered for de-listing from the 303(d) List.

La Jolla Shores at Paseo Grande

303(d) List Site Name	Location	Station	Total Analyzes	No	Yes	Allowable (Yes/Total)	Percent
Pacific Ocean Shoreline, Scripps RA	Paseo Grande	BH-340	6	6	0	na	0.00
		BH-340-100-L	12	12	0	na	0.00
		BH-340-100-R	9	6	3	na	33.33
		BH-340-300-L	9	9	0	na	0.00
		BH-340-300-R	6	6	0	na	0.00
		BH-340-25-L	6	6	0	na	0.00
		BH-340-25-R	6	3	3	na	50.00
		BH-340-50-L	6	6	0	na	0.00
		BH-340-50-R	6	6	0	na	0.00
		BH-340-75-L	27	27	0	0	0.00
		BH-340-75-R	36	36	0	0	0.00
		BH-340-0-M	237	237	0	17	0.00
		TOTAL	504	503	0	24	1.64

Eleven stations were monitored at the El Paseo Grande site during this time: the majority were taken at the sampling site and 75 ft to the left and right.

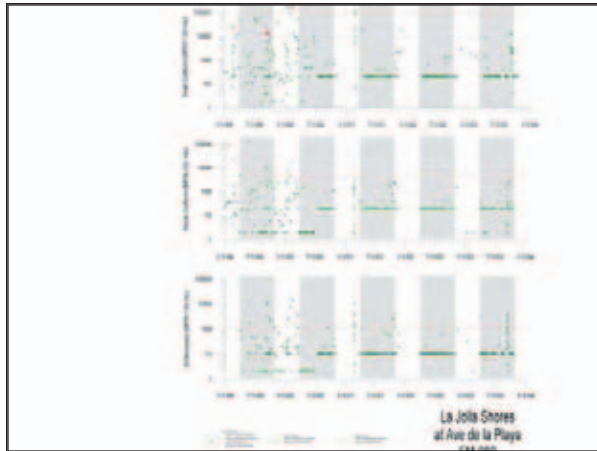


La Jolla Shores at Paseo Grande BH-340

La Jolla Shores at Avenue De La Playa

303(d) List Site Name	Location	Station	Total Analyzes	No	Yes	Allowable (Yes/Total)	Percent
Pacific Ocean Shoreline, Scripps RA	Avenue De La Playa	FM-080-0-M	2	2	0	na	0.00
		FM-080-150-L	2	2	0	na	0.00
		FM-080-150-R	2	2	0	na	0.00
		FM-080-100-L	12	10	2	na	16.67
		FM-080-100-R	12	10	2	na	16.67
		FM-080-150-L	2	2	0	na	0.00
		FM-080-150-R	2	2	0	na	0.00
		FM-080-25-L	2	2	0	na	0.00
		FM-080-25-R	2	2	0	na	0.00
		FM-080-50-L	2	2	0	na	0.00
		FM-080-50-R	2	2	0	na	0.00
		FM-080-50-L	18	17	1	na	5.56
		FM-080-50-R	20	19	1	na	5.00
		FM-080-75-R	39	37	2	3	7.69
TOTAL	434	397	37	33	6.41		

Fourteen stations were monitored at La Jolla Shores at Avenue De La Playa during this time: one at the sampling site (FM-080-0-M), six as far as 150 feet to the left, and seven as far as 150 feet to the right of the site



Mission Bay

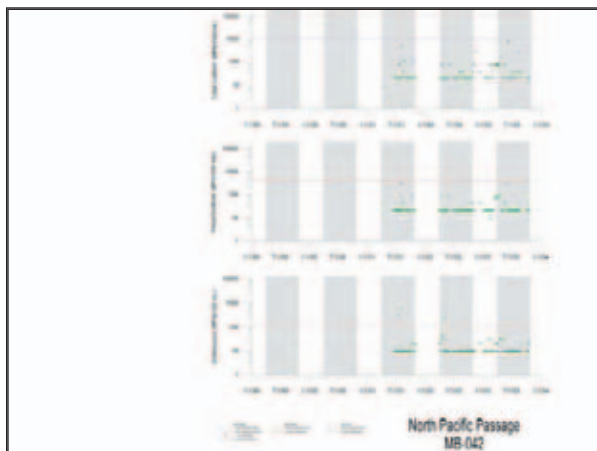
- 4,235 acres
- 27 miles of shoreline
- 303(d) listed for bacteria
- 45 monitoring locations
- Intensely monitored



North Pacific Passage (MB-042)

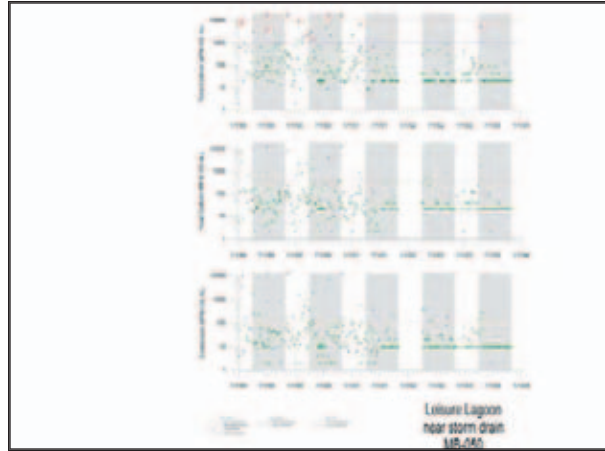
This site is located on the east side of Mission Bay, directly in front of the Hilton Hotel.

303(d) List Site Name	Location	Station	Total Analyses	No	Yes	Allowable (Yes/Total)	Percent (Yes/Total)
Mission Bay	North Pacific Passage	MB-042-0-M	288	265	3	20	1.11



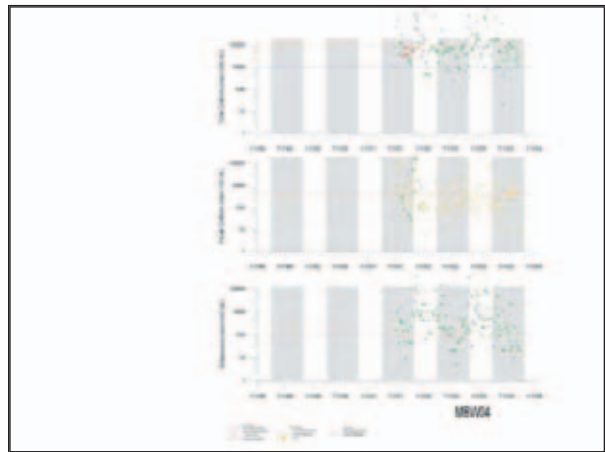
Leisure Lagoon Near Storm Drain

303(d) List Site Name	Location	Station	Total Analyses	No	Yes	Allowable (Yes/Total)	Percent (Yes/Total)
Mission Bay	Leisure Lagoon, near storm drain	MB-100-1-M	203	622	64	50	1.30
		MB-100-100-L	5	0	0	0%	0.00
		MB-100-100-R	3	3	0	0%	0.00
		MB-100-100	6	6	0	0%	0.00
		MB-100-100-S	3	0	0	0%	0.00
		MB-100-100-T	22	22	0	0%	0.00
		TOTAL	632	705	64	1%	1.68



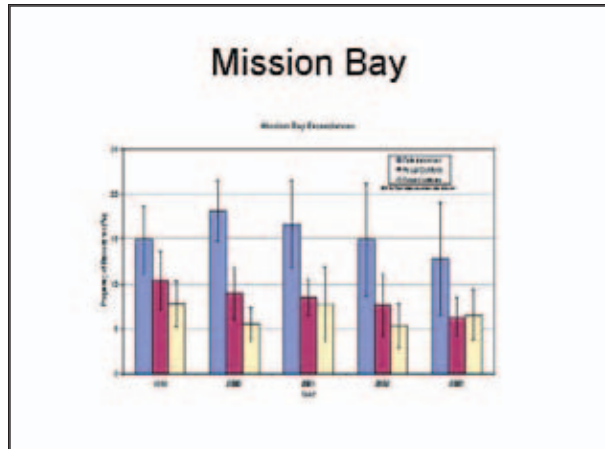
East Side Mission Bay at the mouth of Cudahy Creek.

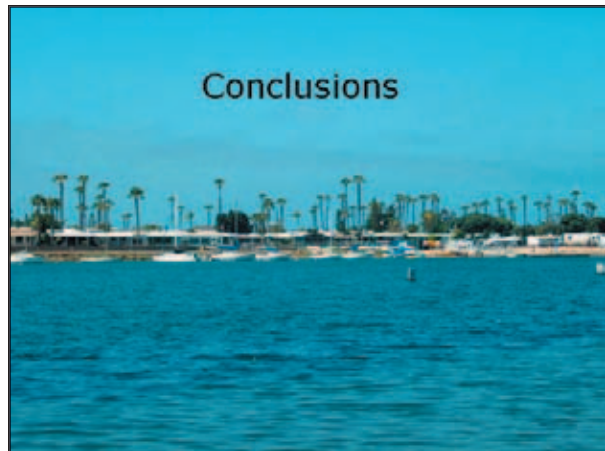
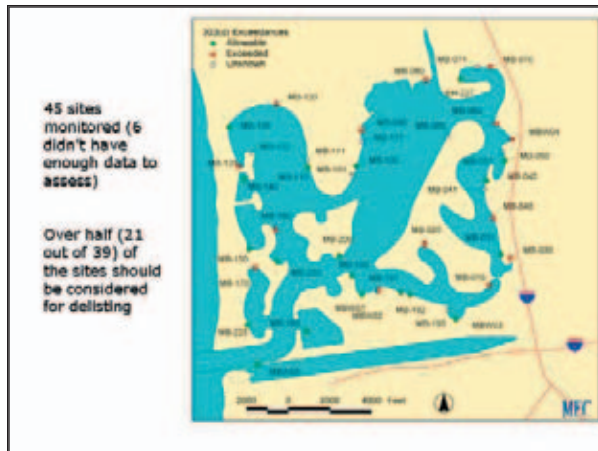
DBS(d) Site Name	Location	Station	Total Analyses	No	Yes	Allowable (Yes/Total)	Percent (Yes/Total)
Missouri Bay	NRW04	NRW04	281	127	154	22	66.2%



Conclusions of Assessment

Beach Area	Location of Impairment	Site Number	Percent (Yes/Total)	Disturbing Consideration
Torrey Pines State Beach	Torrey Pine State Beach at the Mar (Anderson Canyon)	114-300	0.0%	Yes
La Jolla Shores and Pacific Beach	La Jolla Shores at El Paseo Grande	124-300	1.64	Yes
	La Jolla Shores at Camino del Rio	124-300	1.96	Yes
	La Jolla Shores at Volador	124-300	10.71	Yes
	La Jolla Shores at Ave de la Playa	124-300	5.47	Yes
	City Beach at North Park	124-300	20.70	Yes
	South City Beach at Coast Blvd	124-300	0.72	Yes
	Whispering Sands Beach at N. Jolla St	124-300	3.79	Yes
	Windansea Beach at Vista del Mar	124-300	0.00	Yes
	Windansea Beach at Sunset St	124-300	2.50	Yes
	Windansea Beach at Vista del Mar	124-300	0.00	Yes
	Windansea Beach at Panama Ave	124-300	0.00	Yes
	Torrey Pines Golf Club	124-300	1.73	Yes
	Pacific Grove at Grand Ave	124-300	0.00	Yes







Questions and Answers

Q: For both Leisure Lagoon and one of the other sites, it looked like you showed a number of sampling locations within each site. For Leisure Lagoon, for instance, one of the sampling locations had a high number of exceedances of the standard. Have you gone into further analysis of what that means and how to deal with that? How do you justify taking it off the list if you've got ongoing exceedances for specific locations?

Lisa Kay

Basically, it depends on how far away from each other those locations are. That is a policy decision. At this point we are just presenting the information. But, I would like to add that there was a completely different study that looked at sources of bacteria and remediated those sources, and in many instances, sources of bacteria have been remediated or removed in a lot of Mission Bay, and there are ongoing projects to continue that effort.

Q: It does look to be pretty site-specific. When you still have a strong source coming in, and if that data are still accurate, then you probably wouldn't want to delist it.

Lisa Kay

Yes, then you probably would not want to remove it.



“The Hunt for Red *E. coli*” – Bacteria Source Tracking in Lake Darling Watershed

Eric O’Brien

Iowa Department of Natural Resources, Water Monitoring Section

Biosketch

Mr. Eric O’Brien is an environmental microbiologist for the Iowa Department of Natural Resources and University of Iowa. Mr. O’Brien completed his master’s research in Environmental Science at the University of Northern Iowa in May 2003. His primary interest of focus is environmental microbiology, specifically focusing on bacterial source tracking. Before joining the Iowa Department of Natural Resources Water Monitoring Section, Mr. O’Brien also helped coordinate undergraduate water research activities at the University of Northern Iowa. These interests led him to work for the Water Monitoring Section of the Iowa Department of Natural Resources in June 2003. Mr. O’Brien directs most of his efforts toward the ongoing bacterial monitoring of Iowa’s State and County owned beaches as well as tracking of bacterial sources at these beaches.

Abstract



Contamination of Iowa’s surface water by fecal microorganisms threatens human health and results in beach postings that have substantial economic impacts to local communities. The typically high nutrient levels and turbidity in most Iowa surface waters compounds this problem. Lake Dar-

ling, located in southeast Iowa, has been placed on Iowa’s 2002 303(d) list, the list of impaired water bodies, for high levels of indicator bacteria. A Total Maximum Daily Load (TMDL) plan will need to be created for this watershed in the future. Therefore, the state has a vested interest in determining the source of bacteria at the beach and in the lake. The Lake Darling watershed consists of 19.8 square miles, much of which is agricultural (55%). To understand and control fecal contamination problems and to assess human health risks, it is necessary to identify contamination sources and transport pathways. This study used a combination of several source-tracking tools to determine the origin of fecal contamination in Lake Darling and the surrounding watershed. These source-tracking tools included DNA ribotyping, antibiotic resistance analysis (ARA), pathogens analysis and sterols/caffeine/cotinine analysis. By using the libraries created from ribotyping and ARA together, increased discriminatory power was observed compared to each library individually. Additionally, analysis noted pathogens to be present in all tributaries entering Lake Darling during various flow regimes, including low flow conditions, throughout the study. Data from this project have provided insight into areas to target implementation of best management practices to eliminate or control these sources.




Microbial Source Tracking in Lake Darling Watershed

Eric O'Brien
Environmental Specialist Senior
IDNR – Water Monitoring Section



Microbial Source Tracking in Lake Darling Watershed

Eric O'Brien
Environmental Specialist Senior
IDNR – Water Monitoring Section



Funding provided by U.S. EPA through a 319 Nonpoint Source Program Grant to the Iowa Department of Natural Resources.

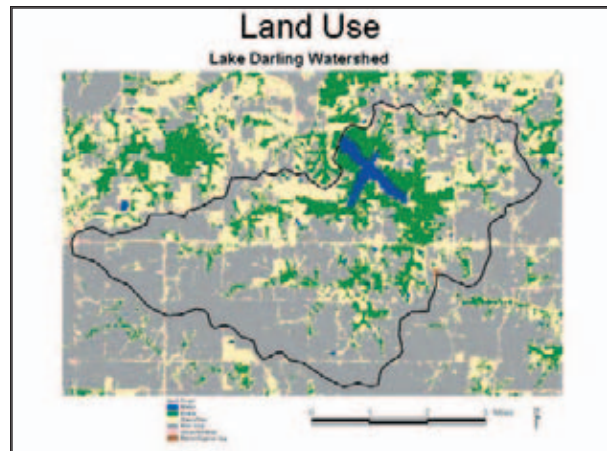
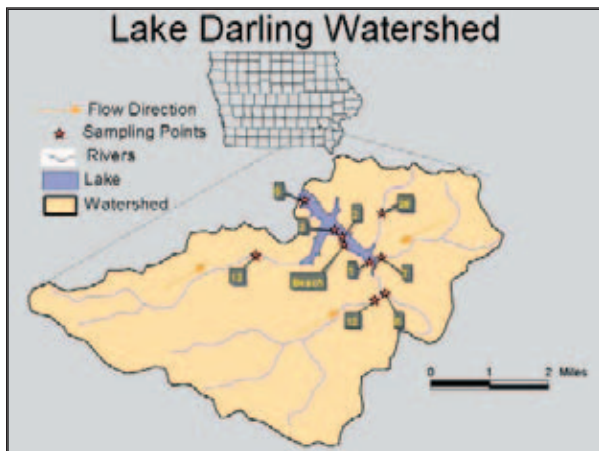



Investigators:
Janice Boekhoff, Nancy Hall, Eric O'Brien, Mary Skopec, Patricia Winokur, Karen Owens



Lake Darling - Background

- On Iowa's 2002 303(d) – Bacteria
- 19.8 mi² watershed
- 55% of land – Rowcrop
- Beach monitored weekly since 1999
 - High levels in April, May, and June
 - Correlate with rainfall events





Source Tracking Methods

- DNA Ribotyping
- Antibiotic resistance analysis
- Fecal coliform & *E. coli* bacteria levels
- Pathogens analysis
- Caffeine and cotinine analysis

Source Tracking Sampling Events

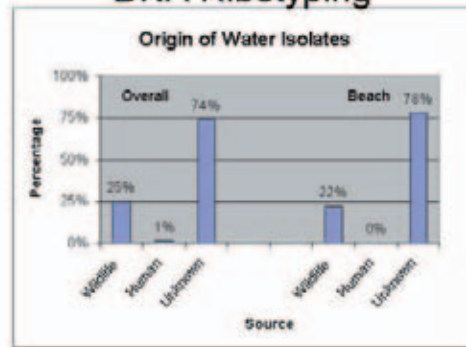
Sampling Date	Flow Regime	Rainfall previous 24 hrs (in.)	Season
6/26/03	Mid-flow	1.4	Summer 03
7/9/03	Mid-flow	0.9	Summer 03
8/6/03	Low-flow	0.0	Fall 03
8/18/03	Low-flow	0.0	Fall 03
9/2/03	Low-flow	0.0	Fall 03
9/15/03	Low-flow	0.3	Fall 03
3/5/04	High-flow	1.6	Spring 04
3/26/04	High-flow	1.2	Spring 04
6/11/04	High-flow	0.6	Spring 04

Fecal Samples

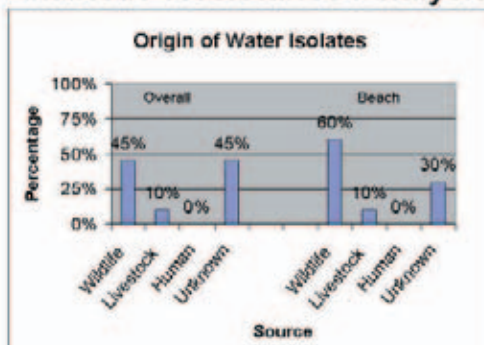
Fecal samples were collected from 5 host classes:

- Cattle
- Swine
- Human
- Goose
- Deer

Results DNA Ribotyping



Results Antibiotic Resistance Analysis



Results

- More unknown sources than known sources suggest the isolate library was either not large enough or not representative of the sources in the watershed
- Future studies are increasing the size of isolate database (especially cattle and swine groups)



Results

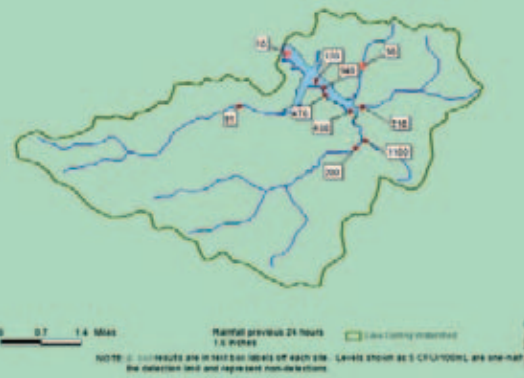
Patterns of High Bacteria Levels

- Higher levels of bacteria were seen after rainfall events
- Sites 7, 8, and 12 (tributaries on south/southeast side of lake) exceeded the bacterial standard 7 of the 9 sampling events
- Lake sites were shown to be high after spring rainfall event on 3/5/04

Lake Darling Source Tracking *E. coli* Results 6/26/03 (CFU/100ML)



Lake Darling Source Tracking *E. coli* Results 3/5/04 (CFU/100ML)



Results Pathogens

- Found *Salmonella* in all tributary sites, at the beach and in all of the lake sites except site 5 (near dam)
- Salmonella* species could not be identified to a particular animal/human group
- Salmonella* species did not correlate with bacteria levels
- No *E. coli* O157:H7 was found anywhere

Lake Darling Source Tracking *E. coli* Results 6/26/03 (CFU/100ML)



Lake Darling Source Tracking *E. coli* Results 3/5/04 (CFU/100ML)





Results Caffeine/Cotinine

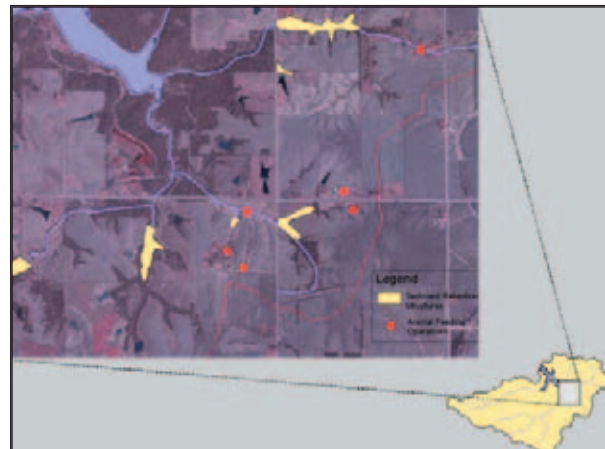
- Caffeine
 - Some caffeine found in site 5 (near dam), but it was just over the detection limit
- Cotinine
 - No cotinine was found in any site
- Caffeine/cotinine results from this project does not indicate human sources

Summary

- Ribotyping, antibiotic resistance and the patterns of high *E. coli* levels in the watershed suggest the sources of fecal contamination are primarily animals present throughout the watershed
- Large number of isolates classified as unknown suggest the library needs more isolates from groups that were not well represented (cattle and swine)
- Caffeine/cotinine results do not indicate the presence of significant amounts of human sources

Outcomes

- Major efforts to control sedimentation
 - Bacteria levels in sediment are very high
 - 9% of land in watershed had sediment control put in during 2004



Outcomes

- Beach exceedances at vulnerable beaches during 2003-2004
 - Statewide rose from 4.5 to 9.5
 - Lake Darling dropped from 5 to 4
 - One of 3 beaches in state to drop

Acknowledgements

- Tony Maxwell and Stan Simmons of the NRCS have collected water and fecal samples and organized the nonpoint source project in the area
- Don Kline and Vance Poulton for assistance in sample collection and use of their boat
- Jeff Hildebrand and Merrill Lucas for their interest, willingness to help and the use of their boat
- Jim Sievers and Paul Brandt for their help with inspecting the sewage lagoons in the area
- The University Hygienic Laboratory (UHL) performed all analyses and reported all results, Mike Schueller and John Miller coordinated the weekly beach sampling and Nancy Hall of UHL provided coordination and expertise in interpretation of the results.



Questions and Answers

No questions.



San Diego Creek Watershed Natural Treatment System

Norris Brandt
Irvine Ranch Water District

Biosketch

(Not submitted)

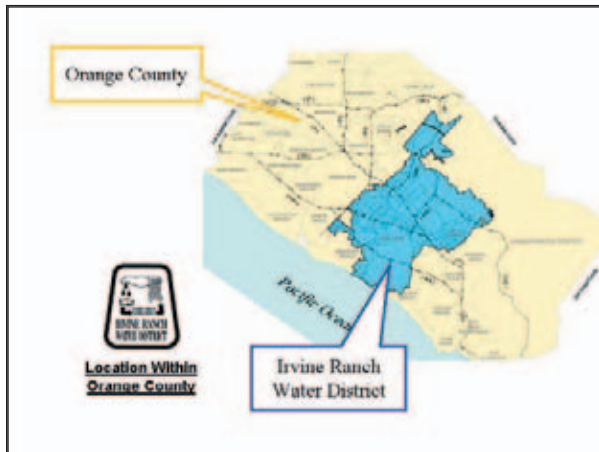
Abstract

(Not submitted)



Irvine Ranch Water District At A Glance

- California Water District
- 20% of Orange County; population 266,000
- Publicly-Elected Board
 - 5 members at large
- Multiple Water Systems
 - Potable Water
 - Irrigation/Recycled Water
- Sewage Collection and Treatment
- Urban Runoff Treatment
- Environmental Stewardship

San Diego Creek Watershed At A Glance

- Primary freshwater source for Newport Bay
- 118 square miles
- City of Irvine and portions of:
 - City of Lake Forest
 - City of Newport Beach
 - City of Orange
 - City of Tustin
 - Unincorporated County
- 4 types of pollutants
 - Nutrients
 - Sediment
 - Pathogens
 - Toxics




Initial "Natural Treatment Site" San Joaquin Marsh

- 320 acres owned and operated by IRWD
- Restoration/enhancement initiated in 1995
- 68 acres of Natural Treatment System Ponds
- Removes about 70% of nitrogen in water pumped from San Diego Creek (75,000 pounds removed last year)
- Removal of 50,000 tons of sediment and 10,000 pounds of phosphorus each year from San Diego Creek desilting basins



Expanding SJM Success to "System" Approach -- Objectives of NTS

- Regional solution to urban runoff
- Treat pollutants from existing urban and natural sources - and new development
- Dry weather (349 days) and "first flush" treatment
- Strategically locate facilities to optimize pollutant removal
- Efficient joint use of public facilities (flood control channels and basins)
- Incremental habitat and open space benefits for NCCP and the watershed

San Diego Creek Watershed Site Map

- 71 Opportunity Sites Evaluated
- Serving New and Existing Communities
- Prioritized by:
 - Effectiveness
 - Availability
 - Cost
 - Constructability
- 31 Sites Proposed for Construction

▲ Type I - Off-line (with quality basins)
■ Type II - In-line (with quality basins)
■ Type III - In-line (with detention within detention basins)
● Site evaluation (subject to NTS Plan)

<h4>Pollutant Removal</h4> <ul style="list-style-type: none">• Pathogens• Nitrogen• Phosphorus	<h4>Pathogen Removal</h4>
<h4>Nitrogen Removal</h4>	<h4>Phosphorus Removal</h4>

Types of Wetlands

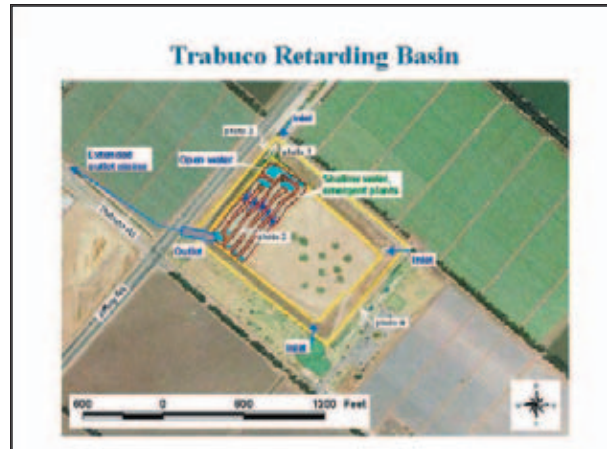
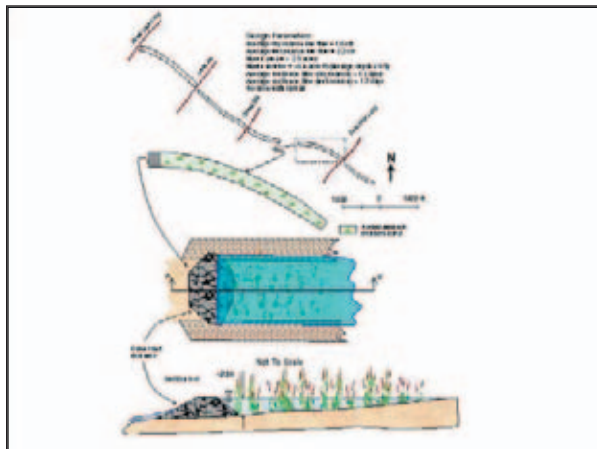
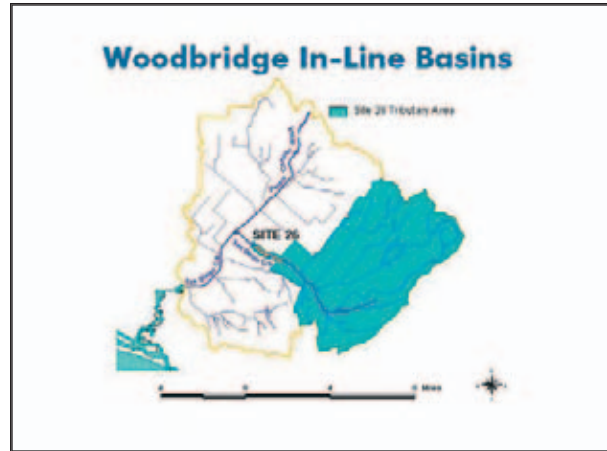
- Type I -- Offline
- Type II -- Inline
- Type III -- Colocated Within Detention Basins

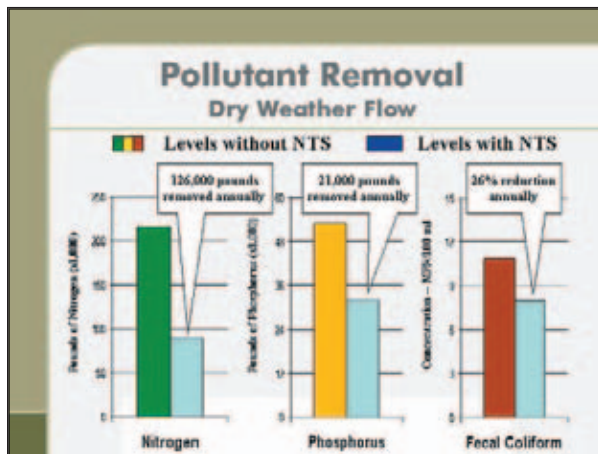
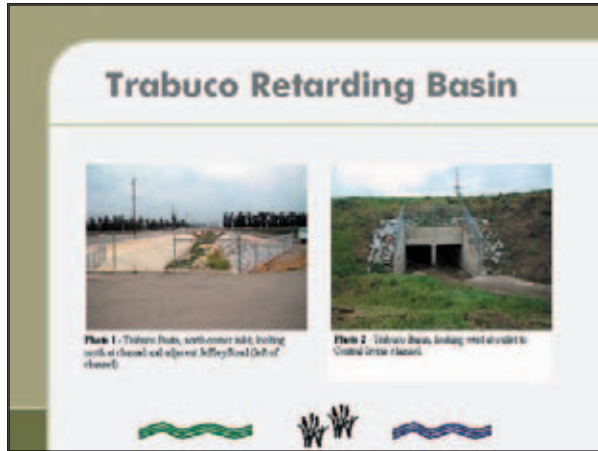
Type I -- Offline

Type II -- Inline

Type III -- Colocated





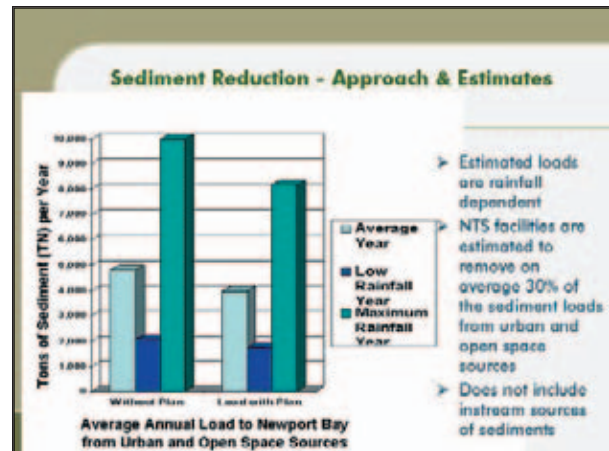
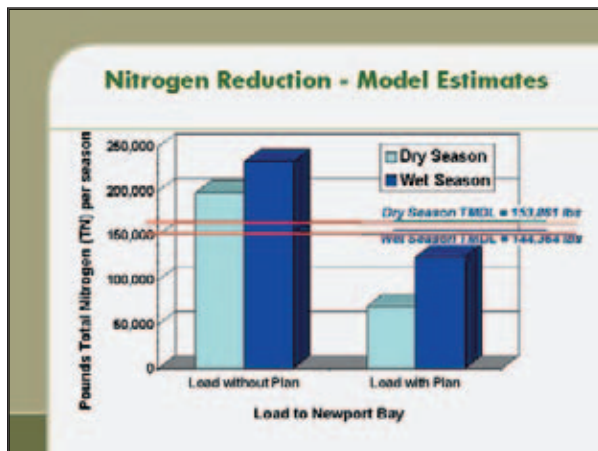


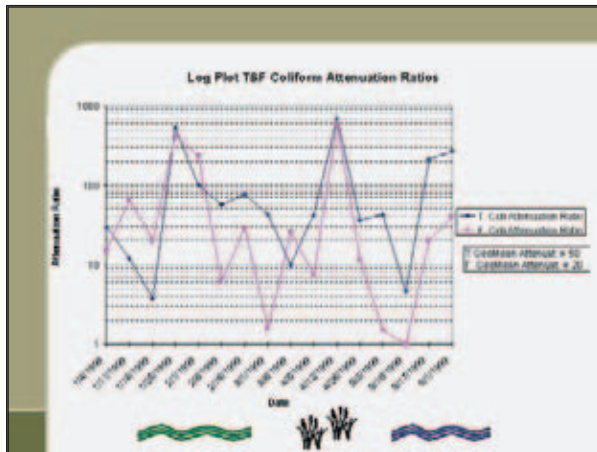
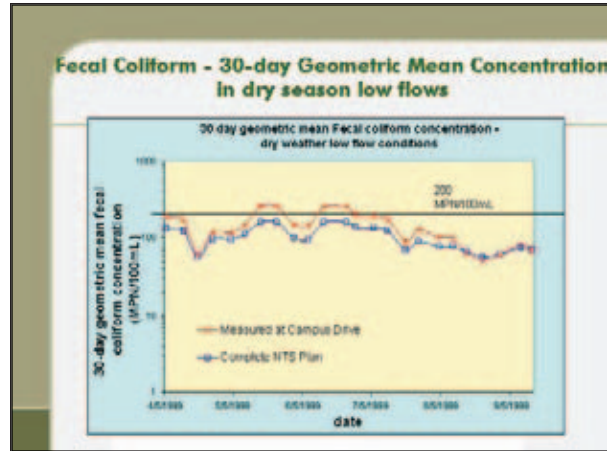
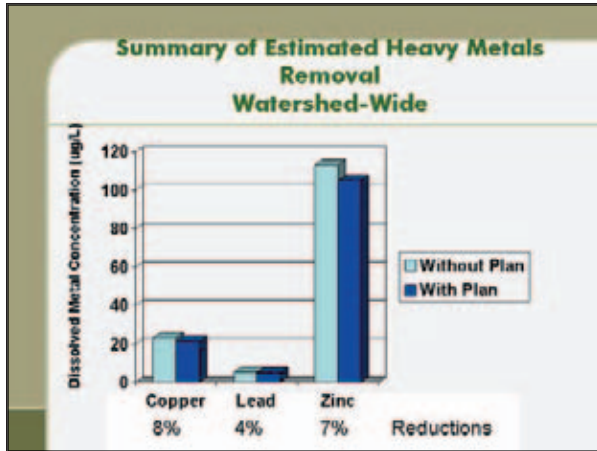
Expected Effectiveness of the NTS Plan

Flow regimes for which the pollutants of concern have been modeled

Constituent	Dry season low flow	Wet season low flow	Storm Events
Sediment			X
Total Nitrogen	X	X	
Total Phosphorous			X
Pathogens	X	X	X
Metals			X
Selenium	X	X	
Pesticides and Toxics			

X = TMDL set and modeled





NTS and Pathogens

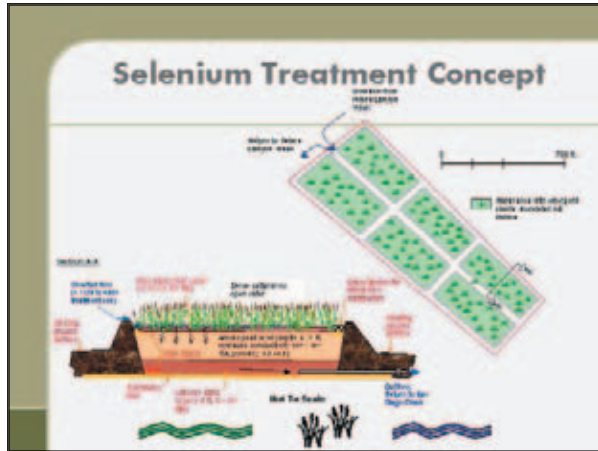
- Experience shows modest removals
- Monitoring as sites are constructed
- Adaptive management--modify or remove unsuccessful sites
- Potential downstream reductions due to removals of other contaminants (ie, nutrients and sediment)

Selenium Sources

- Selenium is from natural sources distributed throughout the watershed
- It accumulated over the centuries in the Historic Swamp of the Frogs (indicated at left)
- Selenium was naturally treated in this historic wetland for thousands of years
- It is now being released through seepage into flood control channels and groundwater dewatering operations in the area

Site 67 - Cienega Site (Selenium treatment)

- Located in Peters Canyon Wash downstream from "Swamp of the Frogs"
- Design partially based on similar operating wetlands in San Francisco Bay
- Treatment concept mimics historic natural processes: sorption in organic rich soils under anoxic conditions
- Pilot treatability studies will be conducted



Ultimate NTS Buildout Costs

- **Construction** **\$41 million**
- **Annual Operation and Maintenance** **\$2 to \$3 million**

***Costs will be incrementally incurred as facilities are constructed.**

Buildout Cost Recovery Model

- **Construction Costs**
 - Developer-donated facilities **55%**
 - State/federal grants **35%**
 - IRWD bond financing **10%**
- **Operation and Maintenance Costs**
 - IRWD water bills **100%**

Schedule

- Master Planning **Spring 2001 - Spring 2004**
- Environmental Review **Fall 2002 - Spring 2004**
- Phase I Design **Fall 2004**
- Phase I Construction **February 2005 - July 2005**

Hot Topics

- **O&M Versus Habitat Preservation**
- **Waters of the US**
- **Bioaccumulation of Toxics**
- **Mosquito Control**
- **Regulatory Framework (TMDLs, MS4, NPDES, Waters of US, Endangered Species)**

Cooperative Effort


- **County of Orange**
- **Watershed Cities -- Irvine, Lake Forest, Newport Beach, Orange, Tustin**
- **IRWD**
- **Landowners**
- **State Water Resources Control Board**
- **Regional Water Quality Control Board**
- **CDFG, USFWS, Corps, EPA, USBR**
- **Coastal Conservancy**
- **Environmental Groups**
- **Residents**



More Information

www.naturaltreatmentsystem.org

Download: **Draft NTS Master Plan**
 Draft EIR
 Brochure
 More . . .





Questions and Answers

Q: Do the subterranean filters that you were talking about do a better job at removing bacteria?

Norris Brandt

No, it's actually really focused on nutrients (nitrogen, specifically) and selenium. I don't know how well it removes bacteria. We didn't really look at pathogens, because we were so focused on the other contaminants. But it would be interesting to check to see if that does occur.

Q: During storms, do those structures get destroyed? I understand that you're in a flood-control structure, so how do you deal with storms and the wet weather?

Norris Brandt

We expect the small rock weirs to be blown out. It's a small volume of coarse sediment that's going to be in the channel. But those are the only ones that are going to be destroyed during that period. Remember, we had the detention basins, and for those, the water rises but it does not flow at a high velocity. So, it rises but does not kill anything, and then it drops back down within about 72 hours at the most.

Q: So, there should be some build-up in the sediments. Do you remove those sediments prior?

Norris Brandt

Yes, there is a whole program that is part of our operation and maintenance (O&M) for that, testing the sediments and making sure we know where we can get rid of them. We are already using some of those sediments for construction materials because it is safe to do so.

Thursday, October 14
1:20 p.m. – 3:00 p.m.

Concurrent Track I:
Identifying and Solving Beach Water Quality Problems
Session Seven:
Remediation Approaches



California's Clean Beach Initiative

Mark Gold, D.Env.

Heal the Bay

Biosketch

Mark Gold, D.Env., is Heal the Bay's Executive Director. Heal the Bay is an environmental group dedicated to making Santa Monica Bay and Southern California coastal waters safe and healthy for people and marine life. Dr. Gold's extensive work with water quality and coastal natural resource topics ranges from sewage treatment, contaminated sediments, legislative and environmental education issues to urban runoff, contaminated fish and wetland restorations. In 1996, working in conjunction with the Santa Monica Bay Restoration Project and the USC Medical Center, he was a co-author of the first epidemiological study of swimmers in runoff-polluted water. He also has co-authored several stormwater, contaminated fish and beach water quality bills and ordinances, and he created Heal the Bay's Beach Report Card®. He is a vice-chair of the Santa Monica Bay Restoration Commission, sits on the State Water Board's Clean Beach Advisory Group and served on the EPA's Urban Wet Weather Federal Advisory Committee. Dr. Gold also was appointed to the California Ocean Trust. Dr. Gold has bachelor's and master's degrees in biology from UCLA, and he received his doctorate from UCLA in environmental science and engineering in 1994.

Abstract

The Clean Beach Initiative was authored by Assemblywoman Fran Pavley, working together with Heal the Bay, in response to California Assembly Bill 411, the state's beach bathing water standards bill. AB 411 requires monitoring of California's most frequently visited beaches. The resulting monitoring demonstrated that there were numerous beaches with frequently high fecal indicator bacteria densities. Reducing bacteria densities, beach closures, and health warnings at California's most polluted beaches became a high priority for funding. This innovative initiative allocates \$80 million to clean up the state's most polluted beaches and to fund rapid indicator research. The major successes have been with simpler projects, such as the nearly 20 dry weather diversions from storm drains into sewers that are now in place. Other funds have been allocated for dry weather runoff mini-treatment plants, such as the one at Moonlight Beach in Encinitas. However the challenges of source identification and abatement have proven too difficult a task at some beaches and water quality problems at many of these locations remain unsolved. Reducing fecal bacteria densities at enclosed beaches with poor water circulation has proved to be particularly difficult. Unconventional bacteria reduction technologies such as treatment wetlands and mechanical water circulation enhancement devices are being considered for funding, but few have been implemented to date. Other regions may learn from California's experiences trying to comply with legislature-mandated project design and construction deadlines, and using a Clean Beach Advisory Group made up of health and water quality experts, to provide project approval, enhancement and monitoring recommendations to California's funding decision making body, the State Water Resources Control Board.



California's Clean Beach Initiative

Mark Gold, D. Env.



Overview of CBI

- Total of \$78M dedicated to State's most polluted beaches
- Capital projects to reduce beach fecal pollution
- \$1.5M for Rapid Indicator Measurement research
- First large appropriation for beaches in CA

Genesis of CBI

- California's beach standards, monitoring & public notification law (AB-411 Assemblyman Howard Wayne)
- Statewide grading system – Heal the Bay's Beach Report Card
- Political Environment:
 - Governor Wilson's Administration
 - Bipartisan support
 - Support from CA Health Departments, SD's Councilperson D. Frye

Genesis of CBI

- AB-411 data collection started in 1999
- Huntington Beach closure in 1999
- Prop. 13, March 2000 - \$2 billion
 - Phase I CBI – Budget bill 2001 \$3.2 million
- Prop. 40, March 2002 - \$2.6 billion
 - Phase II CBI – Budget bill 2003 – \$4.6 million

Key Elements of CBI

Statewide priority list of projects

- Statewide monitoring and grading assisted in prioritization
- \$\$'s assigned to specific beach/project
- Project Criteria
 - Demonstrated problem beach
 - Capital project
 - Reduction in Postings/Closures
 - Durability, O&M for 20 years
 - CEQA Compliance
 - ***Monitoring Program

Key Elements of CBI

Significant funds

- Project funding ranges from 50k to \$3 million
- Typical funding:
 - Diversions – \$0.5 - \$1.5 million
 - Dry-weather treatment - \$0.8 - \$1.5 million
 - Source ID/Feasibility Study – \$0.2 – \$1 million



Key Elements of CBI

Technical Review Panel (CBTF)

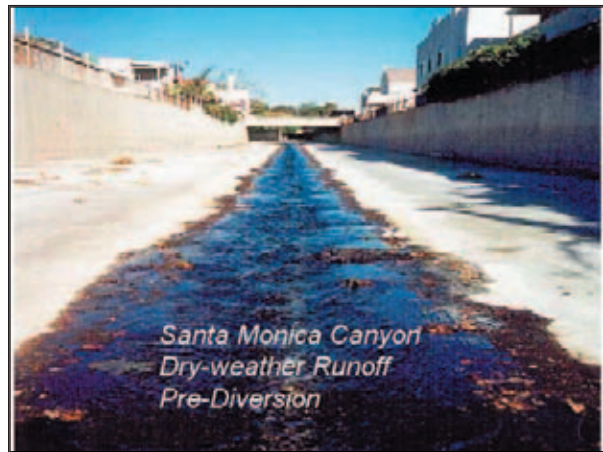
- Expert members representing the coastal beach public health community
 - Local health agencies
 - POTWs
 - Researchers
 - Environmental group water quality scientists
- All projects must be reviewed by CBTF

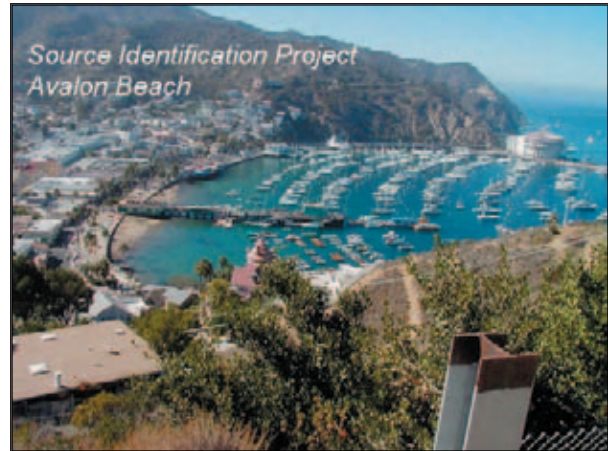
Drawbacks to CBI

- Capital projects only
 - Largely limited to beaches with known sources
 - Beaches where capital abatement projects are difficult:
 - Enclosed beaches
 - Beaches receiving natural freshwater discharge
- Local sponsor must take lead role
 - Some Prop. 13 project funds were not awarded – *Why?*

Effective Projects

- Storm drain diversions
 - 38 dry weather diversions
- Storm drain treatment plants
 - 8 dry weather treatment plants
- Sewer upgrades
 - 10 sewer collection system projects





Problematic Projects

- Enclosed beaches
 - Unidentified sources
 - low circulation
- CEQA-related issues
- Indifferent local agencies



Conclusions

- CBI is an effective model for use nationally
 - Project criteria
 - Technical review: Project proposal to Completion
 - Monitoring: Pre and Post
- Is \$78 million enough?



Questions and Answers

Q: I'm from the San Diego area, and we've had a lot of talk in the past and today about this watershed concept and how what's going on in the watershed is driving beach water quality. So, can you talk a little about some limitations of Clean Beach Initiative (CBI) projects to fund upstream inland restoration projects, as opposed to being focused on cleaning up after the fact?

Mark Gold

Yes, clean beaches projects have been focused more on end-of-pipe solutions. The reality is that if it's a small, concrete-lined channel, and there are a lot of those that are causing pollution problems at beaches, those are the ones that are more easily solved. Upstream pollution abatement projects and source identification projects cost a lot of money, and the incremental improvement for any one project doesn't quite meet the threshold that the legislature passed, which is that you have to have a measurable improvement in the reduction of beach postings and closures. So, because of that, it has been a problem. So, these other funds from these bond measures that are sitting up there at the State Water Resources Control Board are a much better source of potential funds (i.e., Proposition 40, Proposition 50) to reduce upstream sources.

Q: Baby Beach in Dana Point Harbor doesn't have a whole lot of people entering the water. There are a lot of people there, but they are walking between the Ocean Institute and the marina. What would you think about eliminating the beach and turning it into an intertidal rocky zone with field trips and that type of stuff with the creatures that could be using the intertidal rocky zone?

Mark Gold

I think local beneficial use determinations need to be made by the people who live there. For me (running a Santa Monica Bay group), giving an opinion on that would be out of place. That is something that the community in Orange County needs to work with their local regional board and see what happens if there is dedesignation of that direct Recreational 1 use. But, it's not appropriate for me to weigh in on that.



EPA's Clean New England Beaches Initiative and Flagship Beaches

Matthew Liebman, Ph.D.

U.S. Environmental Protection Agency, Region 1

Biosketch

Matthew L. Liebman, Ph.D is an Environmental Biologist at the U.S. Environmental Protection Agency New England regional office in Boston, MA. Dr. Liebman received his B.A in Biology in 1980 from Carleton College in Minnesota and a Ph.D. in Ecology and Evolution from the State University of New York at Stony Brook in 1991. Since 1990, he has worked at the EPA office in Boston as a project manager and scientist in the National Estuary Program, dredged material disposal and monitoring program, and as a water quality specialist. He is the regional coordinator for EPA's BEACH program, nutrient criteria initiative and national sediment inventory. At EPA, Dr. Liebman has conducted or been involved in research efforts in dredged material disposal site monitoring, and impacts of nutrients and bacteria on water quality in streams, coastal waters and beaches.

Abstract

Co-authors: David Turin, Larry Macmillan, Chris Ryan and Warren Howard, EPA Region 1

Taking advantage of the Federal Beach Act, EPA New England launched an initiative in 2002 to enhance our ability to protect public health by reducing beach closures or advisories, while establishing consistent statewide monitoring and assessment programs. In addition to providing grants for monitoring, assessment and public notification at coastal beaches, the goals of the initiative are to

control sources of fecal contamination from storm water and non-point pollution sources; establish "Flagship Beaches" in each of the five coastal New England states; promote high quality monitoring and assessment methods and new technologies; promote information sharing among beach managers; and involve the public and communities in education, monitoring and advocacy. The Initiative raises the profile of coastal beaches as important recreational resources by enhancing existing EPA and state programs with increased financial and technical assistance. Since 2001, the number of closure days for coastal and inland beaches has declined from 2400 to 1900 in 2003. We attribute this decline to improvements in beach management and monitoring and actual improvements in water quality due to investments in remediation. Nevertheless, one in five beaches in New England experiences a closure at some point during the summer.

In New England, the major cause of closures are storm water discharges to beaches located in urban areas, especially at beaches in or near Boston Harbor, Massachusetts, Greenwich Bay, Rhode Island, and western Long Island Sound, Connecticut. Many storm water pipes discharge directly onto the beach, with little or no treatment; some storm water is contaminated with human sources of bacteria, from illicit and improper connections, or from leaks in the systems. This presentation will highlight examples of these problems, and discuss strategies to remediate these difficult problems at Flagship and other beaches in New England.



EPA's Clean New England Beaches Initiative and Flagship Beaches

- Matthew Liebman, EPA New England
- With David Turin, Larry Macmillan, Warren Howard, Chris Ryan, Allison Watanabe and Daberat Perez-Rivera



EPA National Beaches Conference, San Diego, CA October 14, 2004



Clean New England Beaches: It's a Shore Thing

- **Goal:** To protect public health by reducing beach closures in New England, with appropriate and consistent, high quality monitoring
- **Why:** Coastal and Freshwater beaches were closed or posted more than 700 days in summer of 2001
- **How:** Federal Beach Act provided funding (nationally over \$10 million in FY02) to Coastal States for monitoring, assessment and public notification activities

EPA National Beaches Conference, San Diego, CA October 14, 2004



Green Harbor, New London, CT



EPA National Beaches Conference, San Diego, CA October 14, 2004



The BEACH Act of 2000

Beaches Environmental Assessment and Coastal Health Act (Section 406 of the Clean Water Act)

- **Consistent Criteria and Standards for Coastal Recreational Waters**
 - States need to adopt EPA criteria (i.e. enterococci) for pathogen indicators within 42 months (2004)
- **Performance Criteria for Beach Monitoring, Assessment and Notification (The Beach Guidance Document)**
- **Grants to develop and implement State Coastal Beach Monitoring Programs**
 - State programs must be consistent with Performance Criteria
 - A funded mandate

EPA National Beaches Conference, San Diego, CA October 14, 2004



1. Provide Federal money to develop and implement state beach programs

- Ensure states and municipalities monitor water quality at beaches, assess sources of pathogens (perform sanitary surveys), and notify the public of water quality conditions consistent with EPA's nine performance criteria -- all states have met performance criteria
- Press events in all five coastal states usually at Flagship Beaches, with Gov. Whitman, R. Varney and other officials to give away about \$1.1 million granted annually to five coastal states

EPA National Beaches Conference, San Diego, CA October 14, 2004



Press events at Flagship beaches




EPA National Beaches Conference, San Diego, CA October 14, 2004






2. Control non-point and storm water pollution sources that contribute to beach closures

- Eliminate human fecal contamination of storm water
- Major component of NPDES storm water permit -- 6 minimum controls include elimination of illicit discharges -- will look closely at Phase 2 permits for communities with history of problems
- Targeted enforcement where appropriate
 - Keeping track and evaluating discharges to beaches in Massachusetts (Boston Harbor/Quincy, Newport RI and Fairfield County, CT)
- Technical assistance
 - Carson Beach in Boston Harbor
 - Storm water permit training sessions
 - Scheduling another soup to nuts workshop in early 2005
- Put the pressure on local officials to reduce sources of bacteria at beaches with letters


 EPA National Beaches Conference, San Diego, CA October 14, 2004

Keeping the pressure on communities with chronic beach closures

- "Recently it has come to our attention that the Town Beach has been closed to swimming on eight days this summer due to elevated levels of indicator bacteria. We strongly believe that chronic beach closures should be investigated, identified and corrected. One of the goals of EPA's Clean New England Beach Initiative is to assess the sources of bacteria that cause closures, and to ensure that sources of fecal contamination discharged to bathing waters are eliminated....."
- "Enclosed with this letter is some information that may assist you with your efforts to identify and eliminate the sources of bacteria that are causing those beach closures....."
- "We would like to know what additional efforts you are making to ensure that people can swim in clean water at ...your town's beaches.."
- *Sincerely, Robert Varney, Regional Administrator*


 EPA National Beaches Conference, San Diego, CA October 14, 2004

Stormwater, the major culprit








 EPA National Beaches Conference, San Diego, CA October 14, 2004


3. Establish "Flagship Beaches" with States


- Relatively high use, diverse or low income population, history of beach closures, potential for improvement, selected with states
- Highlights good beach management, monitoring practices
- Commitment to target resources and track improvements

CT Rocky Neck SP, Ocean Beach Park (New London)
 ME Ferry Beach SP, Wells
 NH Hampton Beach SP
 MA Salem, Wollaston (Quincy), Provincetown
 RI Goddard SP, Warren, King Park Swim Area




 EPA National Beaches Conference, San Diego, CA October 14, 2004


Map of New England Beaches




 EPA National Beaches Conference, San Diego, CA October 14, 2004

Wollaston Beach Quincy, MA


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Warren Town Beach, RI



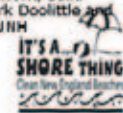
EPA National Beaches Conference, San Diego, CA October 14, 2004



4. Promote high quality monitoring and assessment technologies

- Ensure state laboratories have an established quality assurance management plan, and an approved quality assurance project plan (QAPP) – all states have approved QAPPs as part of performance criteria and several have established regional laboratory networks (e.g. MA and ME)
- Promote research on rapid indicator methods (RI, MA, CT) – funded a RARE project at University of Rhode Island
- Promote Microbial source tracking (MST) to identify potential sources of fecal contamination, establish a network, build capacity at EPA New England – see talk by Mark Doolittle, Jack Paar of EPA (Q-PCR) and Steve Jones of UNH (Ribotyping)

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5. Promote information sharing among beach managers

- Hold "quarterly" meetings with state beach managers
- Topics have included
 - Comparison of beach monitoring programs, including indicator bacteria thresholds, sampling programs, re-sampling and posting decisions
 - Database of beach closures
 - Microbial source tracking methods
 - Beach location data

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Beach closure days in New England

State	2001	2002	2003
CT	195	97	200
ME	16	5	0
MA	647	503	559
NH	0	0	3
RI	530	76	380
Totals	1388	681	1142

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Clean New England Beaches web site



Sanitary Surveys

- Sanitary survey training conducted by all states including one with NSSP in RI
- Sanitary surveys conducted at Flagship and other beaches by states
- EPA staff with interns, conducted preliminary sanitary surveys at Flagship and other beaches to assist municipal health officials

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6. Involve and educate the public and municipalities

- Develop educational material to encourage citizens to bring storm water discharges to local governments attention – fact sheets and web page: www.epa.gov/nr/epa/beaches
- Develop material targeted at municipalities (NPDES permits) to control non-point source pollution affecting recreational waters

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Do people really read the signs?



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Results of Sanitary Surveys

- Most surveys are at coastal beaches in urban or suburban eastern Massachusetts or Rhode Island
- **Major problem is that natural streams have been integrated into the stormwater infrastructure, so point or nonpoint sources are not attenuated**

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Source of bacteria -- a golf course with discharge directly downstream with little attenuation



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Ask town for information, they know about leaks in sewer pipe



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When conducting sanitary surveys, it's important to sample in streams where people swim



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Beach in Beverly, MA, north of Boston

- Small neighborhood beach with moderate use by families in summer
- About 1 closure per year, geomean about 20
- Three storm water outfalls, sampled by local watershed organization finds consistent counts > 1000 enterococci
- Most of town is sewered

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Topographic map of Beverly, MA



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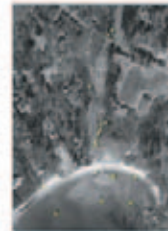
Beverly, MA Beach



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Aerial photo of Beach with sampling stations



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Culvertized stream and sampling large outfall at beach



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Another beautiful Beach in Beverly, MA

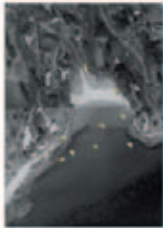


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Aerial and shoreline view of this beach



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Conference, San Diego, CA

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But there are risks at this beach



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Sampling the outlet of a pond discharge to this Beverly Beach – it's good to measure conductivity and salinity of discharges



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October 14, 2004



Findings from Beverly Beach

- Sources – upstream duck pond, with little attenuation by culvert
- Enterococci levels related to rainfall, and to antecedent dry duration
- Tidally restricted salt marsh perhaps promotes some resuspension and environmental growth
- High tide values are higher

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Next steps, challenges and conclusions

- Inform town of results
- Provides information for more targeted sampling
- Consider habitat restoration
- How do you re-integrate the natural courses of streams to provide some attenuation?
- Were "working hard" to reduce beach closures, even coming in on Saturdays!

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October 14, 2004



Some nice beach views from Good Harbor Beach, Gloucester, MA



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Conference, San Diego, CA

October 14, 2004





Questions and Answers

Q (Stephen Jones, University of New Hampshire): One of your last statements was about opening salt marshes and improving tidal flushing, and maybe improving water quality. There is some evidence in New Hampshire where they have been doing a lot of salt marsh restoration, right next to the beaches. During my presentation this morning I showed how we were looking at our beaches and they all have these outlets from the salt marshes. In a couple of instances they have increased the size of the culverts and the flushing in and out of these salt marshes, and the water quality has decreased. So, it may not be as straightforward as it seems. It seems right what you are saying, but we are going to be taking another look at this because they are going to be doing another salt marsh restoration at one of the beaches this spring, and in the upcoming year we are going to be doing some source tracking and microbial work. But be aware that it may not be as straightforward as it seems.

Matthew Liebman

Every place is site-specific, but I'm wondering if in those cases in New Hampshire the levels of bacteria are probably much lower than what we're finding in the Boston area. So, when you say you see a decrease in water quality, it could be a matter of scale.

Q: Yes, but the mechanisms by which this happened—we are not sure what is going on. So, it would be interesting to find out.

Matthew Liebman

We deal with people who protect wetlands all the time, and there is a major issue because people are always complaining about the salt marsh and the wetlands contributing the sources of bacteria to their beach. And, our coastal wetlands people kind of resent that because it implies that we should not protect the wetlands as much. So, it's important to remember that healthy functioning wetlands appear to contribute only small amounts of bacteria to coastal waters.



The Effectiveness of Spatial Distribution Studies in the Development of Successful, Cost-Effective, Targeted Remediation Efforts

Julie Kinzelman
City of Racine

Biosketch


Julie Kinzelman is a microbiologist for the City of Racine Health Department where she has 14 years experience in recreational water quality monitoring and research. Dr. Kinzelman received a BS in Medical Technology from the University of Wisconsin - Parkside, a MS in Clinical Laboratory Sciences from the University of Wisconsin - Milwaukee, and is a Ph.D. Candidate (2005) in Public & Environmental Health at the University of Surrey (Guildford, UK). Dr. Kinzelman is the principal investigator or co-investigator on research initiatives funded by the National Institute of Health, S. C. Johnson Fund, Wisconsin DNR, and Wisconsin Department of Health & Human Services. Her current research activities focus on using public health based monitoring programs to assess the interaction of coastal processes contributing to recreational water quality advisories.

Abstract

An interdependent relationship exists between localized sources of contamination and coastal processes. Both direct and indirect sources of contamination if provided with a suitable mechanism of transport, such as run-off due to rainfall or wave action, can negatively impact surface water quality. An unacceptable amount of swimming advisories over the course of several years prompted Racine, Wisconsin to conduct scientific studies to detect and remediate point and non-point sources of contamination impacting the adjacent Lake Michigan coastal waters. A storm sewer outfall, previously identified as a significant source of *Escherichia coli* and other bacterial indicators, now is pretreated and discharges first-flush storm water (during rainfall events) to a series of infiltration/evaporation beds and incorporates a constructed wetland to provide further filtration. Beach sands are now maintained by mechanical grooming equipment in such a way that the bacterial density is significantly decreased, effectively reducing the number of dry weather advisories previously encountered at this site by 30%. In Racine, beach management strategies are ongoing and continually re-evaluated in light of new research findings. Cost-effective remediation steps have been implemented to reduce the bacterial burden on adjacent surface waters and hence the risk of contracting disease through swimming-related activities. The development of site-specific targeted remediation efforts benefits both public and environmental health.



The Effectiveness of Spatial Distribution Studies in the Development of Successful, Cost-Effective, Targeted Remediation Efforts



Julie Kinzelman
City of Racine Health Department



CAUSE OF FREQUENT ADVISORIES UNKNOWN

ANNUAL ADVISORY EVENTS

YEAR	SITE NORTH	SITE ZOO	BEACH REASON (DAYS)
1994	6 / 6%	21 / 26%	04
1995	51 / 59%	42 / 48%	87
1996	6 / 6%	2 / 2%	95
1997	18 / 19%	30 / 32%	83
1998	16 / 16%	4 / 4%	99
1999	16 / 16%	19 / 20%	94
2000	62 / 68%	39 / 41%	94
2001	17 / 20%	21 / 26%	84
2002	27 / 31%	23 / 25%	87
2003	31 / 32%	25 / 27%	95

* Racine switched from a fecal coliform to an *E. coli* standard in 1999.

NORTH BEACH



- No consistent pattern to advisories
- Not significantly correlated to rainfall
- No combined sewer system
- Occurred dry weather



**Point Source (Direct) Pollution:
English Street Storm Sewer Outfall**

- COLLECTING AREA - ENGLISH STREET STORM SEWER





EVALUATION OF WET WEATHER EVENTS

- 1999 Plumbing Study
- Sampling of English Street Outfall discharge during storm events
- Weekly sampling of Root River @ several points throughout the watershed

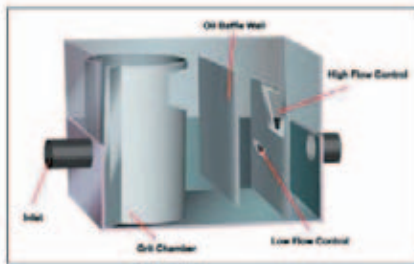


Step 1 - Outlet Relocated



Vortechs System

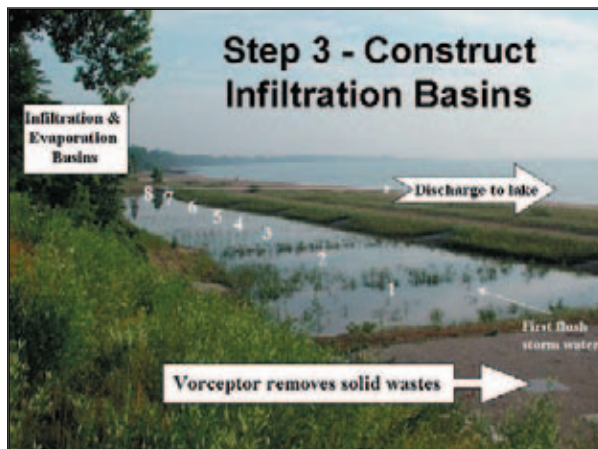
An EPA award-winning design that efficiently removes contaminated sediment, floating oil and debris from surface runoff.



Step 2 - Chamber Installation



Step 3 - Construct Infiltration Basins



Efficacy of Outfall Remediation

E. coli Densities During Rain Events - Vortechs System [First Flush] vs. English Outfall					
Date	IE East Discharge MPN/100 ml	IE West Discharge MPN/100 ml	EO Discharge	Dilution	PPT (in.)
6/26/2003	23870	45110	Not Flowing	1:100	0.01
7/3/2003	18100	Not Collected	22600	1:1000	0.32
8/1/2003	201400	Not Collected	63100	1:100	0.15




**Comparison of *E. coli* Densities
Retention Basin vs. Overflow Outlets**

Date	ORP	IBP	Temp. (ORP/IBP)	PPT-24hrs	Dilution
5/5/2003	1203	161	Not Taken	0	1:10
5/29/2003	210	74	63/59	0.35	1:10
6/5/2003	120	310	58/59	0	1:10
6/12/2003	145	04	60/60	0	1:10
6/19/2003	410	98	68/68	0.14	1:10
6/26/2003	12996	24192	74/73	Trace	1:10
7/2/2003	400	631	82/78	0	1:10
7/10/2003	630	300	68/72	0.03	1:100
7/17/2003	243	259	69/79	0	1:10
7/24/2003	85	85	Not Taken	0	1:10
7/31/2003	52	1	71/81	0	1:10
8/7/2003	521	1	72/83	0	1:10
8/14/2003	455	10	83/81	0	1:10
8/21/2003	504	31	78/84	0	1:10
8/28/2003	880	41	Not Taken	0	1:10
9/2/2003	921	250	72/60	0	1:1
MEAN	458	156	Without 6/26/03		



Water Sedge (*Carex aquatilis*)



- 300% increase in height (10 cm to 31.2 cm)
- Increased in number (50 plants to 67 plants)
- Provided bank stabilization
- Phase II planting (9/14/04) added 100 additional plants





Non-Point (Indirect) Pollution



- 100s to 1000s of seagulls
- Provide a fecal burden
- Potential health risks???

City Ordinances Attempt to Address Indirect Sources of Fecal Contamination



Compliance?

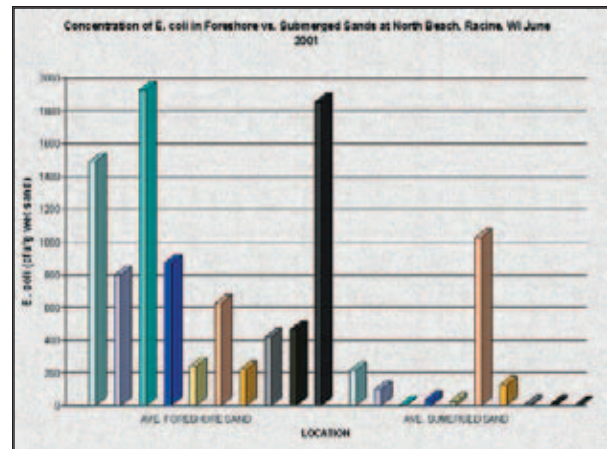


Host Sourcing Studies Are Under Way



Distribution of *E. coli* in Beach Sands

- *E. coli* concentration highest in beach sands
- Backshore sands had little or no *E. coli* (per gram dry weight)
- Highest levels of *E. coli* were detected between the lifeguard stands & the berm crest
- Continually wetted sands had the highest concentration of *E. coli*. [Distance to groundwater]
- Partitioning of core sediments showed a decrease in *E. coli* concentration up to an order of magnitude for every 5-cm increment below the surface

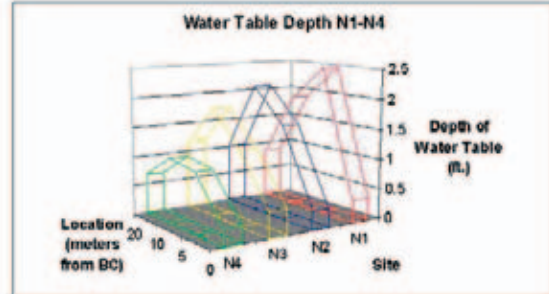




Grade/Slope of Beach



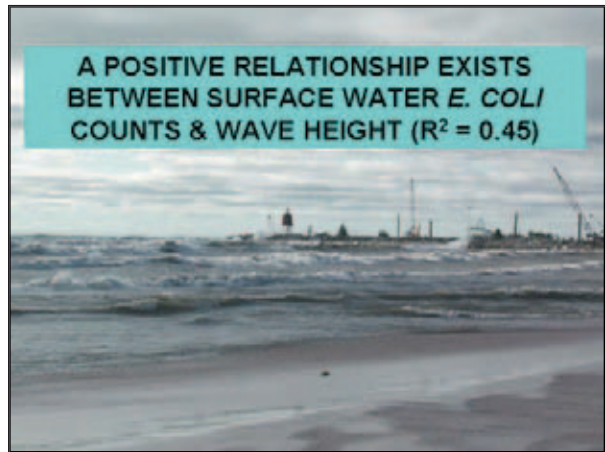
Depth to water table influenced concentration of *E. coli*



CORE PARTITIONING *N2 – Wet Strip Behind Lifeguard Stand*

DEPTH	MASS	WET/DRY	DILUTION	EC MPN/100 ML	EC/G
0-5 cm	43.2	Wet	1:100	11370	263.2
6-10 cm	52.2	Wet	1:100	5560	106.5
11-15 cm	41.3	Wet	1:100	1730	41.9
16-20 cm	36.3	Wet	1:100	100	2.8
21-25 cm	48.9	Very Wet	1:100	<100	0

A POSITIVE RELATIONSHIP EXISTS BETWEEN SURFACE WATER *E. COLI* COUNTS & WAVE HEIGHT ($R^2 = 0.45$)



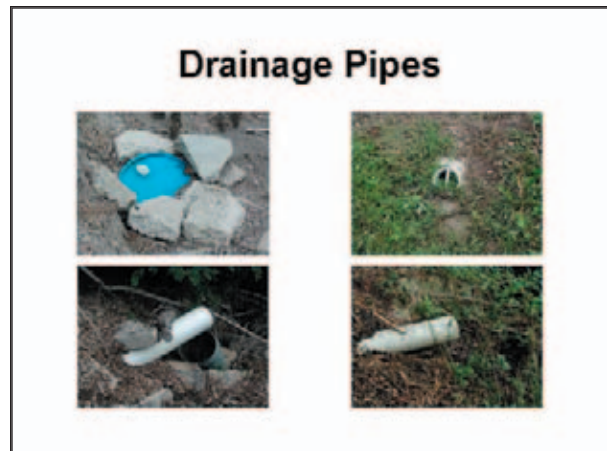
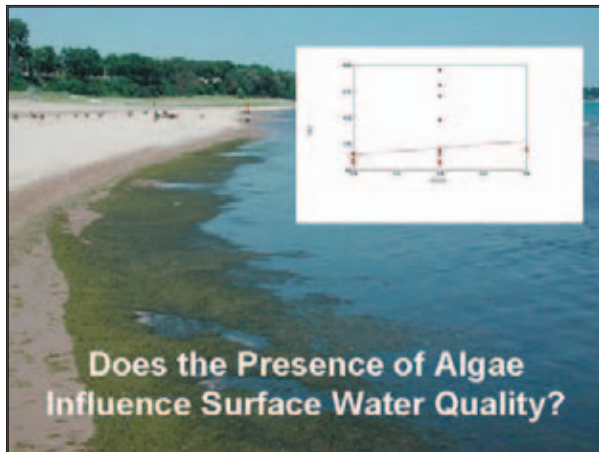
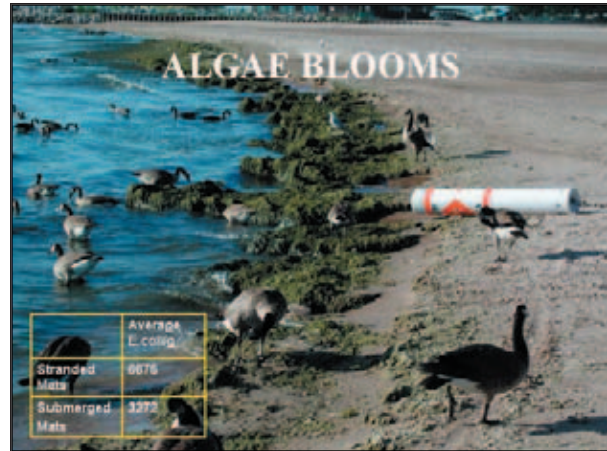
SURF RAKE MECHANICAL BEACH GROOMER

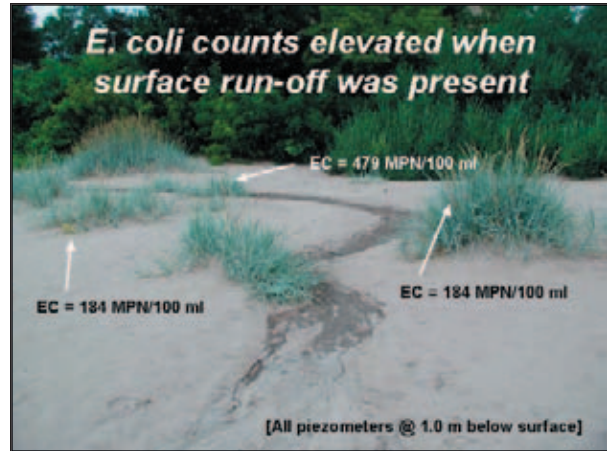
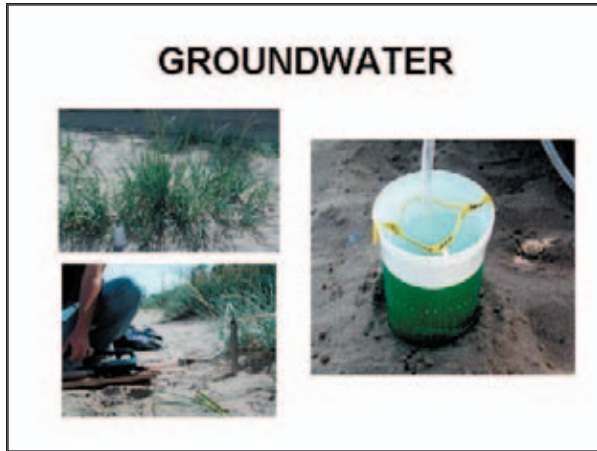


GROOMING STUDIES – 2001/ 2002



- 30 transects & a control area
- Hand-raked (2x/wk or daily)
- Unraked
- 2 weeks
- Bacterial density varied w/rain
- Used Actual Equipment
- 2 Treatments
- Status Quo vs. Deeper Grooming w/o Finisher
- Bacterial Density Varied Based on Sand Condition





Root River *E. coli* Densities - 2004

SITE	MEAN EC MPN/100 ml	RANGE
Johnson Park (R1)	1518	10 - 14,136
Horlick Dam (R2)	1431	10 - 12,997
Cedar Bend (R3)	3705	0 - 12,997
Washington Park OF (R4)	38,856	0 - 198,628
Water Street OF (R5)	18,020	100 - 173,287
State Street Bridge (R6)	1372	63 - 11,199
Chartroom (R7)	1098	20 - 9804





Could Boaters be a Source of Contamination?

- 2003 survey
- 6 marinas
- 1305 boat slips
- 63% occupied (823 slips)
- 2 pumping stations
- \$7.00 to pump regardless of amount
- Pumping stations connected directly to city sewer system
- 8000+ gallons of sewage pumped in 2003
- New head design makes illegal dumping next to impossible
- Illegal dumping occasionally observed by Sheriff's patrol boats



ACKNOWLEDGEMENTS

•City of Racine Departments of Health (Laboratory), Parks & Recreation, and Public Works.

•University Of Surrey, Guildford, UK
•Keep Our Beaches Open (KOBK)

Research funded by:

•WI Department of Health & Family Services
•Sustainable Racine





Questions and Answers

Q: Can you talk about the cost of your Vortechs system?

Julie Kinzelman

For the Vortechs system, including the whole engineering process, the relocation of the outlet, and the installation of the two Vortechs, it was about \$750,000 dollars. We had about \$150,000 through a grant from the Department of Natural Resources, and the city put in about \$600,000 of its own money.



Utilizing Storm Water Monitoring to Assess Beach Water Quality

Jill Lis, R.S.

Cuyahoga County Board of Health

Biosketch

Jill Lis is a Program Manager in the Environmental Health Service Area of the Cuyahoga County Board of Health. Ms. Lis received her B.S. in Environmental Health from Bowling Green State University in Bowling Green, Ohio in 1992. Since then, she has been working as a Registered Sanitarian in the Environmental Health Service Area of the Cuyahoga County Board of Health in Cleveland, Ohio. She has been managing the Bathing Beach Program since 1997, in addition to several other recreational and water quality programs. She is also an active member of the Ohio Environmental Health Association.

Abstract

The Cuyahoga County Board of Health (CCBH) received Beach Act funds in 2003 to re-evaluate its existing program to meet the objectives of the Beach Act. The overall goal was the development of a comprehensive risk-based beach monitoring and public notification program. To aid in the beach classification process, the Lake Erie shoreline was evaluated for the location of storm sewer outfalls and streams in the vicinity of the beaches. A total of 20 locations, 11 storm sewer outfalls and 9 streams, were identified that were accessible for sampling. These locations were sampled once a week during the recreation season for *E. coli* bacteria.

Sampling results revealed that 16 out of the 20 locations have potential to impact beach water quality. Several significant rain events occurred during the 2003 recreation season which may have contributed to elevated bacterial levels; however, elevated concentrations of *E. coli* were identified even during dry weather conditions. The data collected has been provided to the municipalities in which the sampling locations were located for collaboration

in investigating potential sources of pollution. This work is being continued throughout the 2004 recreation season in order to validate the 2003 data.

The CCBH conducts an extensive water quality program, including a Phase II Storm Water Program, in which illicit discharges are detected for their elimination. Fifty-five of the 56 communities within the CCBH jurisdiction are designated Phase II communities that must comply with Phase II Storm Water Management Plans and Programs. A regional storm water program has been developed by the CCBH to assist these communities in meeting their requirements. The program provides communities with educational outreach and participation, illicit discharge detection, MS4 inventories, dry weather flow surveys, water quality monitoring of MS4 outfalls, and investigative activities to identify illicit pollution sources to MS4 systems.

In addition to its Phase II Storm Water Program, the CCBH performs numerous water quality activities. These activities include: identifying and eliminating public health nuisances and hazards in the surface waters within the health district, surveying the watersheds within the health district, educating the public on non-point source pollution, participating in local watershed protection groups and meetings, and supporting the Household Sewage, Semi-Public Sewage, and Parks and Recreation Programs, including the Bathing Beach Program.

The CCBH utilizes a watershed approach in dealing with water quality issues. Cuyahoga County consists of 3 principal watersheds, all of which drain to Lake Erie: the Rocky River Watershed, the Cuyahoga River Watershed, and the Chagrin River Watershed. The overall water quality in Cuyahoga County ultimately affects the beach water quality. In efforts to enhance its role with these issues, the CCBH is actively working towards developing a Watershed Protection Unit, which will address all water quality issues within its health district.



Phase II Storm Water Program as it Relates to the Beach Program

Prepared By:

Jill Lis, R.S., Program Manager
Cuyahoga County Board of Health



CCBH General Health District

- Serves over 830,000 residents
- Represent:
 - 35 cities
 - Excluding the cities of Cleveland, Lakewood, and Shaker Heights (These cities have their own city health department.)
 - 19 villages
 - 2 townships



Watershed Protection Unit

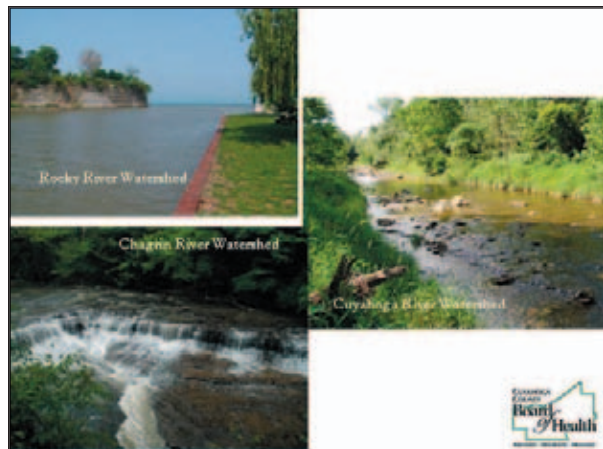
- Storm Water Program
- Household Sewage Program
- Semi Public Sewage Program
- Beaches
- Marinas
- Water Quality Program
- Educational Outreach



Cuyahoga County



3 Major Watersheds: Rocky River, Cuyahoga River, and Chagrin River





Phase II Storm Water Program

- U.S. EPA/Ohio EPA Phase II Storm Water Regulations
 - Effective March 10, 2003
- Requirement
 - Phase II designated communities must develop and implement storm water management plan
 - Cuyahoga County is comprised of 59 communities
 - 58 are Phase II designated communities



Phase II Cont...

- 6 minimum control measures
 - 1. Public education
 - 2. Public involvement
 - 3. Illicit discharge detection and elimination
 - 4. Construction site runoff control
 - 5. Post construction site runoff control, and
 - 6. Good housekeeping



CCBH Involvement

- Household Sewage Treatment Systems (HSTS)
- Water Quality Sampling
- No Guidance from Ohio EPA
- Communities looking for assistance
- Principal Member of Northeast Ohio Storm Water Task Force Committee



Illicit Discharge Detection and Elimination

- Identify any discharge into a community's MS4 system that is not storm water
 - MS4: Municipal Separate Storm Sewer System
 - Conveyance System: can be storm sewer, swale, or ditch



Illicit Discharge Detection and Elimination

- By 2008:
 - Communities need to identify all outfall locations within their MS4 system.
 - All outfalls must be mapped.
 - Communities must have their illicit discharge detection and elimination plan in place.



Illicit Discharge Detection and Elimination Plan

- Sources of discharge into an MS4 other than storm water or ground water must be detected and eliminated.
- Communities must develop this plan in order to meet the Phase II requirements.





Illicit Discharge

- Example:
 - Household Sewage Treatment Systems (HSTS) that discharge are sources of an illicit discharge
- Communities have until 2008 to assess these systems and develop a plan on eliminating these sources of illicit discharge.

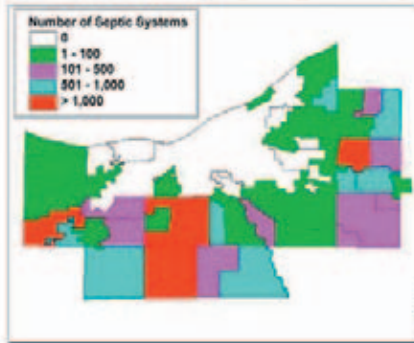


Off-lot Discharging HSTS

- Approved in the State of Ohio
 - Outdated rules / no legislation
 - No general NPDES permit for household septic systems from the Ohio EPA
- One of only a handful of states that approves these systems
- Are now illicit sources of discharges under Phase II Storm Water



Household Sewage Treatment System Distribution In Cuyahoga County



Illicit Discharge Cont...

- Three ways to eliminate HSTS as an illicit discharge:
 - Sanitary Sewers
 - Install an On-Lot HSTS
 - Permit off lot HSTS with Ohio EPA NPDES Permit



Soil Conditions



- Sandy soils
- Only in isolated areas
 - Use of leaching systems

- Heavy clay soils
- Widespread
 - Not suitable for leaching systems





Simple activities impact the State's Water Quality

- Improperly disposing of motor oil
 - Over-using lawn and garden products
 - Leaving pet waste on the ground
- Rain, snowmelt, and over-watering move these household-generated pollutants to rivers, lakes, and streams. This polluted runoff has a major impact on water quality.



Storm Water Program

- CCBH provides the following services:
 - MS4 inventory
 - Dry Weather Screening
 - MS4 outfall sampling
 - Illicit Discharge detection
 - Public Education and Outreach
- Completed MS4 inventories for 20 communities
- Have contracts with 30 communities for dry weather and sampling projects.



Inventory, Dry Weather Screening, and Sampling



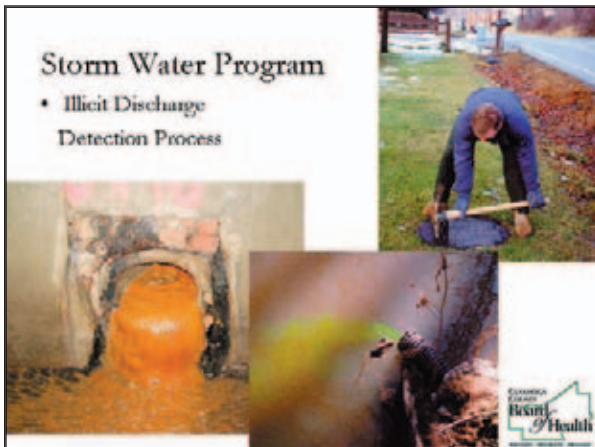
Storm Water Program

- Dry Weather Screening and Sampling



Storm Water Program

- Illicit Discharge Detection Process



HEM1028	
Location:	
Address:	
City:	
State:	
County:	
Watershed:	
MS4 ID:	
MS4 Name:	
MS4 Type:	
MS4 Size:	
MS4 Status:	
MS4 Contact:	
MS4 Phone:	
MS4 Email:	
MS4 Website:	
MS4 Description:	
MS4 Notes:	
MS4 Photos:	
MS4 Maps:	
MS4 Data:	
MS4 Status:	
MS4 Type:	
MS4 Size:	
MS4 Status:	
MS4 Contact:	
MS4 Phone:	
MS4 Email:	
MS4 Website:	
MS4 Description:	
MS4 Notes:	
MS4 Photos:	
MS4 Maps:	
MS4 Data:	

- Database for storm water program






Agency	Agency	Agency	Agency	Agency	Agency	Agency	Agency
...

Agency	Agency	Agency	Agency	Agency	Agency	Agency	Agency
...

Agency	Agency	Agency	Agency	Agency	Agency	Agency	Agency
...



Storm Water Program

- Educational Outreach
 - Adults
 - School-Aged Children
 - Public Officials



Adults

- Homeowner Seminars on HSTS and Non-point Source Pollution
- Public Involvement: Stream Cleanups, volunteer monitoring



School Aged Children




05.26.2004





Public Officials

- Attend Council Meetings
- Educate on Phase II requirements
- Draft Ordinances from Storm Water Task Force Committee




Other Outreach Activities

- Development of an all inclusive webpage
- College program on water quality monitoring of outfalls
- Watershed Festivals
- Printed Materials
 - Brochures
 - Coloring Book
 - Comic Book
 - Candy Wrappers watershed specific




Storm Water Program

- Collaboration:
 - Watershed Organizations
 - County Planning Agency
 - Cleveland Metroparks
 - Regional Sewer
 - Ohio Department of Natural Resources
 - Ohio EPA
 - Communities
 - School Districts



Beach Program Background

- The Ohio Department of Health (ODH) administers the Bathing Beach Monitoring Program for the State of Ohio
 - Program has been in place since the mid 1970's
 - Cooperative effort between the ODH, the Ohio Department of Natural Resources, public and private organizations along the Lake Erie shoreline, and local health departments with beaches in their jurisdictions





Beach Program Background Cont...

- The program aims to encourage local health departments to develop their own monitoring programs, however, local programs are not mandatory
- The program also provides incentive for the development of
 - Predictive models for assessing water quality
 - Pre-emptive warning systems for public notification
 - Programs to identify and eliminate potential pollution sources



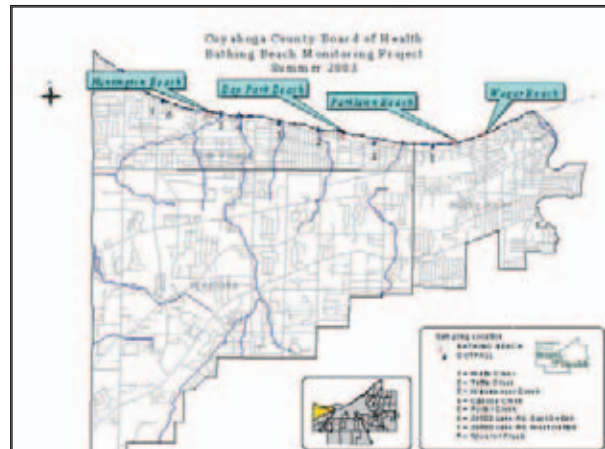
Program Component

- Sanitary Surveys
 - Allows for the determination of site suitability for beaches
 - Surveys focus on items that could impact the health and safety of bathers:
 - Identification of potential pollution sources
 - Storm sewer outfalls, sewage systems, etc...
 - Availability of toilet facilities
 - Other influences, such as breakwalls

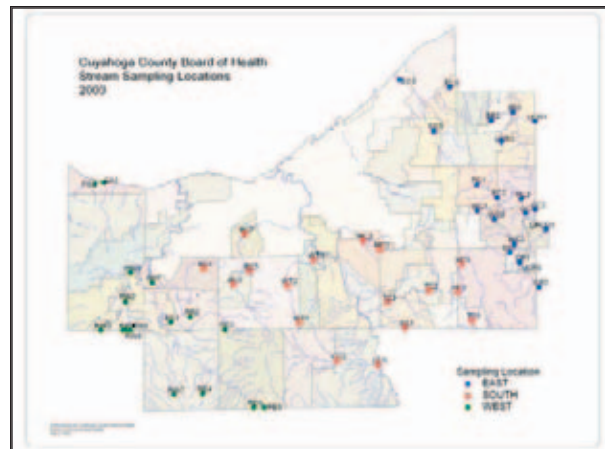
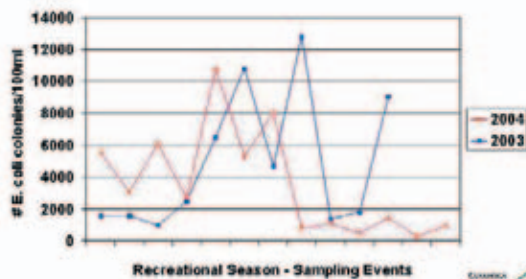


Beach Act Projects

- Summer 2003/2004
- Development of a risk-based beach evaluation and classification plan
 - Partially accomplished through the identification and monitoring of any storm sewer outfalls/streams that were found to be located on, or near, the beaches



29800 Lake Road – East Outfall





Contact Information

- Harry Stark, R.S., M.P.A.
 - Supervisor/Specialist
 - (216) 201-2001 x 1205
 - hstark@ccbh.net

- Jill Lis, R.S.
 - Program Manager
 - (216) 201-2001 x 1240
 - jlis@ccbh.net





Questions and Answers

No questions.



Diversion is the Solution to Pollution, So Far

Cathy Chang, D.Env.

Santa Monica Bay Restoration Commission

Biosketch

Dr. Cathy Chang is a water resource control engineer at the Santa Monica Bay Restoration Commission. Dr. Chang received her B.S. in Physics, her M.S. in Civil & Environmental Engineering, and her D.Env in Environmental Science and Engineering-- all three degrees from U.C.L.A., California. She worked on storm water and urban runoff pollution regulation and policy for several years at the Los Angeles Regional Water Quality Control Board. For the past four years, she has been a staff for the Santa Monica Bay Restoration Commission, where she has completed a comprehensive assessment of storm water programs in Los Angeles County and oversees projects that provide regional solution to storm water and TMDL issues in the Santa Monica Bay Watershed.


Abstract

In the late 1980's, alarmed by the evidence that dry-weather urban runoff is the main cause of bacterial contamination at beaches along Santa Monica Bay, California, Los Angeles County public agencies began testing and implementing various pollution control measures. Many of these measures were fully or partially funded by the Santa Monica Bay Restoration Commission (SMBRC). Measures ranged from source control to end-of-pipe solutions, and included programs to conduct sanitary surveys, detect illicit connections, reduce street washing, extend storm drain outlets beyond surf zones, and divert runoff to sanitary sewers or on-site treatment facilities.

Meanwhile, valuable monitoring data, collected concurrently with project implementation, has allowed agencies to evaluate the feasibility and effectiveness of many of these measures. Currently, diversion of runoff to sanitary sewers appears to be the most effective measure. Pre- and post diversion monitoring data at several project locations indicates a rapid and significant improvement in water quality. Data have also shown that on-site treatment can be equally effective if properly sited and the treatment method is appropriate to the on-site conditions. Failures have also yielded valuable lessons. Even some of the diversions which were highly effective initially, have required modifications to correct deficiencies in their original engineering designs.



Diversion is the Solution to Pollution, So Far



Hyon (Cathy) Chang, D. Env
Santa Monica Bay Restoration Commission

Santa Monica Bay Restoration Commission

One of over 20 Estuaries Projects established by National Estuary Program.

Goals

- Safe, Swimmable, Fishable Bay

Accomplishments & On-going Activities

- Santa Monica Bay Restoration Plan
- On site wastewater management task force
- Epidemiological study
- Storm water pollutant load modeling
- DIVERSION PROJECTS
- Pollution prevention projects
- Public outreach & education
- Habitat restoration
- Stakeholder process

Water Quality at Santa Monica Bay Beaches

- Over 80% beaches with very good-to-excellent water quality during summer.
- Frequent beach water quality postings.
- Water quality exceedances during both dry and wet weather.
- Most water quality exceedances near urban runoff drains.
- Most dry weather problems concentrated at a number of chronically affected sites.
- Wet weather problems are more widespread.

Water Quality at Santa Monica Bay Beaches
Dry Season

Exceedances of Bacterial Health Risk Indicators at Santa Monica Bay Beaches
Dry Season (April 15 - October 15)

Water Quality at Santa Monica Bay Beaches
Wet Season

Exceedances of Bacterial Health Risk Indicators at Santa Monica Bay Beaches
Wet Season (October 15 - April 15)

Dry Weather Urban Runoff Diversions 101

What's being diverted?

- Dry weather urban runoff.

How does diversion system work?

- Divert dry weather flows from chronically problematic storm drains to (sewage) treatment facility.

What are associated costs?

- Capital: \$200K-\$1+M, depending on capacity and other logistics.
- O&M: varies (trash removal, sewer hook-up, occasional pump maintenance).

What are potential benefits?

- Improve dry weather beach quality, protect public health and marine organisms, enhance TMDL compliance, benefit local economy, etc.

Will diversions prevent or eliminate beach closures?

- Most likely not. Beach closures are mainly caused by sewage spills and overflows which often occur during wet weather.



Dry Weather Urban Runoff Diversions 101

What Does It Look Like?

storm drain trash separator unit pump unit sanitary sewer

Overview of Diversions Along SMB Beaches

- Over 20 priority drains diverted along the coast.
- About 10 additional diversions proposed or under construction.
- 2 facilities dedicated solely for treating dry weather flows and 1st flush.
- Total cost of diversions along SMB over \$11 million.
- Total cost of urban runoff treatment facilities ~\$12 million.
- Funding- Prop A, 12, 13, and Clean Beaches Initiatives.
- Most diversions operate only during dry season (7 months).
- A couple of diversions also handle first flush.
- Adoption of bacterial TMDL quickened pace of diversion construction.

Locations of Diversions Along SMB Beaches

Are Diversions Making a Difference?

Pre- and post-diversion beach grades (AB 411: April-October)

LOCATIONS	PROJECT COMPLETION DATE	2001	2002	2003	2004
Melbourn Beach Pier	05/97	A	A	A	A+
Santa Monica Beach (New Pavilion)	1/93	B	A	A	C
Hermina Beach Pier	04/97	A+	A	A	A
Santa Monica Municipal Pier	10/97	B	C	C	B
Venice Beach (Thermes Ave) (Archie)	06/98	A	A+	A	A+
Redonda Beach (Hermosa Street)	01/01	B	A	C	A
Will Rogers State Beach (Over Club Dr.)	01/01	B	B	C	A
Venice Beach (Rimble Ave)	04/01	A	A+	A	A+
Dwightler State Beach (Days del Rey/Cokey Blvd)	04/01	A	A	A	A
Santa Monica Beach (Ashland Ave)	04/01 (09/77)	A	A	A	A
Dwightler State Beach (Imperial Hwy)	06/02	A	A	A	A
Will Rogers State Beach (General Canyon)	4/03	C	C	C	A
Will Rogers State Beach (Santa Monica Canyon)	4/03	F	C	D	D

Are Diversions Making a Difference?

Lessons Learned

- Noticeably improved dry weather beach water quality near many diverted drains.
- Persistent problems at some locations due to: undersizing, pump failures, and other sources
- Diversion is not "cure all" for dry weather beach quality problems
 - Only 3 of 5 most problematic drain systems able to be diverted
 - Success depends on source type, distance to treatment facility, discharge volume, etc.
 - Other sources of problem exist especially near piers (birds, septic tanks, leaky sewer line, other site specific anthropogenic activities)
 - Susceptible to mechanical (e.g. pump) and engineering/design (e.g. undersizing) flaws.



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Summary

Bright Side, So Far

- Best solution so far for addressing problematic dry weather flows with small enough volume and in vicinity of treatment facility
- Effective in improving beach water quality near diverted drains

Limitations

- Not "cure-all" for all dry weather beach quality problems
- High capital cost & continuous maintenance
- Susceptible to mechanical and engineering/design flaws.
- Does not address wet weather water quality problem.
- Does not encourage source control.
- Requires cooperation of multiple agencies.

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Questions and Answers

Q: (Diana Munz). When you are able to see water quality improvements from this, do you just see it immediately downstream of the diversion, or are you able to show reduced postings at the receiving beach?

Cathy Chang

It is tricky in Los Angeles County to talk about postings because when the storm drains have continuous flows they have permanent postings. I assume the postings have disappeared for the permanent ones, where improvements have been seen.

Mark Gold

That is true for some of them. They have reduced postings for the ones that are not permanent and flowing. It has been a pretty positive program.

Q (Steve Hartsel, San Mateo County): Have you done a follow-up epidemiological study that shows the actual health effects of the improvement of the water quality here?

Cathy Chang

No we have not.

Q (Steve Hartsel, San Mateo County): Are there any plans to do so? It seems like it would be the logical thing to do,

Mark Gold

No. With the epidemiology design, it would not be logical. That is because the way the study was designed was comparing swimmers to swimmers. So, the controls were those swimming right in front of the storm drains compared with those swimming 400 yards away in cleaner conditions. So, one would expect that it would be a similar outcome to when you actually remove the pollution source. There is no reason to think that they would be a different population.

Q (Steve Hartsel, San Mateo County): That wouldn't be confirmation to go back and test in the same place and do the same surveys?

Mark Gold

Not for a million dollars, which was the cost of the study.

Thursday, October 14
8:00 a.m. – 9:40 a.m.

**Concurrent Track II:
Changes on the Horizon
Session Eight:
Making Warning Systems More
Rapid: Modeling and Rapid
Methods**



A Regional Nowcast Model for Southern Lake Michigan Using Data Readily Available to Beach Managers

Richard Whitman

U.S. Geological Survey, Great Lakes Science Center

Biosketch

Dr. Whitman is the station chief and research ecologist at the U.S. Geological Survey Lake Michigan Ecological Research Station. Dr. Whitman received his Ph.D. from Texas A&M University in Wildlife and Fisheries Science. He went on to teach at Indiana University NW for 10 years as an associate professor of biology. He became a research biologist with the National Park Service and then the U.S. Geological Survey Great Lakes Science Center, where he has worked for the past 15 years. Dr. Whitman's research interests include sources and occurrence of bacteria contamination in sands and waters of Lake Michigan and the relationship of hydrometeorological and antecedent biological conditions to indicator bacteria contamination in freshwater streams and beaches.

Abstract

In recent years predictive modeling of beach water quality from retrospective empirical local hydrometeorological measurements have become common. Factors influencing recreational water quality are both local (e.g., sewage, hydrodynamics, morphology) and regional (e.g., weather patterns, currents, antecedent conditions). We explore regional factors that help explain *E. coli* concentrations with hopes of later partitioning these from

local effects. *E. coli* data from 55 beaches along 217 km shoreline from Milwaukee, Wisconsin to Michigan City, Indiana were assembled for 2000-2003 in addition to ambient and derived data from national, state and local weather stations, wave dynamic installations and lake buoys. Local *E. coli* spatial correlation was clearly evident. This fine-grain spatial pattern was layered within seven larger scaled geographic zones. Regression demonstrated that rainfall, wind speed, solar radiation, wave height, barometric pressure, and antecedent *E. coli* were important factors. While there were strong seasonal trends and multi-day momentum of *E. coli*, there was only weak daily autocorrelation. Resultant regression models yield coefficients that were several times higher than those predicted by currently used protocols (i.e., 24-hour lag between collection and closure). Discriminant functions correctly classified a beach closure or opening most of the time using these hydrometeorological conditions, whether or not the beaches were aggregated by wind direction, zone or day. These models demonstrate local differences among beaches and the explanatory factors, provide reasonably good real-time predictions, and help explain general hydrometeorological interactions with recreational water quality. All independent factors are readily available on the Internet and through cooperation among beach managers.



Toward a Regional Nowcast Model for Southern Lake Michigan Using Data Readily Available to Beach Managers

October 14, 2004

Richard Whitman

Lake Michigan Ecological Research Station

Porter, IN

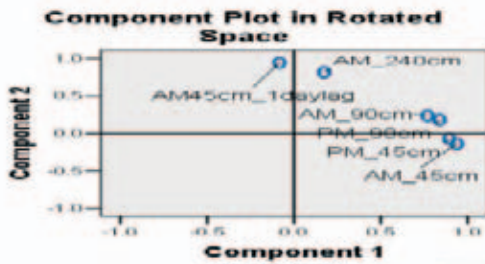


Local Forces

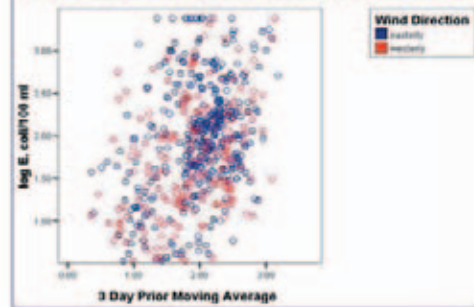
- Morphology
- Shoreline Exposure
- Sources
 - Direct (e.g., sewage, birds, swimmers, streams)
 - Indirect (e.g., re-suspension, sands, algae, runoff)



Factor Loading Plot, PCA, AM & PM Water @ 63rd St.



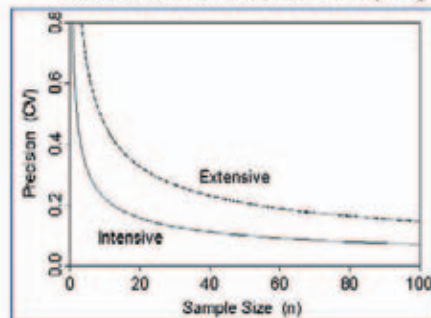
E. coli vs. 3 Day Prior Moving Average
57th, 63rd, Calumet, Rainbow, South Shore Beaches



MDS of *E. coli* Counts at Shallow (S) and Deep (D) Transect Sites (1-5) at 63rd Street Beach Chicago



Number of Samples Required for a Given Sample Precision: Extensive vs. Intensive Sampling





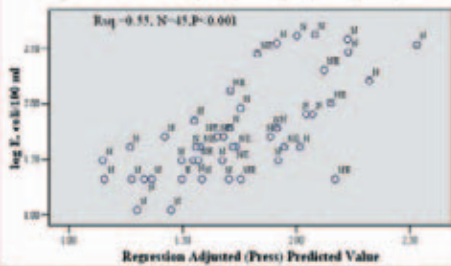
Current 2004 Predictive Modeling Activities

Location	Sponsors	Data Period
Chicago, 63 rd	Chicago/CPD	Summer, 2004
Lake Co., IN	Gary/IDEM	Summer, 2004
LaPorte Co., IN	National Park Service	Summer, 2004
Door County	Door County	Summer 2004
S. Lake Michigan	EPA, Chicago, Gary, USGS	Summers 2000-03

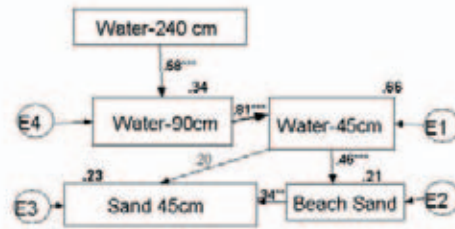


E. coli Predictions For North to Northeast Winds, Ogden Dunes

$$\text{Log } E. coli = 0.535 + 0.015(\text{Color}) + 0.007(\text{Chl.}) + 0.012(\text{Turb.}) + \epsilon$$



Path Analysis of *E. coli* in sand and water, 63rd Street, Chicago (N=66, Chi-square=6.6, df=5, p=0.251)



Cumulative R square above box, Regression Weights above arrows
Regression Weights Significance (*=0.05, **<0.01, ***<0.001)



General Forces

- Currents
- Season
- Diurnal & Lunar
- Weather
- Offshore conditions



Correlations Among Summer, 2000 *E. coli* Concentrations at Three Lake Michigan Beaches

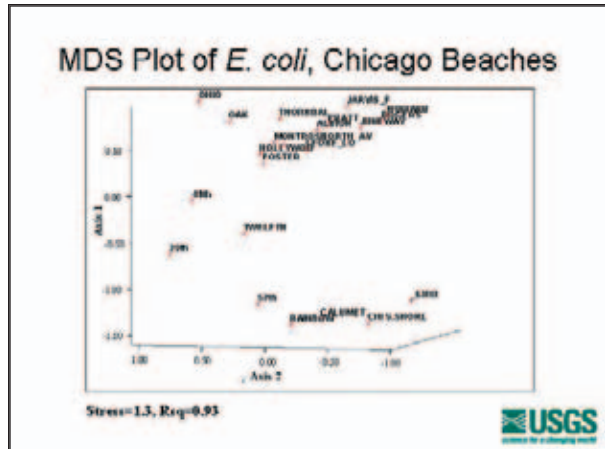
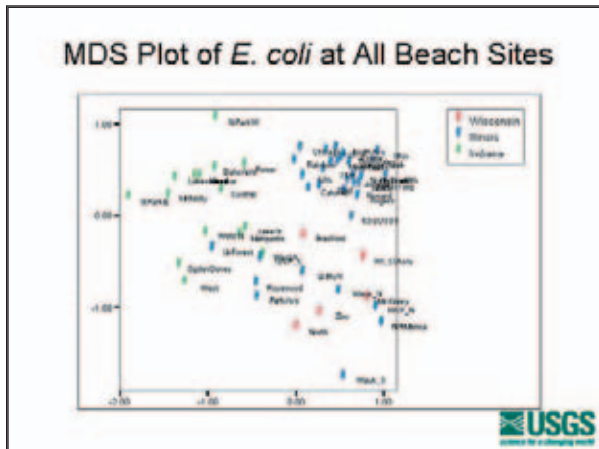
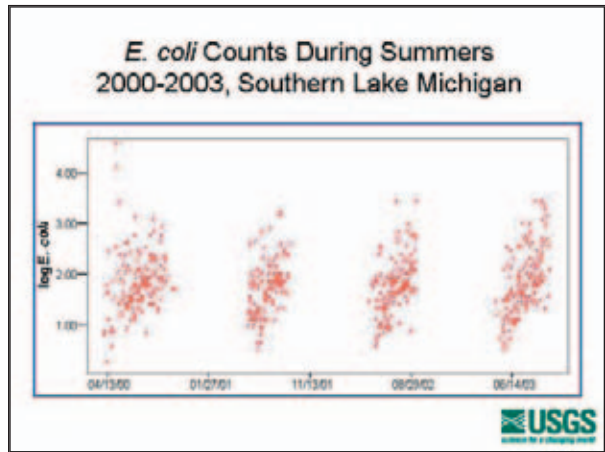
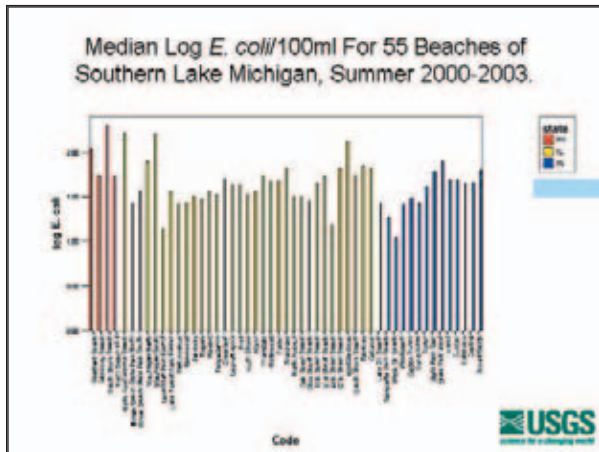


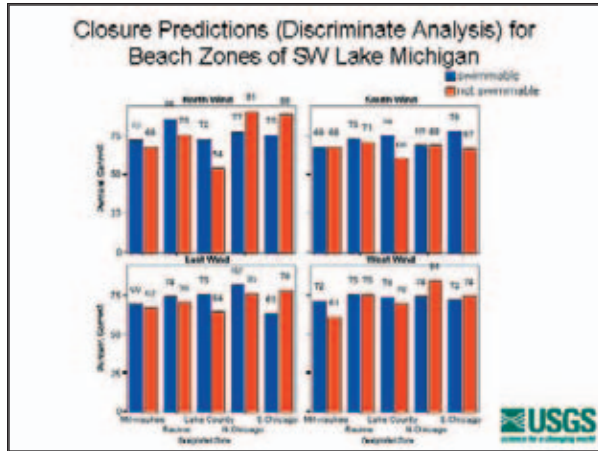
Pearson Correlation (2-tailed)	63 rd Beach	North Beach
West Beach	r 0.564 Sig. 0.002 N 27	0.386 0.047 27
63 rd Beach		r 0.552 Sig. 0.003 N 27





- ### Regional Forecast Model
- 55 southern Lake Michigan beaches
 - *E. coli* data 2000-2003
 - 15 meteorological stations
 - 3 GLERL Coastwatch Station
 - 2 coastal wave/direction gauge US ACOE
 - 1 offshore buoy (NDBC-NOAA)
 - 4 visible (IN, IL), 1 UV (IL) solar radiation
 - ADCP (NOAA, USGS, Chicago)
-
- USGS
science for a changing world





Wind Direction	Function	1
Southerly	EC_3dayPMA	.736
	Rain	.441
	Temp_10PMA	.350
	Stage	.295
	Wave ht.	.264
	Insolation ^a	-.137
	Windspeed ^a	.083
Pressure ^a	.023	

Correlations between Discriminating Variables and Canonical Discriminant Functions

a. Variable not used in the analysis (p>0.05)

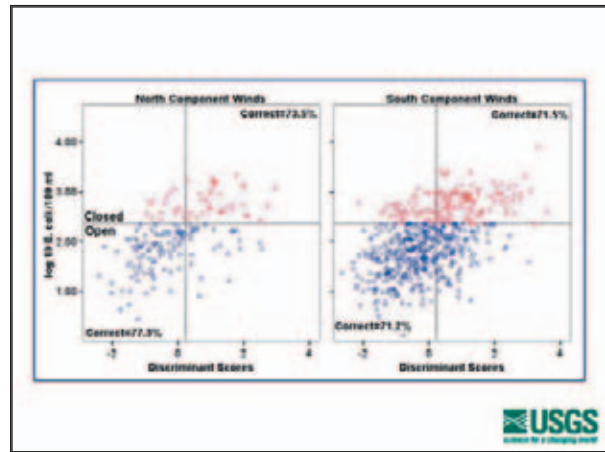
USGS logo

Wind Direction	Function	1
North Component	Wind Speed	.531
	Stage	.529
	Wave HL ^a	.443
	Rain	.436
	EC_PMA	.348
	Insolation ^a	-.134
	Pressure ^a	.050
Temp_PMA ^a	.025	

Correlations between Discriminating Variables and Canonical Discriminant Functions

a. Variable not used in the analysis (p>0.05)

USGS logo



Acknowledgments

Cities of Milwaukee, Racine, Chicago, Gary; National Park Service, Chicago PD, Indiana DNR, Lake County, IL, USGS, EPA, GLERL, US ACOE, IN Dept. of Environment, NWS, State Meteorologists: IL, IN and WI

USGS logo

Richard Whitman
 Lake Michigan Ecological Research Station
 Great Lakes Science Center
 Porter, IN

Phone: (219) 926-8336 ext. 424
 Fax: (219) 929-5792
 Email: richard_whitman@usgs.gov

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Questions and Answers

See Questions and Answers for Greg Olyphant on page 243.



Predicting the Need for Beach Closures in Real Time: Statistical Approaches and their Applicability to the Lake Michigan Shoreline

Greg Olyphant

Indiana University, Department of Geological Sciences

Biosketch

Dr. Olyphant has been a professor of hydrology at Indiana University (Bloomington) since 1980. He has been a member of the Interagency Task Force on *E. coli* (focused on the southern Lake Michigan shoreline) since its inception in 1995. He has published several papers that demonstrate the functional relationships between hydrometeorological conditions and bacterial concentrations in streams and beach waters. He has also served as a consultant to public health officials and park administrators on issues of water quality and methods for posting advisories and closures.

Abstract

A long record of water quality data, from numerous beaches along the Lake Michigan shoreline, has shown that knowing what *E. coli* concentrations were on a given day (day of sample collection) rarely provides an accurate prediction of what the concentrations are on the next day (day

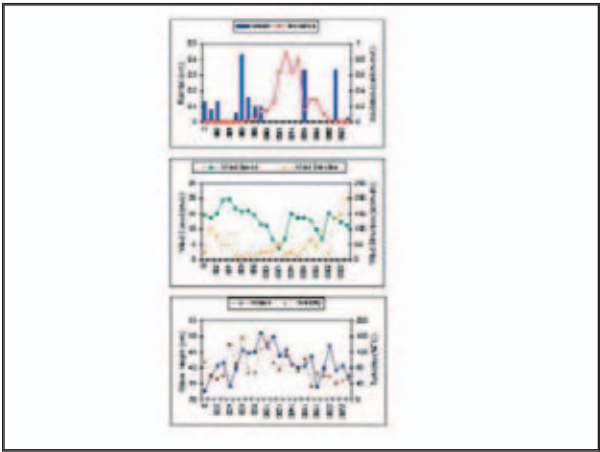
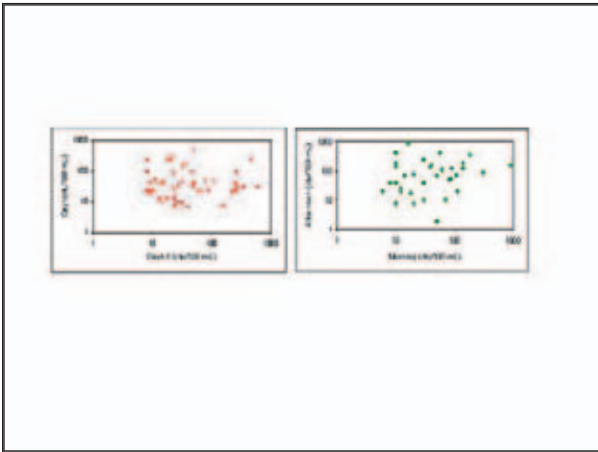
of decision). This is because the concentrations in beach water strongly depend on short-term changes in prevailing hydrometeorological conditions. For example, during stormy periods, increased inflows of contaminated stream water, and stirring of bacterially laden sands in the nearshore zone can cause *E. coli* concentrations to spike for several hours. On the other hand, the concentrations can decline by an order of magnitude during calm weather when suspension is low and bacteria have been exposed to long periods of intense sunshine. A recent pilot study (63rd Street Beach, Chicago, 2000) has demonstrated that by continuously monitoring hydrometeorological conditions, a statistical model can be developed to accurately predict bacterial concentrations in beach water so that real-time decisions can be made about posting warnings and closures. Beach Act funds are being used to test and refine the modeling approach at two additional locations along the southern shoreline of Lake Michigan. An overview of the model formulation and summary of experimental results at the two new study sites will be the main focus of the current presentation.



Predicting the Need for Beach Closures in Real Time: Statistical Approaches and their Applicability to the Lake Michigan Shoreline

Ψ Greg A. Olyphant
 Department of Geological Sciences
 and Center for Geospatial Data Analysis
 Indiana University

gda Center for Geospatial Data Analysis

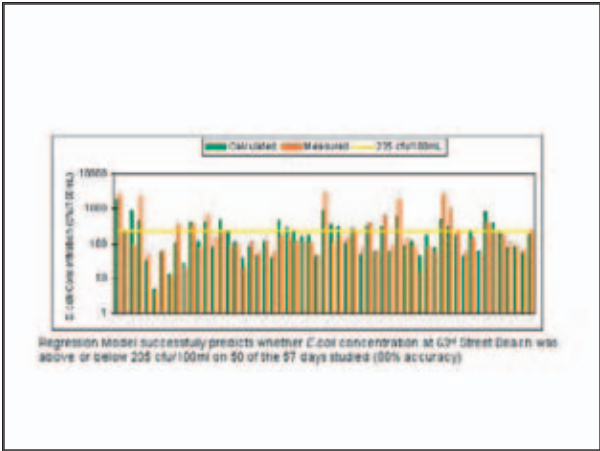


Predictive Model: 63rd Street Beach, Chicago 2000

$$\ln EC = \beta_0 + \beta_1 PV + \beta_2 T + \beta_3 R_{24} + \beta_4 W + \beta_5 P + \beta_6 R_{24} PV + \beta_7 \epsilon + \nu_t$$

Parameter	β_0	β_1	β_2	β_3	β_4	β_5	β_6	β_7
Estimate	5.15	0.06	-2.45	1.44	-0.09	0.23	4.5×10^{-7}	1.0×10^{-1}
St. Error	2.49	0.02	0.34	0.27	0.03	0.07	2.1×10^{-5}	5.3×10^{-4}
t-ratio	2.09**	3.50**	-7.22**	5.39**	-3.20**	3.17**	2.13**	1.91*

Estimated autocorrelation coefficient, $\rho = 0.07$. Multiple correlation coefficient, $R = 0.84$. Total sample size: $N=57$.
 * Estimated regression parameter is statistically different from zero at the 90 percent confidence level.
 ** Estimated regression parameter is statistically different from zero at the 95 percent confidence level.





Some Recommendations that Should lead to an Improved Early Warning System for Beach Closures/Advisories:

- Utilize electronic instruments that can be averaged/totalize over appropriate intervals for real-time forecasting.
- Deploy sensors in or near to the surf zone at each beach of concern.
- Place meteorological towers close to the beach(s) of concern.
- Install a sonde and other appropriate sensors at stream outlets (if one is near by) and monitor *E.coli* to develop a predictive model for outfalls from interior watersheds.
- Monitor beach water twice daily in order to calibrate a model that can distinguish between morning and afternoon conditions.
- Use statistical confidence intervals when assigning health risk warnings or making closure decisions.



Variable/Condition		BSF	IF	BSF-E	BSF-W
Flow (1 hr, 4 hr, 12 hr, 24 hr winds)	ft ³ /sec	✓	✓	✓	✓
Distance Component of Wind (1 hr, 4 hr average)	ft/sec	✓	✓	✓	✓
Alongshore Component of Wind (1 hr, 4 hr average)	ft/sec	✓	✓	✓	✓
Wave Height (1 hr, 4 hr average)	ft	✓	✓	✓	✓
Lake Stage (1 hr, 4 hr average)	ft	✓	✓	✓	✓
Incidence (1 hr, 4 hr average)	ft	✓	✓	✓	✓
Air Temperature (1 hr, 4 hr average)	°C	✓	✓	✓	✓
Water Temperature (1 hr, 4 hr average)	°C	✓	✓	✓	✓
Water Turbidity (1 hr, 4 hr average)	FTU	✓	✓	✓	✓
<i>E. coli</i> Loading by Discharge (1 hr, 4 hr average)	FC/du	✓	✓	✓	✓
Binary Detection of when Sample was Collected	Yes/No	✓	✓	✓	✓

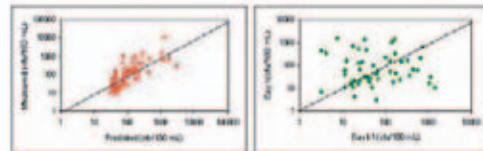
✓ Variable was measured at the study beach.
 * Variable correlated significantly to the predictive (regression) equation.

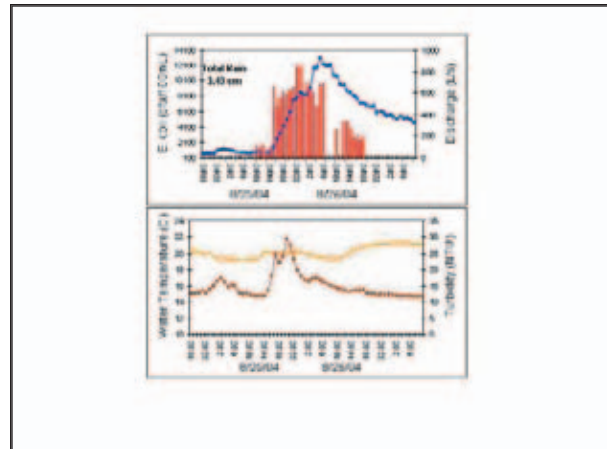
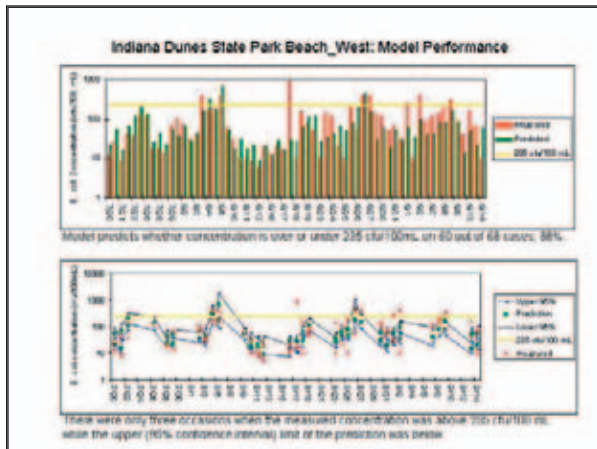
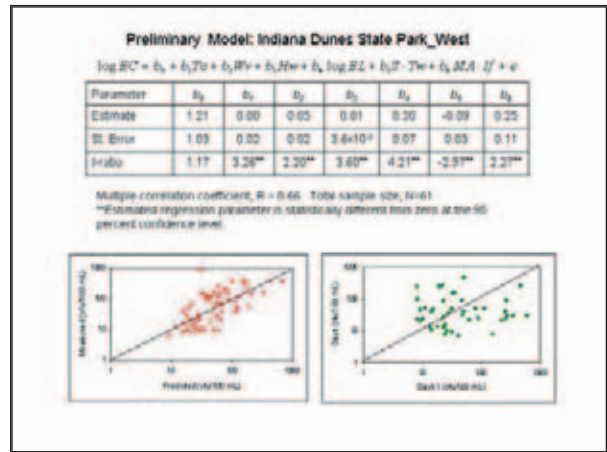
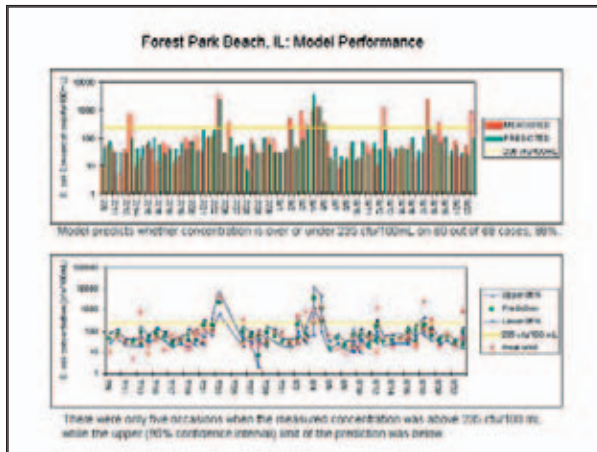
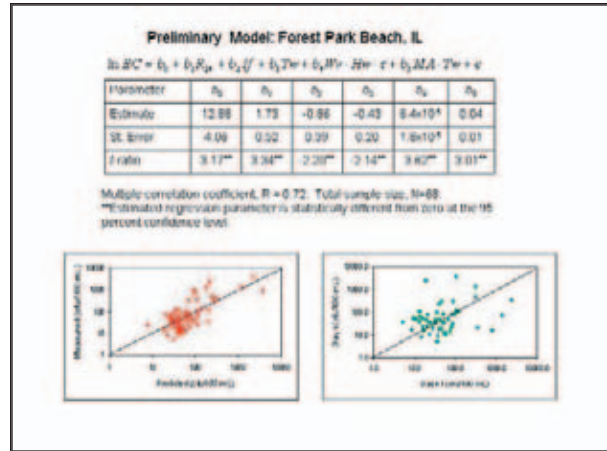
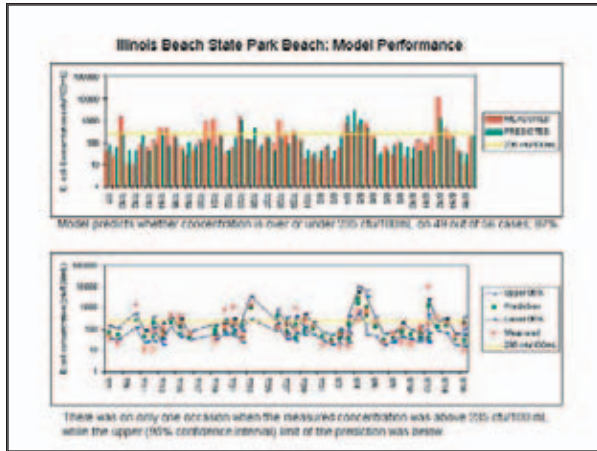
Preliminary Model: Illinois Beach State Park, IL

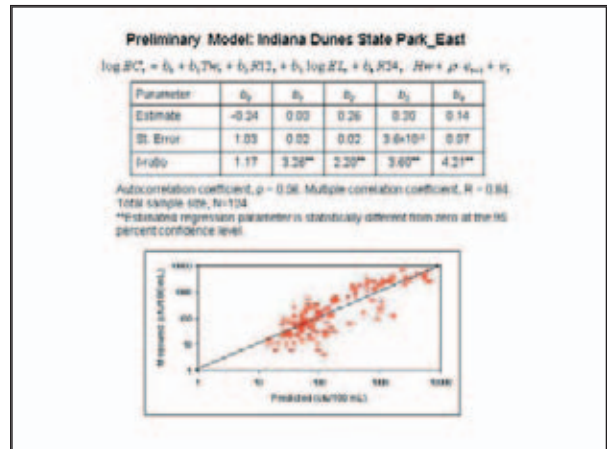
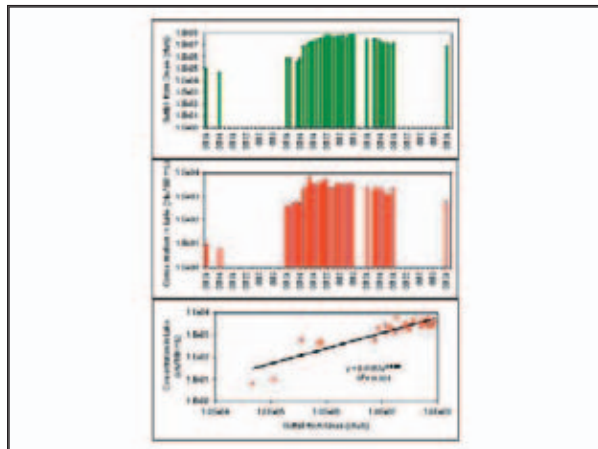
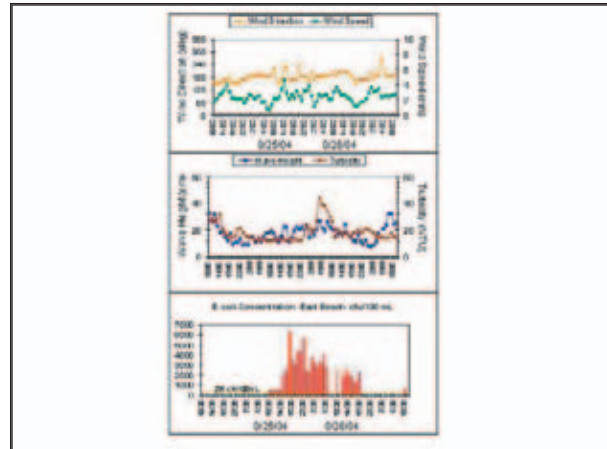
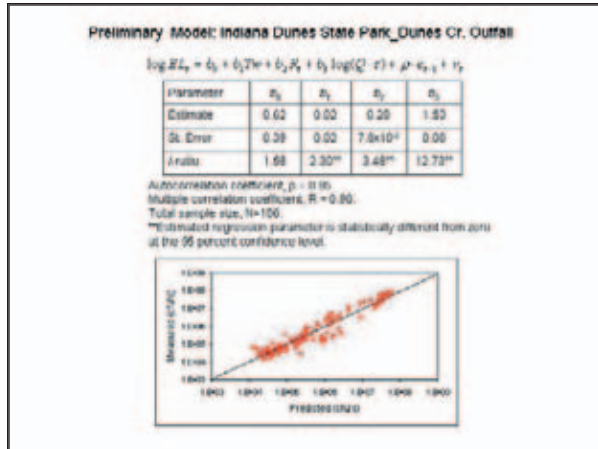
$$\ln_{10} E^*C = b_0 + b_1 T + b_2 W + b_3 W^2 + b_4 U + b_5 T^2 - b_6 W^2 + b_7 W^3 - b_8 T^2 - U^2 + e$$

Parameter	b_0	b_1	b_2	b_3	b_4	b_5	b_6	b_7	b_8
Estimate	0.89	0.04	0.03	0.21	-1.94	-2.9x10 ⁻⁴	0.07		
St. Error	4.99	4.2x10 ⁻¹	0.01	0.10	-1.02	-1.3x10 ⁻⁴	0.04		
F-ratio	0.14	9.83**	2.04**	2.04**	1.89*	-2.25**	2.02**		

Multiple correlation coefficient, $R^2 = 0.72$. Total sample size, $n=56$.
 * Estimated regression parameter is statistically different from zero at the 95 percent confidence level.
 ** Estimated regression parameter is statistically different from zero at the 99 percent confidence level.







PRELIMINARY CONCLUSIONS

- Predicting the need for Closures and/or the Issuing of Health Warnings -Lake Michigan Beaches- cannot be reliably achieved from samples taken a day or even several hours earlier.
- Predictive Models based on continuously measured hydrometeorological variables -"SwimCasts"- provide a better alternative because they are more accurate and can be revised on an "as needed" basis. All such forecasts have uncertainty associated with them and should be based on probabilities.
- Most beaches are not dominated by a river outfall and the water quality (bacterial) in the swimming zone is strongly conditioned by prevailing weather: rainfall, sunshine, temperature winds and waves. The bacterial concentrations in these have a very short "memory" ; i.e. very little autocorrelation.
- Beaches that are immediately down drift of a bacterially infested stream show strong dependence on the outfall of bacteria during storms and the high resulting concentrations can persist for a very long time (many hours/days?).



Questions and Answers

Q: You are located right next to United States Steel and some other steel manufacturers. Do the outfalls from the steel manufacturers located nearby have an effect on the beaches in the national park?

Greg Olyphant

The beach I'm talking about is not as close to the steel plants as the one Richard referred to in a previous slide. The U.S. steel plants are doing a good job of trying to improve their water quality and have invited the *E. coli* Taskforce (an interagency taskforce in Indiana) to incorporate their outfalls into the *E. coli* monitoring data that was collected for several years. I think the biggest culprits are the streams that are draining relatively large watersheds with a variety of land uses in them and have many sources of bacteria.

Q: U.S. Steel rechanneled the Grand Calumet River at one time, early in its history, so that the entire Grand Calumet River consisted of the effluent from the steel plant.

Greg Olyphant

The Grand Calumet is another issue. I thought you were referring to the Little Calumet River.

Q: Yes, I was talking about that too.

Greg Olyphant

The Grant Calumet is definitely another beast far to the west of us, and I haven't had the opportunity to look closely or model any of the beaches affected by its outfall.

Q (Steve Weisberg, SCCWRP): You (Greg) and Richard both made very compelling cases that your modeling efforts provide superior prediction to actual conditions than yesterday's measurements. I have a three-part question. First, do you think your models are sufficiently advanced that you would recommend that people should be using them in place of yesterday's samples for beach warnings? Second, are people using them in that case? Third, if they are not, what do you perceive as the biggest gap that keeps them from moving in that direction?

Greg Olyphant

I'll speak for the five cases that I have been involved in. Yes, I recommend that every beach initiate a monitoring program along with their existing monitoring program for water quality to monitor hydrometeorological conditions with an eye towards developing a forecasting model, but never cease actual water quality monitoring because that will be the basis for improving the ability of the model in the long haul, validating it in cases of possible litigation, and rejecting it if it's bad in the long haul. Basically, I think having one good correlation set in 2063rd beach, I was not very confident. But, having three additional sites this year at similar beaches that gave almost identical results makes me feel far more confident. However, I am not sure at all about ocean beaches because I have not had the opportunity to work in one of those.

Q: (Steve Weisberg) Are people adopting them at this point?

Greg Olyphant

I think Chicago beaches are moving towards predictive modeling. The interagency taskforce in the last meeting that I attended said that this is what we should be pushing. Every municipality



should make the investment for the model, because the overall investment is not that great, but it would allow themselves to have a much more effective basis for warning the public and having a comfort factor of their own in regards to the decisions they are making. In Michigan, people are very uncomfortable with their decisions because they have seen the history of false positives.

Comment (Richard Whitman, USGS): It is difficult to isolate your individual questions because there are political, social, and health concerns that all interact in a manager's mind when he or she asks, "am I going to go with an untested, unvalidated by EPA tool in lieu of something that I know is safe?" If they allow people in the water, then as long as they use the EPA recommended guidelines and results from samples collected yesterday were good, then they feel they are okay legally in terms of protecting the public. I don't know anyone that will throw away the EPA guidelines and switch completely to the predictive mode. I think they will use the model as a supplement to the monitoring.



High Frequency Radar Provides Real Time Data for Enhancing Beach Monitoring Programs

Eric Terrill

Scripps Institution of Oceanography, University of California at San Diego

Biosketch

(Not submitted)

Abstract

(Not submitted)



Questions and Answers

No questions.



Rapid Measurement of Bacterial Fecal Pollution Indicators at Recreational Beaches by Quantitative Polymerase Chain Reaction

Richard Haugland

U.S. Environmental Protection Agency, Office of Research and Development

Biosketch

Dr. Haugland is microbiologist in the Microbiological & Chemical Exposure Assessment Research Division, National Exposure Research Laboratory, Office of Research and Development. He received a B.S. in Biology at Muskingum College and a Ph.D. in Developmental Biology at the Ohio State University. His past research has addressed diverse problems including improvement of nitrogen fixation in crops, biodegradation of hazardous chemicals in the environment, assessment of the microbiological quality of indoor environments, and most recently, water quality monitoring and homeland defense. A common component of all of these research activities has been the application and development new molecular technologies. Dr. Haugland joined the USEPA in 1991. Since then he has authored or co-authored over 20 publications and has received a number of awards for his work including the EPA bronze and gold medals.

Abstract

Previous studies have demonstrated that measurements by the membrane filtration (MF) method of *Enterococcus* fecal indicator bacteria in recreational beach water samples are correlated with swimming-associated gastroenteritis. This relationship currently serves as a basis for recom-

mended guidance by the USEPA on unacceptable health risks associated with swimming in both fresh and marine waters. The MF method, however, requires at least 24 hours for results and during this delay swimmers may be exposed to unsafe waters. The quantitative polymerase chain reaction (QPCR) method is presently being evaluated as a possible alternative to MF. Water analyses using this technology can provide results in approximately 2 hours. In the summer of 2003, studies were conducted by several organizations including USEPA, Office of Research and Development, USEPA Region I, and the Southern California Coastal Water Research Project at both freshwater and marine beaches to determine the correlation between results of the QPCR and MF methods. Two of these studies also tested a newly developed assay for fecal indicator bacteria in the class *Bacteroidetes* and collected data on swimmer illness rates that are being compared with the QPCR and MF results. In recognition of the performance of this method to date, the USEPA Office of Water is considering its use as a reference method in performance evaluations of alternative nucleic acid tests for fecal contamination in ambient waters. This presentation will provide an overview of the QPCR method, describe its present application for beach water quality analysis and discuss the relationship between QPCR and MF measurements of enterococci based on comparative data from several studies.



1

Rapid Measurement of Bacterial Fecal Pollution Indicators at Recreational Beaches by Quantitative Polymerase Chain Reaction

Richard A. Haugland
USEPA, Office of Research and Development,
National Exposure Research Laboratory

Presented at National Beaches Conference,
October 13-15, 2004

2

How Does Quantitative PCR Analysis Work?

Principles of QPCR Analysis

3

PCR: POLYMERASE CHAIN REACTION

DNA Amplification Using Polymerase Chain Reaction

- PCR is now a widely used laboratory method for detecting specific DNA (or RNA) sequences that can originate from specific organisms, e.g. fecal indicator bacteria
- It does this by making copies of these sequences (amplification) in large enough numbers (e.g. Millions) to allow their detection - usually after the amplification is completed.
- Quantitative PCR (QPCR) differs from conventional PCR by detecting these copies with a fluorescent probe directly in the instrument as the reaction proceeds - for this reason it is also often called real time PCR.

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QPCR Growth Curves

- The quantitative capability of real time PCR stems from the direct correlation that has been shown between the starting number of target sequence copies in the sample and the number of amplification cycles required for the instrument to first detect an increase in probe fluorescence associated with the generation of new copies.
- The cycle numbers where the probe fluorescence curves cross a threshold value (red line near the bottom of the figure) that is significantly above the background fluorescence (purple line at the very bottom) are automatically reported by real time PCR instruments.

● 100 pg DNA ● 10 pg DNA
● 10 ng DNA ● 1 pg DNA
● 1 ng DNA ● no DNA

*Cycle Threshold: Cycle # at which growth curve = 20 fluorescence units (significantly above background)

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- Assuming that the recovered quantity of DNA sequences from the target organisms is consistent in the sample extraction process, this same relationship will also hold true for target cell numbers versus cycle threshold values.
- Hence, log-linear standard curves, such as the one shown in this figure, can be generated for the quantitation of target organisms in similarly processed test samples.

Enterococcus QPCR assay Standard Curve

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10 Steps and 2 Hours to Recreational Water Quality Results

Procedures for Quantitative PCR analysis of fecal indicator bacteria



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Step 1. Collect water sample



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Step 2. Filter water sample



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Step 3. Transfer filter to extraction tube containing glass beads, buffer and positive control DNA



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Step 4. Bead mill filter membrane for 1 min to break cells and release DNA



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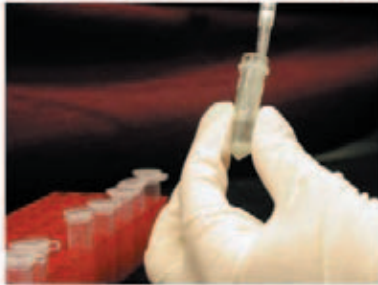
Step 5. Centrifuge briefly



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Step 6. Recover liquid sample with released DNA from extraction tube




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Step 7. Transfer sample to reaction tube containing PCR reagents



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Step 8. Place reaction tube in real-time thermal cycler



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Step 9. Run reaction in thermal cycler



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Step 10. Import run data into spreadsheet and calculate target cells in sample

Sample	Enteric Control	dCT	Calib. dCT	dΔCT	Ratio	Calib. cells	QPCR cells
5A	22.82	26.43	3.61	-4.97	1.36	0.39	1.03E+005
5B	33.56	37.23	3.67	-4.97	1.30	0.41	1.03E+005
5C	32.87	37.09	4.22	-4.97	0.75	0.59	1.03E+005
7A	33.98	28.24	4.84	-4.97	2.81	0.80	1.03E+005
7B	32.87	28.56	4.31	-4.97	2.29	0.80	1.03E+005
2C	33.61	28.99	4.62	-4.97	2.59	0.80	1.03E+005

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Epidemiological and Environmental Assessment Studies

QPCR vs. Membrane Filtration Results

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U.S. EPA, N.E.E.A.R. Study, 2003-2007

- **Two Freshwater Beaches in 2003:**
 - West Beach, Indiana Dunes National Lakeshore, Lake Michigan
 - Huntington Beach, Day Village Ohio, Lake Erie
- **New water monitoring protocol:**
 - 6-8 sampling locations / beach
 - Three sampling visits / day
 - 2-3 days / week (weekends and holidays)
 - 9-10 weeks / beach
- **Survey of swimming-associated health outcomes**
- **Two analytical methods for enterococci in water samples (QPCR and EPA Method 1600 membrane filtration)**

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Southern California Coastal Water Research Project, Mission Bay Study, 2003

- 6 Marine Beaches within an Embayment
- Water monitoring protocol similar to EPA study:
 - 2-5 sampling locations / beach
 - Two sampling visits / day
 - 2-3 days / week (weekends and holidays)
 - 10 weeks / beach
- Survey of swimming-associated health outcomes
- Same analytical methods as EPA study – QPCR and Membrane Filtration for enterococci in water samples

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2004 Rapid Methods Comparison Study

Organized by Southern California Coastal Water Research Project

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Study Design

1. Tested four potential rapid methods (including QPCR) for enterococci in parallel with standard MF and MPN methods performed by several Southern CA water testing laboratories.
2. Matrices were offshore marine water samples spiked with "known" enterococci CFU numbers from sewage or urban runoff and several ambient marine and freshwater samples with high expected enterococci numbers.
3. Potential interferences from humic acids and suspended solids present in some samples
4. Results for rapid methods reported at 2, 4, 6 and 8 hrs.

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Future Advances in Methodology

- Elimination of Interferences
- Improved Positive Control Assays
- Bacteroides assay

26

Conclusions

- QPCR results to date show good correlation with results of the current MF method (particularly at high pollution levels) and also with health data
- The QPCR method may be useful at this time as a warning system for high pollution levels but confirmation with other methods is still recommended
- Results from ongoing epidemiological studies may lead to the development of new criteria for beach closings based on same-day measurements by this method



Questions and Answers

No questions.



Recreational Water Testing by Rapid, High-Throughput Real-Time Quantitative PCR (QPCR) for Fecal Indicators

Jack Paar

U.S. Environmental Protection Agency, New England Region Lab

Biosketch

Mr. Jack Paar, III is Biologist with the US Environmental Protection Agency, New England Regional Laboratory, Office of Environmental Measurement and Evaluation, Ecosystem Assessment Unit, Ecology Monitoring Team, in North Chelmsford, MA. Mr. Paar majored in Oceanography at the US Naval Academy from 1975 to 1977. After honorable discharge from the Navy he transferred to Northeastern University (NU) in Boston, MA and participated in the Co-Op Education Program. Mr. Paar worked as a Student Biologist from 1979 through 1981 in the US EPA New England Regional Laboratory, Lexington, MA Biology Section. Assisting senior biologists in both field assessment and laboratory analysis he gained considerable experience in sediment oxygen demand assessments, whole effluent toxicity testing, and test organism culturing. Upon graduation in 1981 with a B.S. in Biology Mr. Paar worked until 1990 as the Laboratory Manager of NU's Marine Science Center (MSC) in Nahant, MA. While at the MSC he worked as marine aquarist, rocky sub-tidal ecology research diver, research photographer, and diving safety officer. In 1990 Mr. Paar once again joined the ranks of the US EPA as a biologist. For 11 years he served as the EPA NPDES Regional Technical Advisory

Committee Power Plant Assessment Biologist, also concentrating in sediment and aquatic toxicity testing. In 1995 Mr. Paar took over management and coordination of the US EPA Water Microbiology Laboratory and obtained qualification as the Regional Drinking Water Microbiology Laboratory Certification Officer overseeing and auditing the six New England State principal water microbiology laboratories for compliance with Safe Drinking Water Act regulations. Along with his colleagues he helped design biology laboratories in the new US EPA state-of-the-art Regional Laboratory and was one of the principal designers of a one-of-a-kind automated sediment toxicity test chamber. In 2002 Mr. Paar obtained sufficient funding and support to open a new Genomics Laboratory at the US EPA Lab focusing on Microbial Source Tracking and rapid fecal indicator assessment. In 2003 Mr. Paar obtained his certification as a Contracting Officer Representative and began contractor oversight as a Task Order Project Officer. He is currently directing genomic research by the Lockheed/Martin Environmental Service Assistance Team for superfund and non-superfund research, developing high through-put genotypic test methods using Real-Time PCR to quantify and identify dehalogenating bacteria at hazardous waste sites and fecal indicators and pollution sources in fresh and marine surface waters.

**Mark Doolittle**

U.S. Environmental Protection Agency, Northeast Regional Laboratory

Biosketch

Mark Doolittle is Senior Discovery Biologist for Lockheed Martin Environmental Assistance Team working as a contractor to the US EPA at the New England Regional Laboratory in North Chelmsford, MA under the direction of Jack Paar, US EPA Project Officer. Mr. Doolittle received his B.S. in Biology from S.E. Massachusetts University (subsequently re-named U-Mass/Dartmouth), his Masters in Microbiology from University of Tennessee/Knoxville, and completed doctoral graduate work in Molecular Biology at Vanderbilt University in Nashville, TN and in Environmental Sciences at U-Mass/Boston. He was awarded a Fulbright Scholarship to study the interaction of bacteriophage with bacterial biofilms at the University of Saskatchewan in Canada. He has worked in the industrial sector as a staff microbiologist in the Gillette Corporation Personal Care Product Division and in the public sector as a contract environmental microbiologist for the Massachusetts Department of Environmental Protection. As a graduate student at U-Mass Boston, he worked for the Metropolitan (Boston) District Commission (MDC), renamed the Department of Urban Parks & Recreation (DUPR), collecting beach water samples and analyzing them at the Massachusetts Water Resources Authority Laboratory (MWRA) at Deer Island. Several years later, the MDC hired him to manage the water quality monitoring program for the MDC's 19 marine and freshwater beaches during the summer bathing months in which he spent a lot of time trying to identify the sources of fecal contamination affecting the beach. In his current position for almost 2 years, Mr. Doolittle has worked on lab development and field testing of Real-Time PCR assays to quantify genomic DNA of fecal indicators and dehalogenating bacteria at Superfund sites.

Abstract

Current microbiological methods for determining water quality for recreational swimming and bathing at public and private beaches measure the number of culturable fecal indicator bacteria, *Escherichia coli* and Enterococci, per 100-mL volume. These methods which utilize Membrane Filtration (Standard Method 9222, EPA Modified *E. coli*

Method, EPA Method 1600 for Enterococci) and Most Probable Number (Standard Methods 9223 Chromogenic-Fluorogenic Colilert or Enterolert) require incubation periods of 18 to 28 hours in addition to sample transport and processing times before verifiable counts of colony-forming-units (CFU) or Most-Probable-Number (MPN) of *E. coli* and Enterococci can be obtained. Due to the episodic nature of fecal contamination events (e.g., sewer and storm water drainage, etc.) and changes in the natural forces (e.g., wind, tides, river and spring flows, UV radiation, etc.) that transport, dilute, and irradiate surface waters, significant temporal and spatial variation can occur in the concentration of fecal indicators in recreational waters. EPA New England has developed a high-throughput DNA Isolation Procedure and Real-Time Quantitative-PCR Assays for identifying and quantifying *E. coli* in recreational waters. Purified DNA extracted from filter retentates of freshwater samples collected along the Charles River (Boston & Cambridge, MA) & Furnace Brook (Quincy, MA) and of marine samples collected at Carson Beach (So. Boston, MA) and Wollaston Beach (Quincy, MA) were analyzed by PCR and standard culturable assays. Numbers of Genomic Equivalents (GEQs) of *E. coli* were strongly correlated with numbers of culturable *E. coli* present in freshwater samples. Lower, non-optimal correlation was observed for *E. coli* GEQs versus CFUs in marine water samples, most likely due the increased rates of *E. coli* die-off in saltwater and temporal and spatial distance from fecal pollution sources. The log-transformed results of PCR analyses performed with two different *E. coli* PCR primer probe sets, one hybridization probe set (rod-A) and one hydrolysis probe set (uid-A), upon replicate aliquots of DNA extracts of the Charles River water samples, were plotted against results of culturable *E. coli* assays. The regression curves (i.e. equations) for both primer-probe sets were similar but the rod-A set had more consistent performance characteristics with a greater positive correlation factor and a GEQ/CFU ratio closer to 1.0. The robustness, specificity, and consistent performance of the rod-A PCR assay makes it a excellent candidate for implementation, real-time quantitative or MPN (presence/absence) formats used to screen recreational water samples for same-day detection of excessive levels of *E. coli*.



US EPA Region I
New England Regional Laboratory

High-Throughput Q-PCR for Fecal Indicators

Mark Doolittle¹, Jack Paar III², Michael Ferrier², Louie Pounds¹
¹United States Environmental Services & Assistance Team, Lowell, MA
²U.S. EPA Region I, NESL-CRMC, Ecosystems Assessment, N. Chatham, MA

USEPA New England

Disclaimer

Any mention of trade names or commercial products does not constitute endorsement or recommendation for use.

USEPA New England

"Mommy? When will we know if it's safe to swim in the water?"



"Sweetheart, we really won't know until tomorrow."

Solution

Reliable Methods that Inform Bathers of Water Quality the Same Day.

USEPA New England

Current Recreational Water Test Methods

Culturable Assays for *E. Coli* & Enterococci

- Membrane Filtration (24 H Incubation)
- MPN W/ Quantitray (18-28 H Incubation)

Reported Results:

- Informs Bathers of Yesterday's Water Quality

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EPA Region I
Genomics Project Goal

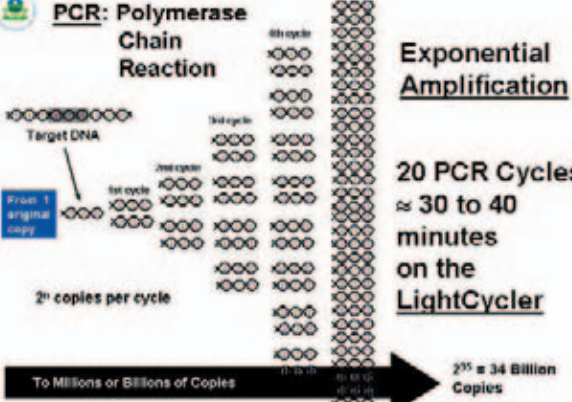
Develop and Validate . . .

- Rapid
- High-Throughput
- DNA Isolation and Quantification
- Fecal Indicator

. . . PCR Methods

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PCR: Polymerase Chain Reaction



Target DNA

From 1 original copy

2ⁿ copies per cycle

20 PCR Cycles ≈ 30 to 40 minutes on the LightCycler

2²⁵ = 34 Billion Copies

To Millions or Billions of Copies

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Technical Hurdles

- *Design & Synthesize Primers & Probes Specific for Fecal Indicators*
- *Consistently Remove or Suppress PCR Inhibitors*
- *Maximize Sample Equivalent Volumes (SEVs) of Concentrated & Purified DNA*
- *Simplify, Standardize & Keep it Quantitative DNA Extraction & PCR Methods (Automate)*

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Region I PCR Instrumentation

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Clean DNA for PCR

Marine & Freshwater Sample

MagNA Pure-LC DNA Isolation Protocol

- **Vacuum Filter** 25 - mL
- **Bead Beat** in Lysis Buffer (MagNA Lyser)
- **Digest** 65°C (*Omit Boiling!!*)
- **Pellet** Cell Debris
- **Transfer** Lysate to MagNA Pure LC
- **Extract / Purify** DNA
- **Elute** 100 - μ L DNA extract (Conc. Factor = 250X)

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Field Sample Filter Lysate Extract

USEPA New England

MagNA Pure Principles

A. sample
B. Cell lysis
C. Magnetic beads added
D. N. acid binds to beads
E. N. acid washed
F. N. acid separated from beads

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100- μ L Purified Sample DNA

USEPA New England



LightCycler Glass Capillaries

plastic stopper
plastic pipetting chamber
glass capillary
outer diameter: 1.55 mm
max. volume: 20 µl

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Fluorescence Detection

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Processing & Analysis Times

- Sample filtering & DNA Extraction

MagNA Pure Compact (8)	1.5 h	---
MagNA Pure LC (32)	---	2.5 h
- Loading of PCR reactions: 0.25 - 0.5 h
- PCR thermocycling & data analysis: 0.75 - 1.0 h
- Total Elapsed Time (8 - 32 samples): 2.5 - 4.0 h

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E. coli Primers & Probes

rod-A (mrd-B)

- Hybridization (FRET) Probes
- (FAM anchor & TAMRA sensor)
- 181-189 bp amplicon
- single-copy cell division gene

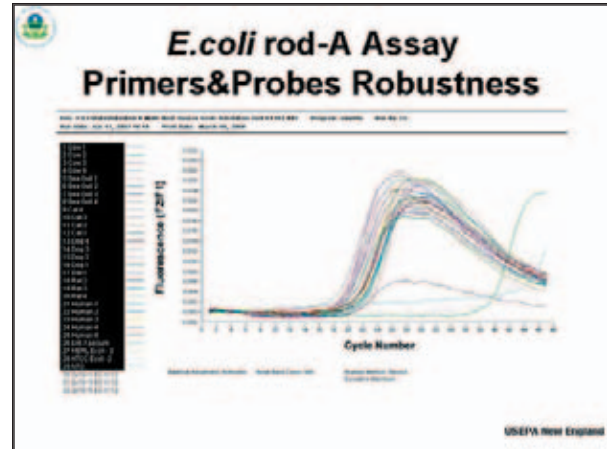
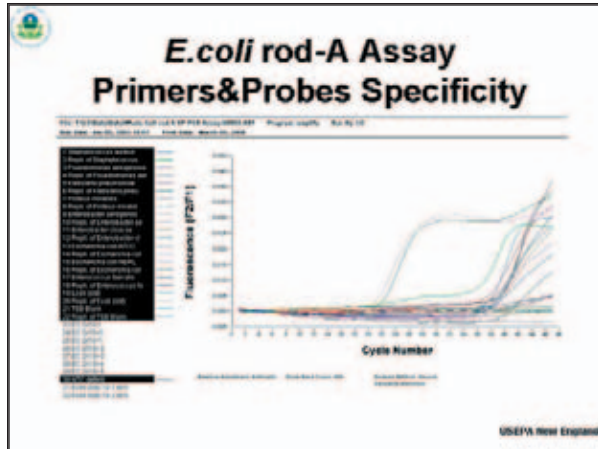
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E. coli rod-A Assay Standard Quantitation Curves

USEPA New England

E. coli rod-A Standard Curve

USEPA New England



- ### E.coli rod-A Assay Attributes
- PCR Reactions
 $\leq 20\text{-}\mu\text{L}$ of $100\text{-}\mu\text{L}$ Eluate (SEV = 5 mL)
 - MDL= 20 Genomic Equivalents(GEQs) of *E.coli* (≈ 10 cells)/100-mL
 - GEQ:CFU ratio $\approx 2:1$
- USEPA New England

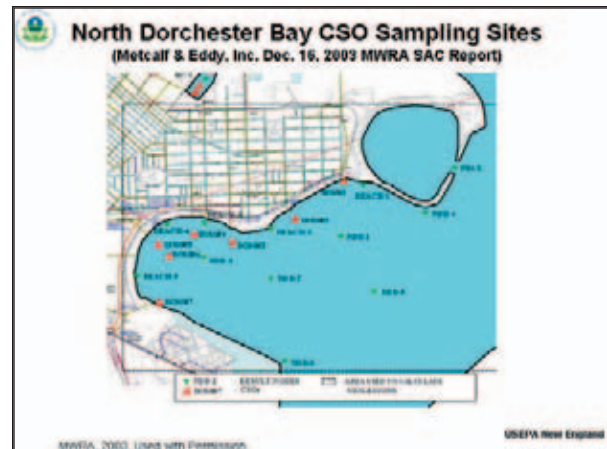
- ### Field Validation Challenges for E.coli rod-A Q-PCR Assay In Marine and Freshwater...
- Consistently & Accurately Isolate & Quantify *E.coli* DNA with High-Throughput
 - Correlate Q-PCR Results with
 - Rates of GI illness (i.e., EPI studies)
 - Culturable *E.coli* Results (Gold Standard) & Safe Recreational Water Quality Limits
- USEPA New England

EPA Recreational WQ Standards Fecal Indicators (CFU/100-m)

Upper Confidence Limit (%)	75	82	90	95
Fresh				
<i>E. coli</i> SDM*	235	298	406	576
GeoMean (8 %)	126			
ENT. SDM	61	89	108	151
GeoMean	33			
Marine				
ENT. SDM (19 %)	104	158	276	500
GeoMean	35			

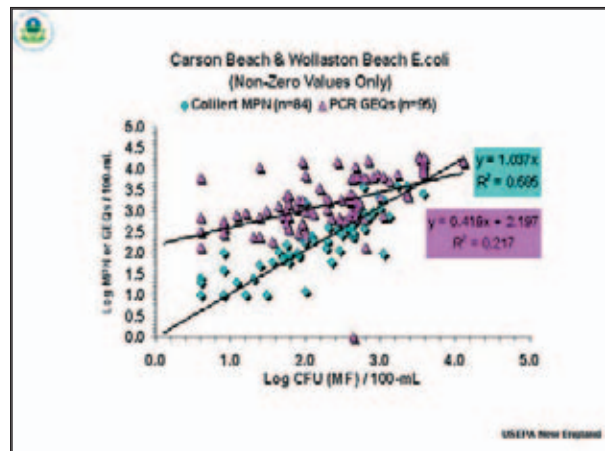
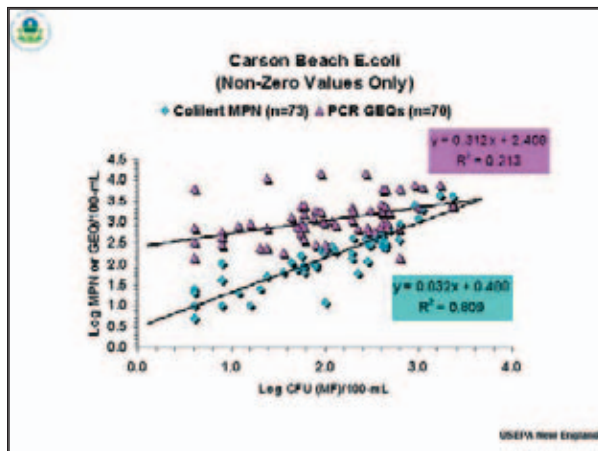
*SDM – Single Day Sample Maximum

USEPA New England

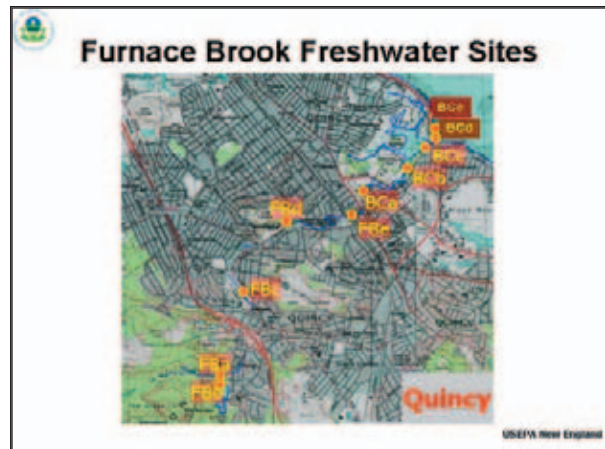


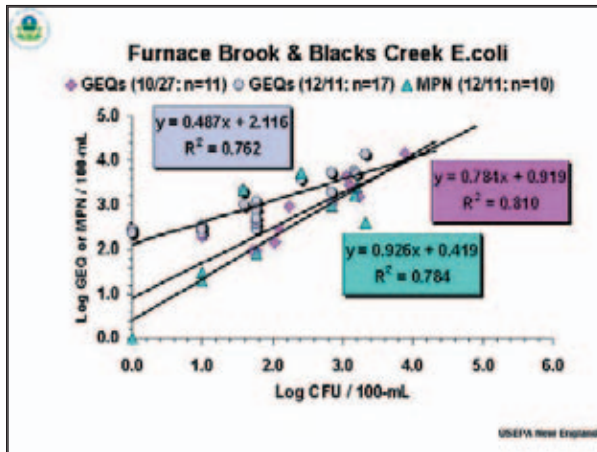
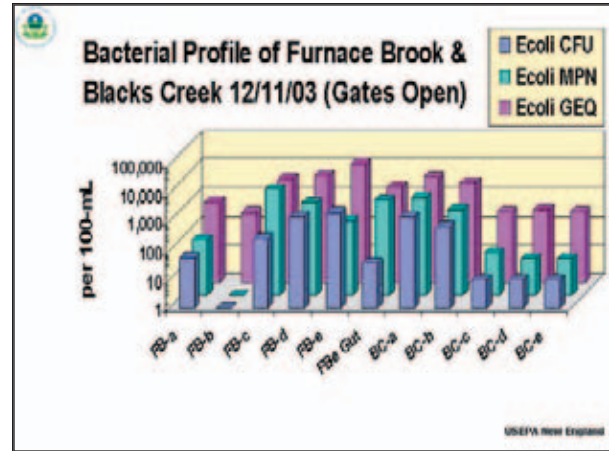
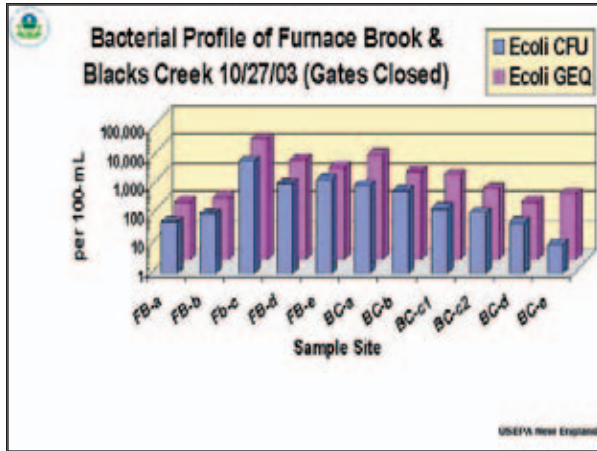


- ### Q-PCR Comparison to "Gold Standards"
- #### *E. coli* (EC)
- Membrane Filtration (Modified mTEC)
Colony-Forming-Units (CFU)
 - Idexx Quantitray 2000 (Colilert)
Most Probable Number (MPN)
 - MagNA Pure LC DNA Isolation & Q-PCR
Genomic Equivalents (GEQs)
- USEPA New England



- ### Summary of Marine Results
- Observed low correlation between Q-PCR (GEQs) & *E. coli* CFU & MPN
 - Consistent with rapid die-off in saltwater
 - *E. coli* Q-PCR Assay may still correlate with EPI Study results & Enterococcus CFU & MPN
 - *E. coli* Q-PCR Assay may have utility along with Enterococcus Q-PCR
- USEPA New England





Charles River Testing Objective

Compare Q-PCR Quantification of *E. coli* GEQs in DNA Extracts using

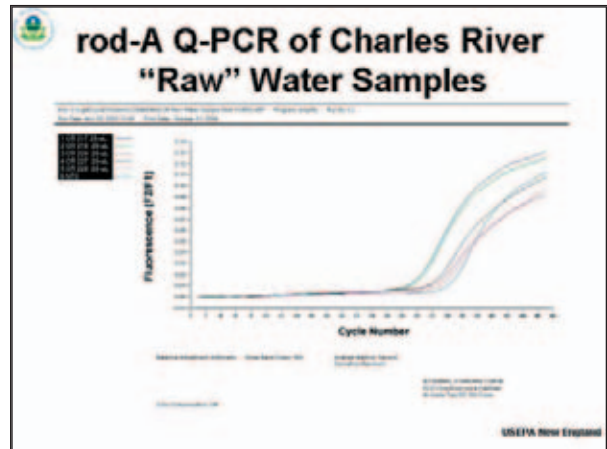
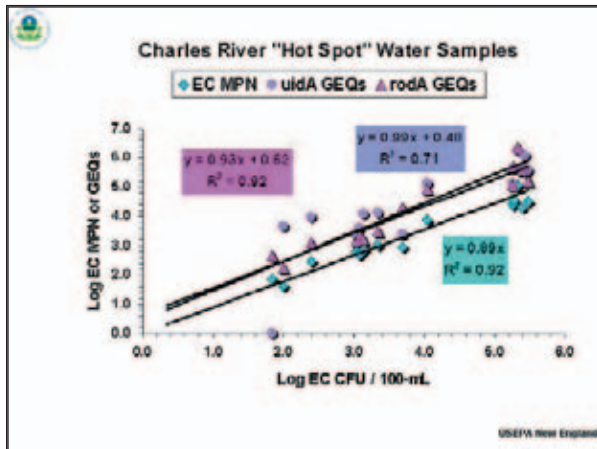
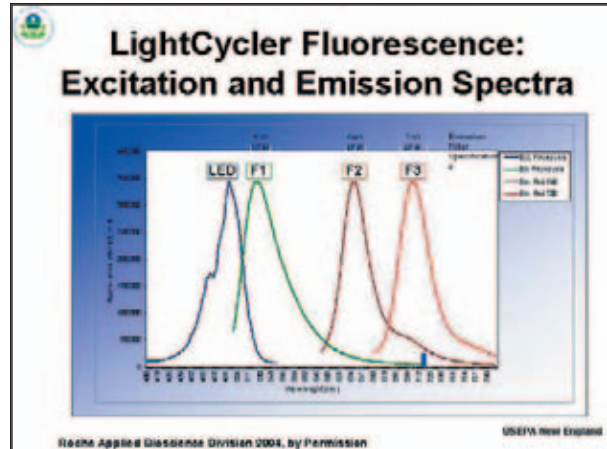
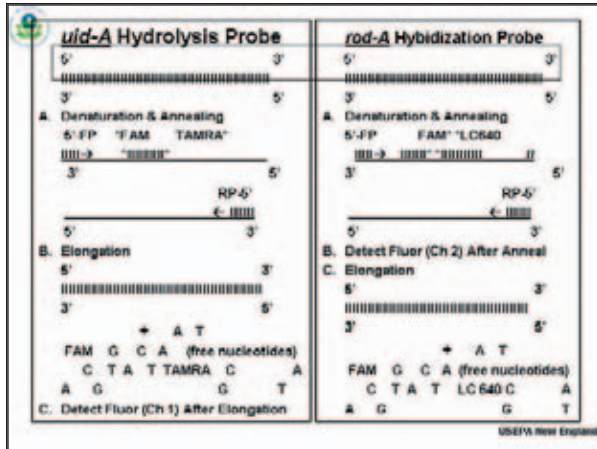
rod-a Hybridization Probe Set
&
uid-a (TaqMan) Hydrolysis Probe Set

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E.coli Primers & Probes

<i>uid-A (gus-A)</i>	<i>rod-A (mrd-B)</i>
<ul style="list-style-type: none"> • Single Hydrolysis (FAM-TAMRA) Probe • 117 bp amplicon • B-glucuronidase gene • Specific to <i>E. coli</i> & <i>Shigella</i> spp. 	<ul style="list-style-type: none"> • Two Hybridization (FRET) Probes • (FAM anchor & TAMRA sensor) • 181-189 bp amplicon • single-copy cell division gene

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CR "Raw" Water Q-PCR GEQs

CR No.	CFU /100-mL	CFU /20-uL	GEQ /20-uL	GEQ /100-uL*
217	144,000	28.8	200	1000
218	173,000	34.6	177	885
224	17,800	3.6	35	175
227	1,700	0.34	43	215
228	4,200	0.82	26	130
RS	235	0.05	>0.5	2.5

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- Summary of Freshwater Results**
- Good Correlation between *E. coli* MF (CFU) & Q-PCR (GEQ)
 - Exceedances of Safe Recreational Water Quality Limit for *E. coli* may be quantifiable by Q-PCR within 1-2 hr by assaying water sample directly
- USEPA New England



Value of PCR for Recreational Water Testing

Pre-screening of samples by Q-PCR will enable:

- Decisions to
 - perform or omit MF or Quantitray assays
 - adjust sample volumes of MF & MPN Assays
 - post beaches the same day
 - re-open a closed beach a day early
- Additional same-day sampling to verify persistence of contamination
- Continuous screening

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Q-PCR Pre-Screening for Beaches (for Discussion Only)

	AM	PM
PCR Action Level	> PCR Action Level CLOSE BEACH	Q-PCR and MF/MPN
500 GEQ		
250 GEQ (= GM 126 CFU)	< 500 GEQ Beach Open. No MF or MPN	Q-PCR Screening
MDL		

USEPA New England

Acknowledgments

Dr. Matt Liebman, EPA Region I, BEACHES Program
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 US EPA ORD Regional Applied Research Effort FY 04

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Charles River PCR (GEQ) Ratios

Sample Collection Date	uld-A		rod-A		GEQ	
	UKs	per CFU	per MPN	UKs	per CFU	per MPN
21-Jul-2004	Mean	15.0	26.2	Mean	2.6	3.4
	SD	17.8	38.0	SD	2.1	1.6
	n	9	9	n	9	9
18-Aug-2004	Mean	3.9	12.8	Mean	4.0	13.8
	SD	4.6	6.7	SD	3.9	6.8
	n	7	7	n	7	7

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Charles River F-LFBs & F-LFMs

Unit	Stat	GDs Per		GDs Per		Stat	GDs Per		GDs Per	
		25-mL	(PCR reps)	25-mL	(Filter reps)		25-mL	(PCR reps)	25-mL	(Filter reps)
F-LFB	Mean	1,061,300		1,061,350		Mean	660,600		664,463	
	SD	360,768		430,901		SD	84,617		28,904	
	RPD	34.9		41.4		RPD	6.9		3.0	
	n	4 (2)		2		n	5		2	
CR204-FLFM	Mean	943,733		943,733		Mean	566,120		500,517	
	SD	169,590		51,336		SD	131,601		78,837	
	RPD	17.9		6.4		RPD	23.1		13.8	
	n	4 (2)		2		n	8		2	
CR208-FLFM	Mean	2,173,733		2,097,194		Mean	1,792,950		1,792,950	
	SD	434,417		851,212		SD	247,291		282,066	
	RPD	30.0		26.9		RPD	13.4		16.7	
	n	6 (2)		2		n	4		2	

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Dilution of PCR Inhibition

E.coli PCR Assay Sample Name	Sample Vol. (µL)	Calc. # EC GEQs/ Sample Vol.	Cross Pl (Cp)	Calc. # EC GEQs/ 100-mL
CB-017-F-102603	10	11	34.68	338
CB-017-F-102603-5ul	5	10	34.71	360
CB-017-F-102603-2ul	2	10	34.68	2,400
CB-004-F-102703	10	0	> 43	0
CB-004-F-102703-5ul	5	4	35.03	354
CB-004-F-102703-2ul	2	1	37.64	340
CB-021-M-102703	10	0	> 43	0
CB-021-M-102703-5ul	5	9	34.89	364
CB-021-M-102703-2ul	2	39	32.76	3,360
CB-019-M-102703	10	8	35.03	334
CB-019-M-102703-5ul	5	7	35.18	372
CB-019-M-102703-2ul	2	12	34.43	2,500

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EC DNA Recovery

2500 EC				
AC'd Comp Sample	Mean % Rvry	SD	n	EC Bkgrd
FB	38.6	8.9	12	33
WB	50.2	10.5	12	36
CB	53.3	9.1	12	168
LB	22.1	10.8	12	14

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EC DNA Recovery

QA/QC	E.coli		
	Mean % Rcvry	Range of % Rcvry	n
Filter-LFM	241.0	2 - 785%	13
Filter-LFB	135.3	41 - 279	11
PCR-LFM	66.0	0 - 117	8
PCR-LFB	N/A	N/A	
	CV	Range of CV	n
Lab Filter Dups	0.53	0.00 - 1.35	12
Lab PCR Rcn Dups	0.20	0.00 - 1.27	12
Field Sample Dups	0.47	0.00 - 1.12	9
Temporal Field Dup	0.37	N/A	1

USEPA New England



Questions and Answers

No questions.

Thursday, October 14
10:20 a.m. – 12:00 p.m.

**Concurrent Track II:
Changes on the Horizon
Session Nine:
New Health Risk Indicators**



Comparative Testing of Rapid Microbiological Indicator Methods for Marine Recreational Water Monitoring

Stephen Weisberg

Southern California Coastal Water Research Project

Biosketch

Dr. Stephen Weisberg is Executive Director of the Southern California Coastal Water Research Project (SCCWRP) where he specializes in the design and implementation of environmental monitoring programs. He serves as chair of the Southern California Bight Regional Monitoring Steering Committee, which is responsible for developing integrated regional coastal monitoring for the Southern California Bight. He also serves on the Steering Committee for the US Global Ocean Observing System (GOOS), the National Oceanographic Partnership Program's Ocean Research Advisory Panel, the Alliance for Coastal Technology Stakeholder's Council, the State of California's Clean Beaches Task Force, the National Research Council Committee on Waterborne Pathogens and on Technical Advisory Committees for the University of Southern California Sea Grant Program and the Southern California Wetlands Recovery Program. Dr. Weisberg received his undergraduate degree from the University of Michigan and his Ph.D. from the University of Delaware.

Abstract

Current methods for enumerating indicator bacteria require an incubation period of 18 to 96 hours, during which time contaminated beaches remain open. Several technologies that have the potential to produce results in less than four hours are under development. Here we evaluated four of those technologies, including immunomagnetic capture with ATP quantification, flow cytometry, dual wavelength fluorimetry, and quantitative PCR (Q-PCR). Fifty-four blind samples encompassing a range of bacterial concentrations and matrix complexity were processed and compared to values obtained by standard culture-based methods performed at six reference laboratories. Each method was evaluated for speed, accuracy, sensitivity, precision, robustness across different matrices, as well as ease of use.



Questions and Answers

No questions.



Assay and Remote Sensor Development for Molecular Biological Water Quality Monitoring

Kelly Goodwin, Ph.D.

National Oceanic and Atmospheric Administration (NOAA), Atlantic Oceanographic & Meteorological Laboratories, Ocean Chemistry Division

Biosketch

Dr. Kelly Goodwin is a Principal Investigator with the National Oceanographic and Atmospheric Administration (NOAA) at the Atlantic Oceanographic and Meteorological Laboratories (AOML) in Miami, Florida. Dr. Goodwin received a B.S. degree in Neurobiological Sciences from the University of Florida. She received M.S. ('90) and Ph.D. ('96) degrees in Environmental Engineering Science from the California Institute of Technology in Pasadena. She received a minor in Oceanography from Caltech during a program in residence at the Scripps Institute of Oceanography ('93). From 1995-1998, she served as a National Research Council Postdoctoral Associate at the U.S. Geological Survey in Menlo Park, CA working on the microbial biogeochemistry of halocarbons. In 1999, she returned to Florida as a researcher with NOAA's joint institute with the University of Miami, the Cooperative Institute of Marine and Atmospheric Studies (CIMAS). She entered federal employment with NOAA in 2003 and became adjunct faculty to the University of Miami's Rosenstiel School of Marine and Atmospheric Science. Her research interests include development and application of biotechnology to improve coastal water quality monitoring.

Abstract

Molecular tools are a promising means to provide rapid and accurate monitoring of coastal water quality. We are developing three nucleic acid hybridization assays to identify and monitor nuisance organisms (bacterial and algal) in coastal waters. A microplate assay returns a rapid colorimetric result and provides moderate throughput at relatively low cost. A Luminex Xmap™ system rapidly provides high throughput and the potential to screen for a large number of targets simultaneously. Electrochemical detection is a cutting edge technology suitable to the size, power, and cost requirements of remote sensing. An overview of the development and application of these technologies will be presented.



Assay and Sensor Development for Molecular Biological Water Quality Monitoring

Kelly D. Goodwin, Ph.D.
NOAA/AOML
Ocean Chemistry Division



Assays Need Improvement

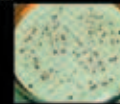


Harmful Algae:

- require extensive microscopic expertise
- hard to distinguish closely related species
- samples fragile & hard to preserve

Fecal Contamination:

- labor intensive
- take too long
- measure indicators vs. pathogens



Molecular Biological Approach



- ✓ Sensitive
- ✓ Specific
- ✓ Microscope & Culture Independent

molecular probes for:

- ✓ red tide dinoflagellates (*Karenia brevis*)
- ✓ sewage-indicating bacteria

potential targets:

- ✓ toxin genes
- ✓ pathogens (vs. indicators)

Goals:

- ✓ provide early and accurate detection
- ✓ source tracking capability
- ✓ improve temporal coverage
- ✓ aid ecological research
- ✓ supply management tool
- ✓ fast, convenient, economical

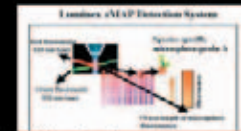
Microplate Assay

	Coli	Enti	Bdis
<i>Escherichia coli</i>			
<i>Enterobacter aerogenes</i>			
<i>Klebsiella pneumoniae</i>			
<i>Enterococcus faecalis</i>			
<i>Pseudomonas aeruginosa</i>			
<i>Bacteroides distans</i>			
<i>Bacteroides vulgatus</i>			
blank			

- ✓ Specific
- ✓ Detect Multiple Targets
- ✓ Medium Throughput, Relatively Low Cost

please see poster

Luminex



xMap™ technology, 100 assays performed in a single microplate well

- ✓ High Specificity
- ✓ Quickly Detect Many Targets
- ✓ High Throughput
- ✓ Flexible and Relatively Inexpensive vs. Microarrays

please see poster



Electrochemical Assay Development

- ✓ highly sensitive
- ✓ no fluorescent label
- ✓ compact sensors
- ✓ easily digitized signal


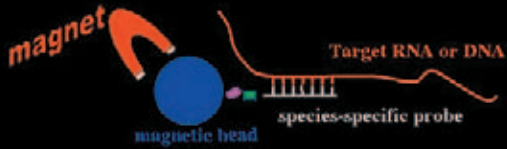




Disposable Electrodes

- ✓ Fast
- ✓ Inexpensive
- ✓ Miniaturized

Scheme 1: Magnetic Bead Separation


magnet

magnetic bead

species-specific probe

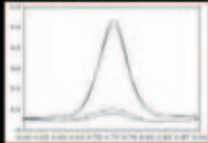
Target RNA or DNA

Detect DNA With a Pencil

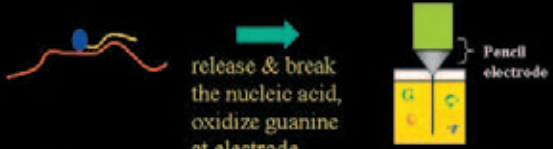


pencil electrode:

- ✓ carbon electrode
- ✓ modified mech. pencil
- ✓ can use a variety of detection methods
- ✓ can use a variety of probes

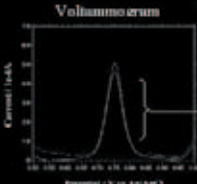


Direct Detection by Guanine Oxidation (no amplification)



release & break the nucleic acid, oxidize guanine at electrode

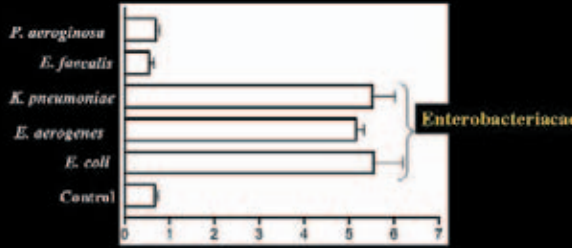
Pencil electrode



Voltammogram

Height is proportional to guanine concentration

Probes Work as Designed and Direct Detection using Crude CELL Lysate Demonstrated



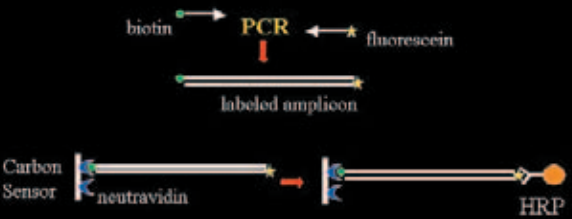
Species	Current (µA)
<i>P. aeruginosa</i>	~0.5
<i>E. faecalis</i>	~0.5
<i>K. pneumoniae</i>	~6.5
<i>E. aerogenes</i>	~6.0
<i>E. coli</i>	~6.0
Control	~0.5

Current (µA)

Guanine Oxidation

Enterobacteriaceae

Scheme 2: Detection of amplified DNA



biotin → PCR ← fluorescein



labeled amplicon

Carbon Sensor ← neutravidin → HRP

plug into instrument

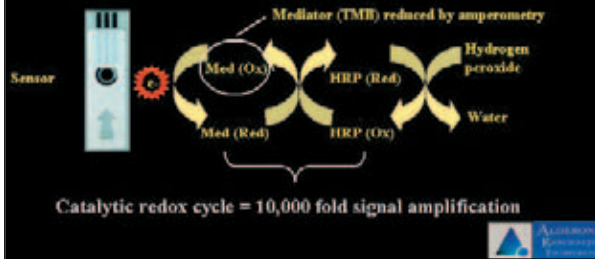
MEASURE CURRENT

Rapid Detect

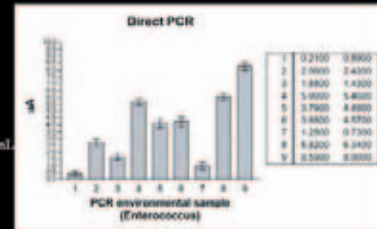


Simple, Sensitive & Versatile Sensor is target independent



Detection of Enterococcus in Environmental Samples (Water and Beach Sand)

- 1= no template
- 2= ES3 =338 CFU/100mL
- 3= KW1_7 =6 CFU/100mL
- 4= PS1 =1200 CFU/100mL
- 5= FS3 =338 CFU/100mL
- 6= FS3 =338 CFU/100mL
- 7= KW1_1000 =2 CFU/100mL
- 8= ES1 =706 CFU/100mL
- 9 = gDNA of *Enterococcus faecalis*



Encouraging Results

- ✓ Rapid and sensitive detection
- ✓ Simple and flexible methods
- ✓ Detection from environmental samples
- ✓ Inexpensive instrument and reagents
- ✓ Multi-target assays using 8-well and 96-well sensor strips
- ✓ Handheld or
- ✓ Potential to deploy on remote platform

Potential Uses

Detect presence of organisms for:

- Pathogen monitoring
- Source tracking
- Invasive species
- Geographic/ecological studies
- Reduced burden of microscopic examination or culturing

Challenges

- ✓ Appropriate target
- ✓ Detection limit issues
- ✓ Quantification
- ✓ Integration into remote platform



In-situ Sensor Development






- ✓ remote nucleic acid extraction
- ✓ quantify targets
- ✓ real-time data relay
- ✓ couple to environmental monitoring



high risk, high reward





Thanks To:





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Chris Scholin



ALDERON
BIOSCIENCES
INCORPORATED

NOPP/NSF
CICEET
NOAA OHH
NIEH/NSF OHH



Questions and Answers

No questions.



Quantification of Enterovirus in Seawater in Imperial Beach, CA using Real-Time RT-PCR

Rick Gersberg

San Diego State University, School of Public Health, Coastal and Marine Institute

Biosketch

Dr. Richard M. Gersberg is currently a Professor (and Head of the Division) of Environmental and Occupational Health in the Graduate School of Public Health at San Diego State University (SDSU), and Director of the Coastal and Marine Institute at SDSU. He has an M.S. degree in biology from the University of Houston, and a Ph.D degree in microbiology from the University of California, Davis. Dr. Gersberg specializes in water quality research, and has broad experience working with both chemical and microbiological pollutants and risk assessments.

Abstract

A real-time reverse transcriptase-polymerase chain reaction (RT-PCR) method utilizing the MGB Eclipse Probe System Kit (Amersham Biosciences) was used to detect and enumerate enteroviruses in ocean water samples were taken at the Tijuana River mouth (near the San Diego, California-Mexico border) and Imperial Beach pier (0.85 mile north of the Tijuana River mouth in San Diego, California) during rain events and dry weather. The samples consisted of 1-4 L of ocean water. Viruses were then concentrated by filtration through a negatively charged filter followed

by elution with sodium hydroxide. Following RNA extraction, RT-PCR, which included cDNA synthesis and real-time RT-PCR, was carried out on samples (in triplicate) using a BioRad iCycler real-time PCR system.

During rain events, the seawater samples appeared to contain inhibitors that effected real-time RT-PCR amplification; however diluting the cDNA samples diluted the inhibitors to such an extent that successful amplification could be achieved. For some of the samples, cDNA amplified by conventional RT-PCR, was cloned and sequenced to determine the specific type of enterovirus present in the samples. The relationship between indicator bacteria (fecal coliform and enterococci) densities and enterovirus concentrations was also determined to assess the validity of the bacteria indicator system for predicting viral levels in recreational beach waters of the U.S. influenced by contaminated runoff from Mexico. By relating the PCR-quantified densities to infectivity, our data were then evaluated in terms of a human health risk assessment for swimming or surfing at Imperial Beach, CA. The high sensitivity and high throughput capability of real-time RT-PCR should be useful in routine monitoring of viral pathogens in recreational beach waters for the assessment and protection of public health.



Questions and Answers

Q (Clay Clifton, County of San Diego Department of Environmental Health): When you say that the presence of the enterovirus was relatively low at Empirical Beach during dry weather, how did you define dry weather and what time of year were your samples taken?

Rick Gersberg

We had a dry summer, where it had not rained for a long period of time. So, we collected our samples during June, July, and August.



Rapid Detection of Enteroviruses in Environmental Samples using Real-Time Quantitative Reverse Transcriptase PCR

Rachel Noble

University of North Carolina at Chapel Hill, Institute of Marine Sciences

Biosketch

Dr. Rachel Noble is an Assistant Professor at the University of North Carolina at Chapel Hill, Institute of Marine Sciences in Morehead City, North Carolina. She previously held a joint appointment between the University of Southern California's Wrigley Institute for Environmental Studies and the Southern California Coastal Water Research Project and focused her work there on regional assessment of water quality along the Southern California shoreline, and detection of enteroviruses in storm-water impacted areas of the coast. In July of 2001, she moved from the West Coast to the East Coast, and there has focused upon the use of molecular techniques, such as Quantitative Polymerase Chain Reaction (Q-PCR) for identification of sources of fecal material in estuarine, coastal, and freshwater environments, for use in assessment of microbiological water quality. Dr. Noble's research currently focuses on the quantification of enteric human pathogens in a variety of environments, including recreational areas, shellfish beds, and commercial fishing areas. She is interested in relating the presence of known human pathogens such as enteroviruses, *Vibrio vulnificus*, and *Salmonella* sp., to levels of fecal coliforms, *E. coli*, and enterococci in recreational waters in order to better protect human health. Other current research foci are basin-scale determinations of pathogen persistence, fate and transport in estuaries, and the impacts of nutrient loading and eutrophication on pathogen survival and ecosystem health. Dr. Noble has also recently been involved in the development of real-time detection of both pathogens and indicators as tools for creating accurate hydrologic and probability-based models of estuarine and coastal systems.

Abstract

Routinely conducted water quality analyses neither provides indication as to the source of fecal contamination, nor do they relate directly to potential public health risk of those in contact with recreational waters. With the advent of new molecular techniques, human viral pathogens, such as enteroviruses, can be used as tools to identify the presence of human fecal contamination in aquatic environments, providing useful source tracking information and data for inclusion in microbial risk assessments. A Quantitative Reverse Transcriptase Polymerase Chain Reaction (QRT-PCR) approach has been developed to detect and quantify enteroviruses from environmental samples. The approach is more sensitive and rapid than traditional cell-culture based approaches and has been well tested in a variety of aquatic systems, providing quantification of human enteroviruses over a wide dynamic range (from as few as 1 to more than 1 million PFU equivalents) in less than 4 hours. Beyond method development, an important facet of this work has been to determine the relationship between the detection of genomic enteroviral RNA versus intact infectious viral particles, by conducting 1- and 2-step QRT-PCR assays on enterovirus genome equivalents versus infectious stocks of poliovirus seeded into environmental samples. Our results suggest a consistent ratio of genome equivalents to PFU, and that while the 1-step assays are slightly less sensitive, the use of the 1-step approaches are recommended because of the advantages of decreased operator handling of sensitive RNA samples, lower risk of cross contamination (due to handling), and more rapid results.



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Rapid Detection of Enteroviruses in Environmental Samples using Real-time Quantitative Reverse Transcriptase PCR

Rachel T. Noble
UNC Chapel Hill Institute of Marine Sciences

UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
INSTITUTE OF MARINE SCIENCES

Recreational water quality

- Viral pathogens thought to be responsible for significant portion of waterborne disease
- Lack of relationship among bacterial indicators and viral pathogens
- Currently used bacterial indicators provide little or no information on source of contamination
- Technological advances mean that viral pathogens can be detected and quantified by molecular methods
- Human viral pathogen quantification allows microbial risk assessment

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Protocol development: Main issues

- Concentration of sample to allow detection: Identify filter capable of high recovery
- Optimize recovery: Develop extraction approach that reduces inhibitory compounds
- Identify ways to reduce time required: 1-step vs. 2-step QRTPCR
- Need assay to be truly quantitative: Development of RNA internal standard
- Real world testing

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Quantitative RTPCR

Cepheid Smart Cycler II®

- Primers/probe set design with S. Foul
- RT for synthesis of cDNA
- PCR for amplification of cDNA

Fluorescence vs. Cycle Threshold graph showing multiple curves crossing a horizontal threshold line. A vertical line marks the cycle threshold for one curve.

Standard Curve graph showing Log Concentration (log₁₀ copies/ml) vs. Threshold Cycle. The equation is $y = -0.33x + 12.07$, $R^2 = 0.99$.

- Increase in fluorescence is directly proportional to the amount of target cDNA in sample and indicated by cycle threshold
- Standard curve generated by plotting threshold cycle vs. log concentration and unknown values interpolated

Quasi-labeled Fluorescence Probes

Single stranded oligonucleotide labeled with two different dyes

TaqMan Assay

1. Quenching of the fluorescence. 2. Emission of the fluorescence.

Specific sequence forward and reverse primers and probe anneal to target DNA.

Taq DNA polymerase creates new DNA strand and hydrolyzes the probe bound to the target DNA

Release of reporter dye causes an increase in fluorescence which is measured by the optics of the QPCR machine

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SAMPLE & DATA ANALYSIS

Water Sample (> 250 ml < 5 liter) → Filtration (11A Millipore 0.45 µm) → Filter w/ captured material → RNA extraction (Extraction Kit, Qiagen RNeasy) → Purified RNA Extract (50 µl)

Data used for:

1. PA (presence/absence)
2. Quantification of load
3. Source tracking
4. Input into whole watershed models

Standard Curve Allows Quantification of Unknowns

Internal standard to determine RT efficiency and PCR inhibition

QRTPCR (Interpolation)



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Filtration efficiency: Enterovirus

- Speed of filtration vs. ability to capture organisms of interest
- Seed samples with known amount of virus and conduct QRTPCR

	Seawater		Freshwater	
	Recovery efficiency	RT	Recovery efficiency	RT
Type HA Long	24.6%	4.98	26.7%	4.99
GEFF Filter Long	26.2%	4.97	24.2%	4.97
A&E Filter Long	14.6%	4.95	4.8%	4.82

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Filtration efficiency

- Trade-off between time spent filtering vs. sample recovery
- Have tested filters available on market, plus flocculation and precipitation techniques
- Have determined that the Type HA Millipore filter works best for collection of enteroviruses (small single stranded RNA viruses) from environmental samples
- Extract whole filter instead of eluting material

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Optimizing the extraction approach

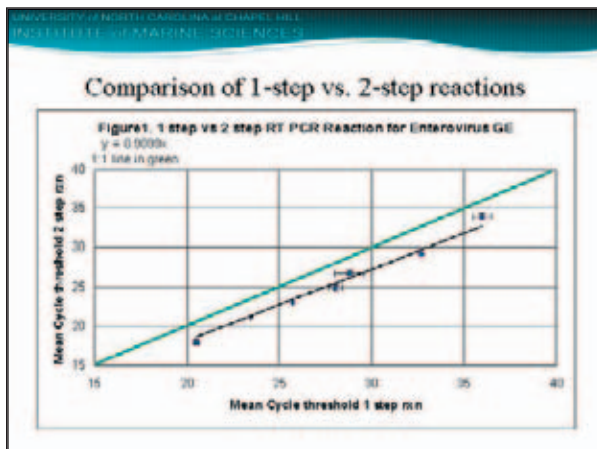
- Extraction protocols tested with a wide range of estuarine, freshwater, and seawater samples
- Extraction protocols tested in conjunction with filter choices

Type HA: Gagey Qiagen/PVP	28.1%
GEFF: Gagey Qiagen/PVP	22.8%
Type HA: Gagey Easyspin + Silica	18.8%
GEFF: Gagey Easyspin + Silica	13.1%
Type HA: Gagey Qiagen	11.4%
GEFF: Gagey Qiagen	7.3%

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1-step vs. 2-step RTPCR?

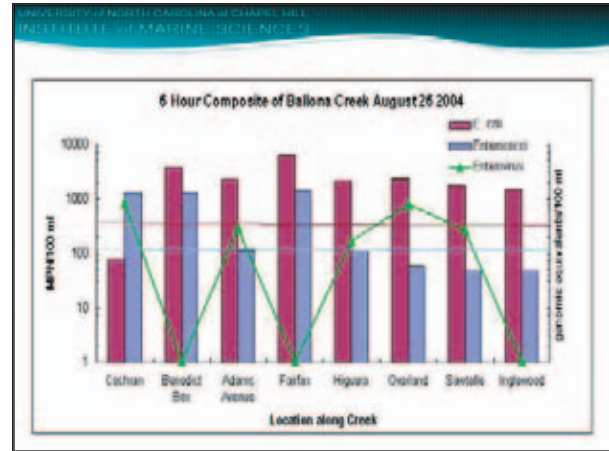
- For enterovirus detection, the first step of the sample analysis is "reverse transcription"
- This step can be conducted separately from the PCR reaction (2-step) or preceding the PCR reaction (in the same tube, called 1-step)
- 1-step = 1 tube, faster reaction
- 2-step RT-PCR is typically more sensitive than 1-step RT-PCR, but 1-step RT-PCR is faster
- Is the difference significant?



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1-step vs. 2-step QRTPCR

- Compared the use of 1-step vs. 2-step QRTPCR approaches for the quantification of enteroviruses
- 1-step is faster and less prone to contamination, but not quite as sensitive as 2-step kits
- Components such as PVP and carrier material have been tested to improve the detection of enteroviruses by RTPCR
- Difference between 1-step vs. 2-step approaches is small (<8% variability and detection limits within 5 g.e.)
- Time advantage is large (saves PCR cycling time and reagent transfer time)



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From sample to result

- 5 hour total assay time
- General breakdown: sample collection, filtration, and extraction 2.5 h, and RT + thermal cycling 2.25 h
- Highly specific
- Wide dynamic range, detects from 0.5 PFU or 5 genomic equivalents to 10^6 PFU or 10^7 g.e.
- Competitive internal RNA standard for quantification
- MS2 and enterovirus transcripts to assess extraction efficiency and creation of standard curve





Questions and Answers

Q (Stephan Weurtz, University of California at Davis): We have also adopted a system to take enteroviruses and we are using quantitative PCR. One of the differences is that we use a hollow-fiber ultrafiltration method that uses 100 liters. Your starting volume tends to be 5 liters or less. You also went to a very contaminated watershed. Do you think that you'll be able to catch the viruses, which are always going to be present in lower numbers than the indicators, using such a small starting volume?

Rachel Noble

You ask a very good question. One of the biggest issues that we have dealt with is the examination of hollow-fiber applications for concentrating the water samples is the volume. What is the final volume of actual material from the hollow fiber system?

Q (Stephan Weurtz): In the field we filter down to about 1.5 liters. Then we take that to the lab and through a second smaller version of the unit we end up with about 50 milliliters.

Rachel Noble

This kind of application is exactly what I conducted through a large part of my graduate work and dissertation work at USC, in Jed Fuhrman's lab. Basically, the issues are that we have been really moving our method toward something that is rapid. I'm sure that your recovery levels are higher than ours. But, the idea is that we are taking a small filter and a small volume, and from that we are able to get the final material that comes off of that filter extracted into a final volume of 50 microliters. So, while our filtration efficiency is not 100 percent, the loss of things beyond that, through the extraction procedure and onto the PCR allows us to have similar overall recovery rates as what you would find with hollow fiber and all the other ultrafiltration techniques. The trade-off is rapidity. I don't know how long it takes for you to do your 100 liter filtration, but there are obvious trade-offs. If you really want to understand whether or not you have a presence of enteroviruses in cleaner water samples, and you want to have a high recovery rate, you need to apply a larger volume filtration. In Ballona Creek (heavily contaminated), that is not necessary, but it is certainly necessary in other more pristine estuarine and coastal environments. This is just one way of going about things. There are other choices to be made, depending on what your question is.



Male-Specific Coliphages as Indicators of Fecal Pollution in Coastal Recreational Waters

Greg Lovelace

University of North Carolina at Chapel Hill, Department of Environmental Sciences and Engineering

Biosketch

Mr. Greg Lovelace is an environmental biologist and field laboratory manager in the Department of Environmental Sciences & Engineering, School of Public Health, for the University of North Carolina at Chapel Hill. The field laboratory is located in the coastal town of Beaufort, North Carolina. Mr. Lovelace received his B.S. in Zoology from North Carolina State University in Raleigh. He worked as a laboratory technician for the City of Raleigh in the municipal sewage treatment plant and then joined the research team of Dr. Mark D. Sobsey. He has remained with Dr. Sobsey's team for the past 27 years. For the majority of that time, he has been Dr. Sobsey's sole researcher on the coast of North Carolina, performing research on microbial contamination of groundwater, shellfish, and shellfish-growing waters.

Abstract

Microbial standards for recreational waters are based on levels of indicator bacteria. Because viruses are more resistant to sewage treatment methods and more persistent in marine waters than indicator bacteria, there is an urgent need for an indicator of viral contamination in recreational waters. Male-specific coliphages have properties

that make them useful indicators to characterize recreational waters: They are easy to detect using simple microbiological techniques; they are usually detected relatively quickly (12-24 hours); and they can be separated into human and non-human groups.




In a previous study we examined levels of somatic and male-specific coliphages in samples of water from six estuarine areas along the central NC coast collected from paired sites situated near to and more distant from point- and non-point sources of fecal pollution. Geometric mean levels of *E. coli* and enterococcus were predictably higher at sites nearer to pollution sources, and the same was generally true for levels of both types of coliphages. Coliphages were good indicators of fecal contamination, and when serotyped, they predicted human sources or both human and non-human sources of fecal contamination.

The aim of a current study with sampling stations in coastal marine waters of the USA is to further improve, validate and apply coliphage detection methods in estuarine recreational waters, including bathing beaches. The results so far indicate that the methods of coliphage detection work well in the estuarine waters tested. The ability to detect and quantify fecal contamination based on coliphage detection and quantification is being further investigated.




Male-Specific Coliphages as Indicators of Fecal Pollution in Coastal Recreational Waters

Mark D. Sobsey
 Greg L. Lovelace
 Amanda Freeman

First, a little history



Early Microbiologists
(Lewis 1981:146)

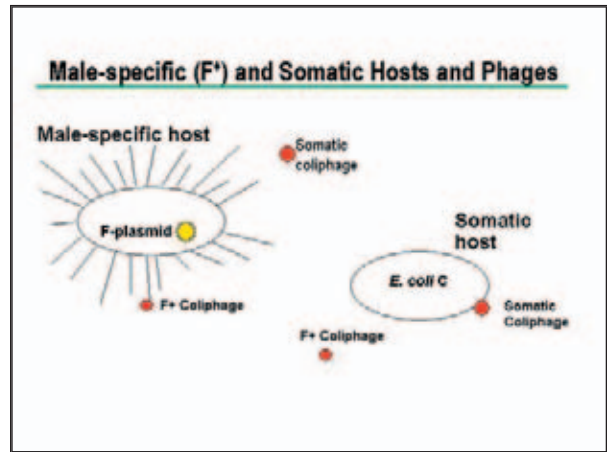
Coliphage Families

<p>A Myoviridae ds DNA cell wall 95x65nm T2</p> <p>C Podoviridae ds DNA cell wall 47 nm T7</p> <p>E Levviridae ss RNA sex pilus 25 nm MS2</p>	<p>B Styloviridae ds DNA cell wall 54 nm λ</p> <p>D Microviridae ss DNA cell wall 30 nm φX174</p> <p>F Inoviridae ss DNA sex pilus 810x5nm φ</p>
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Somatic (F)
Infect through receptors on host cell wall


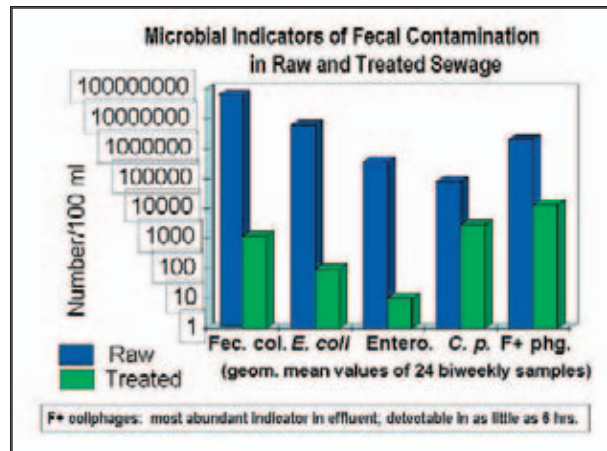
Male-Specific (F*)
Infect through receptors on host F pill

IAWPRC (1991). Water Res. 25(5):529-545.



Male-Specific Coliphages as Indicators of Fecal Pollution in Coastal Recreational Waters

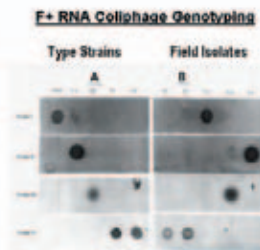
- Question: Can coliphages be used to identify sources of human fecal contamination?
- Question: Can the EPA groundwater coliphage methods be adapted to detect coliphages in estuarine and sea waters (and shellfish)?
- Question: Are coliphage concentrations indicative of proximity to or impacts from fecal waste sources?
- Question: Are coliphage methods practical and accessible?
- Continuing and future work



F+ Coliphage Genotyping by Oligonucleotide Probe Hybridization Assays

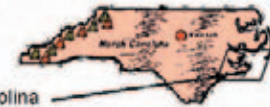
- Oligoprobes
 - Groups I, II, III and IV F+ RNA
 - Also, three groups of F+ DNA
 - fd, M13 and CH prototypes
 - Label with non-radioactive probe
- Hybridization assays
 - F+ RNA: Hsu et al., 1995; Beekwilder et al., 1996
 - F+ DNA: Vinje et al., AEM, Oct. 2004



Study #1: Somatic and Male-specific Coliphages as Fecal Indicators

Can coliphages be used to quantify and identify sources of human fecal contamination?

- Double Agar Layer (DAL) assay technique for male-specific coliphages and somatic Salmonella phages
- Male-specific host - *S. typhimurium* WG49
 - Later study: *E. coli* Famp
- Somatic host - *E. coli* C
 - Later study, CN13



- "Crystal coast" of North Carolina
- Three point-source (P) and three non-point source (NP) study sites

Analysis of Bacteriophage Concentrations in Water Collected from Closed, Transition and Open Stations by the Kruskal-Wallis Nonparametric ANOVA Test

Bacteriophage Group / Station Type	No. of Samples	Mean of Counts	P-value
VOC49			
Closed	41	66.341	0.0115
Transition	34	47.515	
Open	31	48.899	
F+RNA			
Closed	41	64.585	0.0386
Transition	34	48.829	
Open	34	50.412	
ECC			
Closed	41	64.622	0.0438
Transition	34	50.368	
Open	34	48.829	

Genotyping and Serotyping of F+RNA Coliphages

Field Site	Total Isolates	Unknown	Group I (MS-2)	Group II (GA)	Group III (GB)	Group IV (GP, FI)
North River	310	7	8	295	0	0
Foster's Bay	3760	0	3666	160	46	0
Calico Creek	67,221	164	6532	57,846	2680	0
Jumping Run Creek	1496	0	1464	32	0	0
Taylor's Creek	4043	0	69	4852	2	0
Goose Creek	11,645	0	11,645	0	0	0

F+ RNA Coliphage Serotyping and Genotyping

Calico Creek and Taylor's Creek (P)

- majority were Type II (GA)
- impacted mostly by human fecal contamination; sewage treatment plant effluent

North River (NP)

- majority of were Type II (GA)
- impacted mostly by human fecal contamination: likely seepage from individual septic tanks

Foster's Bay (P), Jumping Run Creek and Goose Creek (NP)

- majority were Type I (MS-2)
- impacted by animal and human sources of fecal contamination

Study #1 Summary

- Type II F+ RNA coliphages, indicative of human sources, were detected at stations receiving both known point- and suspected non-point source input of human fecal waste
- Type I F+ RNA coliphages, indicative of a combination of human and non-human sources, were detected at stations thought to receive suspected non-point source input of human and non-human fecal waste

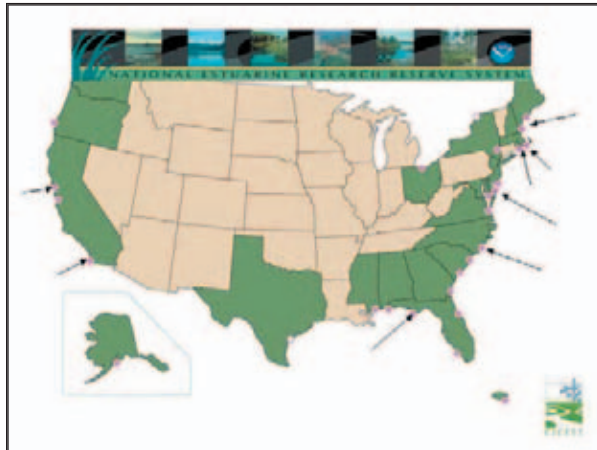


Study #1 Summary

- Coliphage occurrence and concentrations related to proximity to fecal contamination sources, based on sanitary survey and growing water classification for shellfishing
- F+ coliphage concentrations were predictive of human enteric virus contamination; enteric bacteria were not – data not shown

Study #2: Use of EPA Groundwater Methods for Coliphage Detection in Estuarine Water

- 3 detection methods: Single Agar Layer (SAL) assay, Direct Membrane Filtration (DMF), and MPN Enrichment
- Male-specific host - *E. coli* Famp
- Somatic host - *E. coli* CN13
- Estuarine water from eight geographically diverse sources in the National Estuarine Research Reserve System



Question: Are the methods effective in detecting coliphages in estuarine water?

Table 1: Detection of coliphage in water samples using all methods

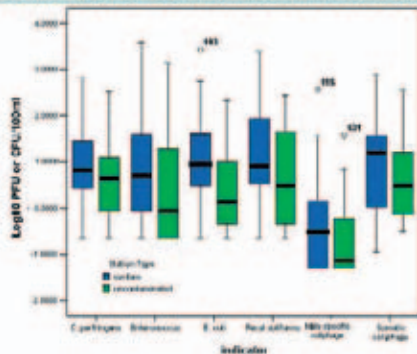
samples	all coliphages	somatic coliphages	male-specific coliphages
total	46	46	46
#positive	46	46	27
%positive	100%	100%	59%

Table 2: Detection of coliphage in water using three different assay methods

	Somatic Coliphage			Male-specific Coliphage		
	Direct Membrane Filtration	Single Agar Layer	MPN Enrichment	Direct Membrane Filtration	Single Agar Layer	MPN Enrichment
total	46	46	46	46	46	46
#positive	35	41	41	14	7	25
%positive	76%	89%	89%	30%	15%	54%



Question: Are indicator concentrations the same near to and remote from fecal waste sources?



Indicator Levels at Contaminated Versus Uncontaminated Sites

Wilcoxon Signed Ranks Test Results ($\alpha=0.05$)

Indicator	Z	Uncontaminated vs. Contaminated	Significance
<i>C. perfringens</i>	-2	-3.122	significant
<i>Enterococcus</i>	-2	-3.728	significant
<i>E. coli</i>	-2	2.658	significant
Fecal coliforms	-2	-2.111	significant
Male-specific phage	-2	-1.388	significant
Somatic phage	-2	-1.655	not significant





Summary and Conclusions:

- The EPA-approved groundwater methods appear to work well for samples of estuarine water, with the MPN Enrichment Method detecting the greatest numbers of both somatic and male-specific coliphages
- There appear to be significant differences in levels of bacterial and F+ coliphage viral indicators at contaminated versus uncontaminated sample sites on a site-specific basis; studies are continuing



Proposed Plans for Dissemination of Coliphage Methods

Most effective field methods to be packaged into kits for coastal zone water quality managers, recreational water and shellfish sanitation programs to provide them with better microbial tools to:

- help protect bathers and shellfish consumers from human fecal contamination, including enteric virus exposures (Human enteric virus studies in progress),
- to more specifically identify and distinguish human and animal fecal contamination impacts,
- to better assess and manage coastal development and its impacts and other environmental changes contributing to fecal contamination.



Ongoing Study Questions

- Among study sites, do water samples from stations near fecal contamination sources have the same or different concentrations of fecal indicators and enteric viruses than the stations remote from fecal contamination sources?
- Are particular groups of F+ RNA, F+ DNA and somatic coliphages reliable and consistent indicators of human or animal fecal contamination sources?
- Can coliphage methods be made practical and rapid for routine field use?
 - Simple coliphage culture and genotyping methods
 - Real-time quantitative (RT-PCR)



Greg Lovelace
greg_lovelace@unc.edu
<http://ciceet.unh.edu>



Questions and Answers

Q (Jack Skinner, Stop Polluting Our Newport): My background in internal medicine. When testing patients for f-specific phage in the stool specimen, it is extremely rare to find it. It is almost like the ecology is different. Whereas, the enteroviruses multiply in the gut, but they do not multiply after they leave the body. How do you explain this?

Greg Lovelace

I have no explanation for that.

Q (Jack Skinner): The only thing I can think about is that it multiplies within the sewage system, but it is not really from human fecal material because there is nearby E. coli where it can replicate. But, I do not understand how you can quantify human (entero) viruses and correlate them with a number of f-specific phage because there is never any f-specific phage found in the human stool samples.

Greg Lovelace

You are right, and I don't know why that is. In response to your comment on male-specific coliphages multiplying in the sewage treatment system, I don't think they do that but I don't have proof of that right in front of me. If you would like to talk about this later, I can talk to Dr. Sobsey and we can try to answer your question.

Q (David Turbow): With the exception of the somatic coliphages, the concentrations were higher at the contaminated sites than at the uncontaminated sites. Why are the somatic coliphages an exception?

Greg Lovelace

I'm not sure. We are only half-way through the study, and that may change once we get more data.

Q (Clay Clifton, County of San Diego Department of Environmental Health): In one of the last slides you showed, was the correlation between the existing indicators and f+ male-specific coliphages good or bad? Since you said the research is continuing, I'm assuming the correlation was not good.

Greg Lovelace

Yes. We are finding that the male-specific coliphages do not correlate well with the bacterial indicators.

Q (Clay Clifton): Have you tested the coliphage alongside of any of the existing indicators in any of the epidemiology studies that were conducted over the past year or two?

Greg Lovelace

Yes. The Mission Bay epidemiology study that Jack Colford will be talking about did incorporate both the somatic and the f+ coliphages.

Thursday, October 14
1:20 p.m. – 3:00 p.m.

**Concurrent Track II:
Changes on the Horizon
Session Ten:
Quantifying Swimmer Risk**



EPA National Epidemiology Study

Timothy Wade, Ph.D.

U.S. Environmental Protection Agency

Biosketch

Dr. Tim Wade is an Epidemiologist with the US EPA in the Office of Research and Development, National Health and Environmental Effects Research Laboratory, Human Studies Division in Chapel Hill, North Carolina. Dr. Wade received his Ph.D from the University of California at Berkeley and is currently a postdoctoral researcher in USPEAs Human Studies Division. He has been a lead scientist on several large studies of the health effects of contaminated drinking water and recreational waters. Dr. Wade is also a principal investigator and lead epidemiologist of several studies examining the health effects of arsenic in drinking water being conducted in the Inner Mongolia region of China.

Abstract

The National Epidemiological and Environmental Assessment of Recreational Waters (NEEAR) is a multi-year study of recreational water conducted by the United States Environmental Protection Agency and the Centers for Disease Control and Prevention (CDC), designed to evaluate new rapid indicators of recreational water quality and to determine their relationship to health effects. These studies are the first to evaluate the relationship between health effects

and rapid indicators of recreational water quality. This presentation will summarize data collection efforts and preliminary analyses for the Great Lakes beach sites. We conducted studies at three Lake Michigan beaches and a Lake Erie beach during the summers of 2003 and 2004. Interviewers asked beach-goers about swimming and other activities. Ten to 12 days after the beach interview, interviewers telephoned each household to ascertain health symptoms experienced in the days following the beach interview. At each beach water samples were collected at several transects at two depths, three times a day. Samples were tested for enterococci using the standard method (Method 1600) and for enterococci and Bacteroides sp. using novel methods including quantitative polymerase chain reaction (QPCR). Several other potential rapid methods of evaluating water quality were also evaluated. During 2003, at the Lake Michigan Beach, interviews with 2877 individuals were completed. At the Lake Erie beach, interviews with 2840 individuals were completed. The relationships between health symptoms and the traditional and rapid indicators will be fully evaluated and presented in detail. Updates on the summer of 2004 data collection efforts and analysis will also be presented.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.



The National Environmental and Epidemiologic Assessment of Recreational Water:

The relationship between novel indicators of water quality and health

Timothy J. Wade
National Beaches Conference
October 14, 2004

BEACHES Act of 2000 from Congress

1. Determine microbial indicators for beach water quality
 2. Develop efficient protocols for monitoring
 3. Assess human health risks
 4. Provide guidance to beach managers
- Final Goal: New risk-based water quality guidelines & rapid monitoring methods for recreational waters.

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Research Question

Is there an association between illness and recreational water quality as measured by rapid methods of determining water quality?

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STUDY APPROACH



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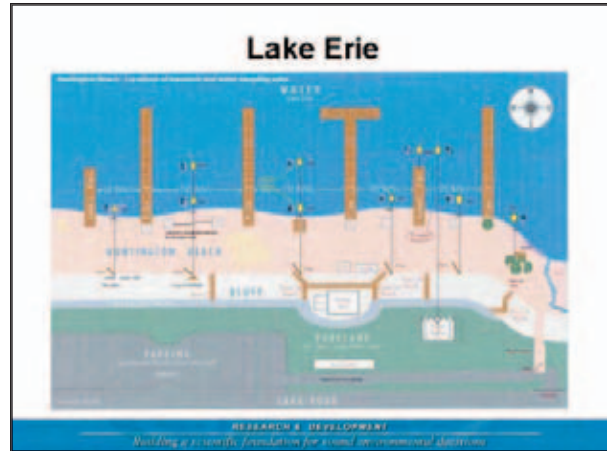
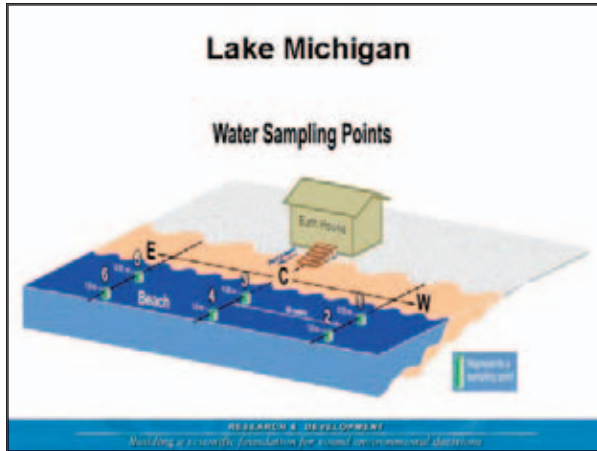


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Water Sampling Strategy

- Taken 3 times daily
 - 8:00 AM
 - 11:00AM
 - 3:00 PM
- Two depths
 - .3 meters
 - 1.0 meter
- Modified sampling scheme according to beach area

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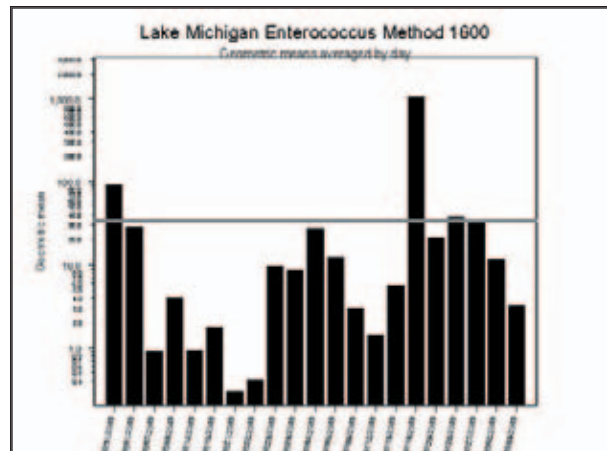
- ### Water Quality Measures
- **Enterococci Method 1600**
 - Current standard
 - Colony forming units 24-48 hrs
 - Intestinal tract bacteria, warm blooded animals
 - **QPCR: Enterococci and Bacteroides**
 - Quantitative (real time) polymerase chain reaction
 - DNA based technology
 - Two hours
 - Intestinal tract bacteria
 - Bacteroides, 2-3 log higher density, anaerobe, dies in environment
 - Measured in cell equivalents (QPCRCE)
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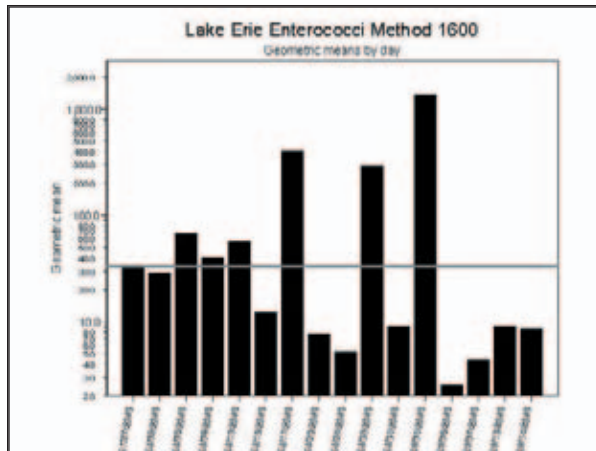
- ### Exposures and Health Outcomes
- **Exposures:**
 - Any contact with water ("any contact")
 - Immersed body in water ("body contact")
 - Head under water ("head under")
 - **Outcomes**
 - Gastrointestinal illness (GI), skin rash, earache, eye irritations, respiratory illness (URI)
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2003 Data Collection

Lake Michigan	Lake Erie
• May 31-August 3	• July 27-September 14
• 20 days of surveying	• 16 days of surveying
• 2877 completed interviews	• 2840 completed interviews
• 67% completion rate	• 60% completion rate

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Survey Results: Swimming

	Lake Michigan	Lake Erie
Any contact	75%	46%
Body contact	58%	27%
Head under	42%	18%
Water in mouth	19%	12%
Gagged on water	6%	3%
Swallowed water	7%	4%
Wave riding	9%	5%

Lake Michigan: Adjusted Odds Ratios for Swimming

	GI	URI	Eye	Rash	Earache
Any contact	2.22*	1.09	1.09	2.35*	1.40
Body contact	2.54*	1.06	1.19	2.44*	1.72
Head under	2.37*	1.09	1.25	2.42*	2.29*
*p<0.1					

Lake Erie: Adjusted Odds Ratios for Swimming

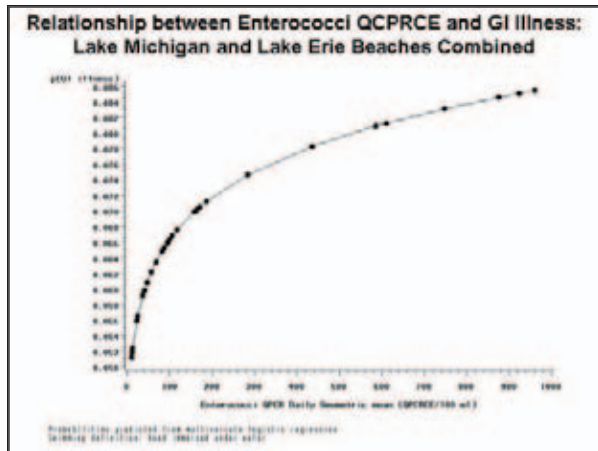
	GI	URI	Eye	Rash	Earache
Any contact	1.43*	1.08	0.65	1.23	1.74
Body contact	1.62*	1.03	0.62	0.86	1.46
Head under	1.68*	1.11	0.51	1.00	1.36
*p<0.1					

Lake Michigan: Trends for GI illness

	Enterococcus Method 1600		Enterococcus QPCR		Bacteroides QPCR	
	OR*	P-trend	OR**	P-trend	OR**	P-trend
Any contact	1.11	0.43	1.78	0.04	0.66	0.11
Body contact	1.08	0.62	1.94	0.04	0.64	0.12
Head under	0.96	0.82	2.16	0.03	0.58	0.12
	100 unit increase		100 unit increase		100 unit increase	

Lake Erie: Trends for GI illness

	Enterococcus Method 1600		Enterococcus QPCR		Bacteroides QPCR	
	OR*	P-trend	OR**	P-trend	OR**	P-trend
Any contact	1.07	0.62	1.71	0.07	1.55	0.15
Body contact	1.58	0.15	2.06	0.07	1.95	0.08
Head under	0.95	0.90	1.51	0.37	2.10	0.09
	100 unit increase		100 unit increase		100 unit increase	



Risk of GI Illness: Method 1600
GI illness among Swimmers above vs. below daily geometric means of 33 cfu:

Enterococci (cfu/100ml)	GI illness-Yes	GI illness-No
≥33	70 (13.0%)	470 (87.0%)
<33	200 (10.6%)	1684 (89.4%)

OR (adjusted)=1.28 (95% CI: 0.95-1.73) p=0.11

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Data Collection 2004

- Two Lake Michigan beaches
- Over 15,000 completed individual interviews
- GI illness-7%
- Swimming:
 - Any contact - 69%
 - Body contact- 52%
 - Head under - 37%
- RR for body contact (unadjusted)
 - 1.29 (95% CI 1.14-1.45)

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Summary and Conclusions

- QPCR appears to be a promising predictor of gastrointestinal illness in fresh water
- First rapid indicator to be correlated with health effects
- Trends were not observed for respiratory illness
- Trends were not observed for rash, earache, and eye ailments, but more data may be necessary

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Future Directions

- Better define risk to high risk groups such as children
- Evaluate other potential rapid indicators such as chemicals associated with sewage
- Studies in marine waters

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The NEEAR Study Team

USEPA NHEERL:
Timothy J. Wade, Rebecca L. Calderon, Elizabeth Sams, Ann H. Williams

USEPA NERL:
Kristen Brenner, Alfred Dulour, Larry Wyner, Richard Haugland

Centers for Disease Control and Prevention
Michael Beach

USEPA Field Team:
Joel Hansel, Holly Wirick, Ed Hudgens, Mike Schmitt, Mike Ray, Danelle Lobbelle, Gina Andrews, Scott Rhoney, Beth Leamond, Walter Breyer

University of North Carolina:
Caroline Smith Hoffman

Westat Corp.
Karen Della Torre, Kurt Patrizi, Robert Clickner, Steve Dietz, and others too numerous to name

The Beaches Interviewers

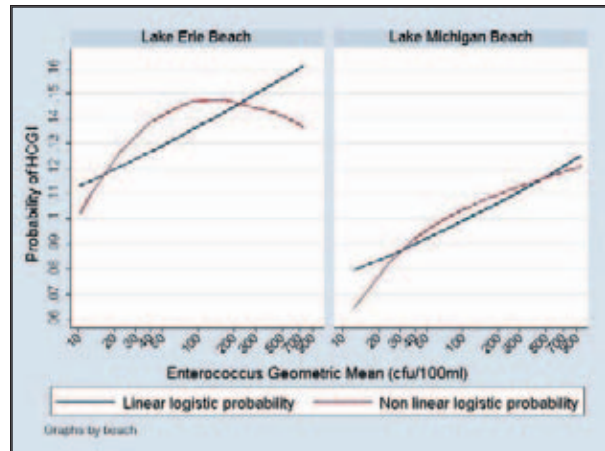
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Acknowledgements

- Richard Whitman and staff, United States Geological Service, Porter Indiana
- Great Lakes Scientific, Inc. Stevensville, Michigan
- Cuyahoga County Sanitary Engineering Division, Cleveland, Ohio
- EMSL Analytical, Inc. Laboratory, Westmont, New Jersey.
- Indiana Dunes National Park Service
- Cleveland Metroparks
- Cuyahoga County Board of Health

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Interactions by Beach, Time in Water

	Estimate	p-value
Beach/QPCR CE	1.05	0.33
Time in Water/QPCR CE	1.01	0.08
Trend at 5 min ^a	1.16	0.71
Trend at 30 min	1.46	0.23
Trend at 60 min	2.49	0.02

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Questions and Answers

Comment (Katherine Field, Oregon State University): Concerning the non-detect level of the bacteroidides assay, that assay was actually designed as a tacman assay using an ABI machine. It was done that way at your original request because that is what you were originally doing. However, you then used it in the field in a completely different way in a different type of assay. That is why it didn't work very well. Anybody who would like to get some more recent information about the sensitivity of that, we have a more recent publication in Applied Environmental Microbiology that just came out this month.



Mission Bay Epidemiology Study

Jack Colford, MD, Ph.D.

University of California at Berkeley, School of Public Health

Biosketch

Dr. Colford is Associate Professor of Epidemiology in the University of California, Berkeley School of Public Health. Dr. Colford is a graduate of the Johns Hopkins School of Medicine (MD 1985) and the UC Berkeley School of Public Health (Epidemiology, 1996). He completed a residency in Internal Medicine and a fellowship in Infectious Diseases at the University of California, San Francisco. He was Chief Resident in Medicine at Stanford University Hospital. He is board-certified in both Internal Medicine and Infectious Diseases. He is the sole instructor in semester-long courses in advanced epidemiologic methods, intervention trial design, and meta-analysis and has received several teaching awards. He has taught for many years as a visiting professor each summer at the University of Michigan (meta-analysis) and the University of Zurich, Switzerland (epidemiologic methods). He has published numerous peer-reviewed articles on the health effects of waterborne diseases. While on sabbatical at WHO-Geneva last year, he co-authored a monograph published by the World Bank evaluating all published evidence of efficacy of water, sanitation, and health interventions. He is the Principal Investigator of four triple-blinded, randomized controlled trials of drinking water and health effects funded by the National Institutes of Health, the Centers for Disease Control, and the Environmental Protection Agency, and the University of California.

Abstract

Most epidemiology studies to establish health risk of recreational swimming have been conducted at locations where human sewage point sources are the primary source of fecal contamination. Here we conducted a study of health outcomes from swimming in Mission Bay (San Diego), CA where nonpoint runoff and animal waste are the primary fecal sources. We enrolled beachgoers, interviewed them about health conditions on the day of exposure and 14 days later, and collected water quality samples at sites linked spatially to participants' location in the water. Both traditional (enterococcus, *E. coli*, total coliform) and novel candidate indicators (Bacteroidetes, coliphage, virus and traditional indicators measured using molecular rapid detection methods) were sampled four times each day at multiple locations on each of six beaches within Mission Bay. A total of 12,458 participants were enrolled and 8,790 (71%) completed the entire study. The principal health outcome was highly credible gastrointestinal illness (HCGI). Logistic models were used to analyze the data. We found an increased risk of HCGI illness among swimmers compared to non-swimmers (OR 1.31, 95% CI 1.01-1.71, $p=0.045$), but did not find associations between traditional microbial indicators and health. Preliminary analysis suggests that there was association with some of the novel indicators, though. The lack of association of traditional indicators with health outcomes emphasizes the importance of research into alternative indicators, particularly at sites where non-point sources are prevalent.



Mission Bay Epidemiology Study

Water Quality
Southern California Coastal Water Research Program
Ken Schiff, Stephen Weisberg, John Griffin

University of North Carolina (coliphage)
Mark Sobsey, Jan Vinje, Greg Lovelace, Doug Wall

Epidemiology
University of California, Berkeley
Jack Colford, Sukhie Sandhu
Sriresh Anunajadai, Catherine Wright

Environmental Protection Agency
Tim Wade

Harvard University
Alan Brookhart

Background

- Many prior epidemiologic studies of recreational water
- All but one of these focused on sewage-dominated waters
- Mission Bay in San Diego gave us a unique opportunity for a different study setting
 - Limited circulation and mixing
 - Heavily used aquatic park
 - Receives numerous nonpoint sources
 - Non-human sources are dominant
 - Urban storm drains
- More than 100 days of beach postings in 1998

Research Questions

- Did swimming in Mission Bay during the summer of 2003 affect the risk of an individual's subsequent development of health outcomes 10-14 days later?
- Did the levels of traditional microbial indicators correlate with symptoms?
- Did the levels of novel microbial indicators correlate with symptoms? (coliphage—male and somatic, Bacteroides, viruses)

Overview of design

- Prospective cohort (8,790 participants, 99% of goal)
- Health questions asked (in person) on day of exposure and 10-14 days later (by phone)
- Microbial indicators (most) measured throughout the day and linked to actual participant's swimming location
- 17% of water samples had enterococcus value > 104

Health outcomes by category

- Gastrointestinal:
 - nausea, vomiting, diarrhea, cramps, HCGI-1, HCGI-2
- Respiratory:
 - cough, cough with phlegm, nasal congestion, sore throat, SRD
- Dermatologic:
 - rash, scrapes
- Non-specific:
 - fever, chills, earache, ear discharge, eye irritation

Exposures to water

- Water exposure vs. health outcomes
 - Any water contact (yes/no)
 - Among swimmers:
 - Face under water (yes/no)
 - Time in water (continuous, categorical, & per 100 min increase)
 - Shoulders in water (yes/no)
 - Swallowing water (yes/no)
 - Water in the mouth (Y/N)
 - Gag or cough from water (Y/N)
 - Amount of water swallowed (continuous)



Microbial indicators

- Enterococcus
- Total coliforms
- Fecal coliforms
- Total:fecal ratio (derived value)
- *E. coli*
- Bacteroides
- *Enterococcus faecalis*
- Male-specific phage (composite daily sample)
- Somatic phage (composite daily sample)

Covariates used in adjusted models

- Age, gender, race
- Allergies, subsequent swimming, contact with shells
- Dug in sand, buried in sand, touched algae
- History of chronic GI illness, contact with GI illness
- Used insect repellent, sunblock
- Showered after beach
- Ate eggs, ate raw foods, ate other food at beach
- History of chronic respiratory disease
- Household income level

Analysis

- Logistic regression models used to estimate odds of illness in exposed group vs. odds of illness in unexposed group (i.e. odds ratio "OR")
- Adjusted models estimated using covariates (prior slide)

Participant characteristics (N=8790)

- Age
 - 0-5: 14%
 - 5-12: 21%
 - 12-30: 27%
 - 30-55: 34%
 - >55: 4%
- Race
 - White: 26%
 - Black: 4%
 - Hispanic: 60%
 - Other: 10%

Symptom frequency (n=8790)

420-Sore throat	243-HCGI1
414-Eye irritation	223-Nausea
386-Cramps	213-Cough
373-Diarrhea	192-Earache
381-SRD	187-Vomiting
311-Fever	74-HCGI2
289-Skin rash	38-Ear discharge

Adjusted models for odds ratio Any water contact *p<0.05

Gastrointestinal	Miscellaneous
1.29 Diarrhea	*1.97 Rash
1.11 Cramps	1.26 Eye irritation
0.98 HCGI1	1.08 Fever
0.90 HCGI2	1.00 Earache
0.85 Nausea	0.42 Ear discharge
0.83 Vomiting	
Respiratory	
0.97 SRD (sig resp disease)	
0.82 Sore throat	
0.73 Cough	



Adjusted models for odds ratio
Water on the face
*= $p < 0.05$

Gastrointestinal	Miscellaneous
*1.33 Diarrhea	*2.10 Rash
1.24 Cramps	1.26 Eye irritation
1.13 HCGI1	1.08 Fever
1.21 HCGI2	1.05 Earache
1.27 Nausea	0.72 Ear discharge
1.02 Vomiting	
Respiratory	
1.00 Sore throat	
0.02 SRD (sig resp disease)	
0.73 Cough	

Water on the face, by age
Odds ratios for health outcomes
*= $p < 0.05$

	Diarrhea	HCGI-1	Sore throat	Rash	Eye irritation
0-5	1.08	1.29	1.25	2.33*	0.69
5-12	2.29*	1.26	0.57	3.04*	1.64
12-30	1.99*	1.09	1.37	1.79	1.63
30-55	0.97	0.90	0.90	1.09	0.88
>55	Too few	Too few	Too few	Too few	Too few

Enterococcus (30 unit change)
Odds ratios for health outcomes
*= $p < 0.05$

	Diarrhea	HCGI-1	Rash	Eye irritation	Sore throat
In (daily geo mean-beach)	0.62	0.73	2.21	0.63	1.04
Max (daily-beach)	0.94	0.97	1.54	0.76	0.99

Coliforms, Total and Fecal (50 unit change)
Odds ratios for health outcomes
*= $p < 0.05$

	Diarrhea	HCGI-1	Rash	Eye irritation	Sore throat
Total coliforms-In (daily geo mean-beach)	0.42*	0.62	2.95	0.57	1.13
Fecal coliforms-In (daily geo mean)	0.30	0.73	1.65	0.57	1.10

Bacteroides Diarrhea – odds ratios-adjusted

Overall	0.48 (0.22, 1.02)
Age 0-5	0.84 (0.10, 2.77)
>5 - 12	0.56 (0.13, 2.43)
>12 - 30	0.31 (0.06, 1.70)
>30 - 55	0.49 (0.11, 2.20)
>55	Too few

Male-specific phage

	Diarrhea-NO N(% of column)	Diarrhea-YES N(% of column)	HCGI-1-NO N(% of column)	HCGI-1-YES N(% of column)
<0.10	3391 (95.2%)	195 (4.8%)	3944 (96.8%)	132 (3.2%)
0.12	87 (95.6)	4 (4.4%)	89 (97.8%)	2 (2.2%)
0.25	4 (100%)	0 (0%)	4 (100%)	0 (0%)
0.31	20 (100%)	0 (0%)	20 (100%)	0 (0%)
0.49	6 (100%)	0 (0%)	6 (100%)	0 (0%)
0.77	37 (90.2%)	4 (9.8%)	37 (90.2%)	4 (9.8%)



Did swimming in Mission Bay during the summer of 2003 affect the risk of an individual's subsequent development of symptoms 10-14 days later?

- Yes but only for a few health outcomes and age groups
 - Of 14 health outcomes measured, rash was the only symptom consistently elevated
 - Of the symptoms examined, only two (diarrhea and rash) were significantly elevated for any age group
 - 5-12 year olds: increased diarrhea, rash
 - Those with higher degrees of water exposure had an increased risk after covariate adjustment for diarrhea
 - Compared to other studies, the risk of illness overall was lower both among those exposed to water and those not

Did the measured levels of any microbial indicators correlate with symptoms?

- Traditional indicators
 - Enterococcus. No
 - Fecal coliforms. No
 - Total coliforms. No
- Non-traditional indicators
 - Male-specific phage. Yes (but very small sample).
 - Bacteroides. No
 - Enterococcus Q-PCR. No

Microbial indicators and illness

- No relationship was found between traditional indicators and illness
 - Current water quality thresholds were not predictive of illness in Mission Bay in our study
- Mission Bay is a unique system relative to the other studies upon which standards were originally set
 - MB is enclosed, has long circulation times, and is non-point source
- Because of these difference, it is difficult to extrapolate these results to other settings



Questions and Answers

Q: You had 17 percent that exceeded the 104. How high were those exceedances?

Jack Colford

Ken Schiff, who is here, directed that aspect of the study.

Ken Schiff

The values were in the hundreds to the tens of thousands.

Q: I assume you looked at different sites throughout Mission Bay. Did you see differences between the east and west sides of the bay?

Jack Colford

Yes, there were differences in some of the beaches. But, I can't remember specifically what they were because the numbers were so small.



Risk Perception Bias and Self Reported Symptoms

Jay Fleischer, Ph.D.

NOVA Southeastern University, College of Osteopathic Medicine, Master of Public Health Program

Biosketch

Dr. Jay Fleischer received a B.S. Degree in Environmental Health Science from the City University of New York, an M.S. in Environmental Science from the City University of New York, an M.S. in Epidemiology from Columbia University's School of Public Health, and a Ph.D. in Environmental Epidemiology /Biostatistics from the Institute of Environmental Medicine, New York University. Dr Fleisher holds faculty positions at both NOVA Southeastern University and the Center for Research into Environment and Health, Leeds University (United Kingdom). Dr Fleisher's main research interest is in the spread of infectious illness via contaminated recreational / potable waters and has been active in this area for the past 20 years. The focus of Dr Fleisher's research has been in the health effects of exposure to waters contaminated with domestic sewage, indicator organism variability, indicator organism – pathogen relationships, risk assessment, statistical water quality sampling protocols, assessing compliance, setting of microbial water quality standards, population health burden assessment, risk perception, and risk vs current standards. Dr Fleisher has advised numerous international committees, organizations, and government agencies on various aspects of these recreational water quality issues. In addition Dr Fleisher authored over 35 peer reviewed publications and 5 book chapters dealing with these water quality issues.

Abstract

Background

Epidemiologic studies of water associated illness sometimes have to rely on self-reported symptoms of the outcome illness(es) under study. Individual participant's perception of risk, in theory, can affect the validity of self-reported symptoms.

Methods

The magnitude and effect of possible "risk perception bias" was evaluated as part of a series of randomized trials designed to assess infectious disease transmission via exposure to marine recreational waters with modest sewage contamination. All study subjects were blinded to both their individual indices of exposure and the outcome illnesses under study.

Results

Of the five outcome illnesses studied, the effect of "risk perception bias" only affected one: Skin Ailments. Although analysis of crude rates of skin ailments showed the exposed group (bathers) to be 3.5 times more likely to report skin ailments relative to the non-exposed (non-bathers), when the data was stratified by any perceived health risk of bathing in such waters, this association was shown to be spurious in nature. Bathers having pre-conceived notions of any health risk due to the exposure were 10.63 times more likely to report skin ailments relative to the unexposed (non-bathers) (95% CI 2.36-47.8, $P = 0.0002$), while bathers without any pre-conceived notion of risk were no more likely to report skin ailments relative to non-bathers (OR = 0.60, 95% CI 0.11-3.24, $P =$



0.71). Further stratification by exposure grouping showed bathers with pre-conceived notions of excess risk to be 4.78 times more likely to report skin ailments relative to bathers without any notion of excess risk (95% CI 1.04-21.86, $P = 0.03$), while among non-bathers those with pre-conceived notions of risk were 3.70 times less likely to report skin ailments relative to non-bathers without any pre-conceived notion of risk (95% CI 0.70-19.60, $P = 0.10$).

Conclusions

This study shows that “risk perception bias” can be strong enough to lead to spurious associations in the presence of self-reported symptoms, and should be controlled for in future epidemiologic studies of recreational water associated illnesses and other water associated environmental exposures where the use of self-reported symptoms cannot be avoided.



Perception Bias and Illness Associated with Bathing in Marine Waters

Randomized Trials of Bathers exposed to marine waters contaminated with various degrees of sewage contamination

- **Goals:** To substantially improve upon previous Prospective Cohort Designs by minimizing following sources of bias:
 - minimizing misclassification of exposure
 - Strengthening measures of outcome illness
 - Eliminate possible selection bias by use of randomisation
 - use all data collected in Analysis
 - Improve estimates of Enterococci via use of replicated determination of every sample taken
 - Control for "Perception Bias"

Study Location

- Four year research programme
- Four beaches which passed EU Imperative Standards chosen by the DETR Committee.-



Recruitment

- Healthy adult volunteers were recruited in local shopping centres
- **Publicity was given in the local media"**
- Volunteers signed a subject information sheet-



Interviews

- 1-3 days prior, on the day and at 7 days post-exposure
- Medical examinations
- Clinical samples collected-



Questionnaire



- 4 part questionnaire
 - 1-3 days before
 - Exposure day
 - 7 days post exposure
 - 21 days post exposure-




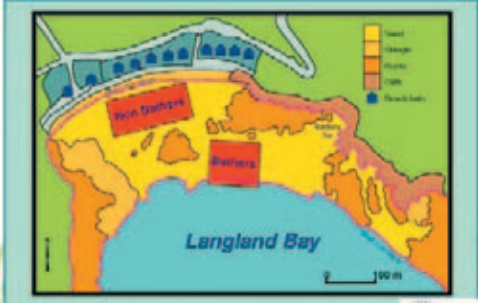


Randomisation

- Randomisation
 - Status reported to volunteers on arrival at the beach-



Beach logistics-



Beach supervision - non-bathers

- Roped off area
- Questionnaire
-



Beach Supervision - Bathers

- One-to-one observation and recording
- Safety cover essential
- Head immersion
- Roped off area to define bathing zones
- Sampling in the zones within 10m and 15 minutes of exposure-



Interviews-





The 'Free Lunch-








Exposure Measurement

- Water quality measured every 30 minutes
- Synchronous sampling
- Six locations 20m apart
- Three depths
- Five bacterial indicators-

Exposure Measurement-

Post-Exposure Interviews

- 7 days post-exposure

Questionnaire administered

- Further medical-






**Table 1
QUESTIONS USED TO ASSESS THE PRESENCE OF POSSIBLE PERCEPTION**

1. Do you consider water-related activities dangerous?
YES ___ NO ___


If YES, which of the following water-related activities do you consider dangerous: (Can check more than 1)

Dinghy Sailing ___ Canoeing ___ Wind Surfing/Sailboating ___
Scuba/Shorkeling ___ Water Skiing ___ Surfing ___
Swimming/Bathing ___ N/A ___



2. Have you heard anything regarding the way beaches are maintained in the U.K.?
YES ___ NO ___
If YES, has the information been positive or negative?
POSITIVE ___ NEGATIVE ___
If NEGATIVE, how often do you worry about this issue?
Not at all ___ Somewhat ___ Very Much ___ N/A ___


3. Have you heard anything regarding the cleanliness of bathing waters in the U.K.?
YES ___ NO ___
If YES, has the information been positive or negative?
POSITIVE ___ NEGATIVE ___
If NEGATIVE, what specific problems have you heard about? (Can check more than 1)
Oil spills ___ Objects floating in water ___ Health risk ___
Chemical pollution ___ Sewage pollution ___ N/A ___



4. Have you ever refused to go bathing for any of the following reasons: (Can check more than 1)

Beach too dirty ___ Water too dirty ___
Surf/waves too rough ___ Fear of becoming ill ___

5. Have you ever become ill soon after bathing in waters in the U.K.?
YES ___ NO ___
If YES, was it any of the following illnesses: (Can check more than 1)
Headache ___ Toothache ___ Ear ache ___ Diarrhea ___
Vomiting ___ Fever ___ Common cold-like symptoms ___
Sore throat ___ Eye irritation ___ N/A ___





6. Have you ever gone to the beach feeling ill?
 YES ___ NO ___

If YES, were you suffering from any of the following symptoms:
 (Can check more than 1)

Headache ___ Toothache ___ Ear ache ___ Diarrhea ___
 Vomiting ___ Fever ___ Common cold-like symptoms ___
 Sore throat ___ Eye Irritation ___ N/A ___

7. Did feeling ill on these occasions prevent you from entering the water to go bathing?
 YES ___ NO ___ N/A ___

8. When at the beach, do you bathe or enter the water:
 On EVERY visit to the beach ___ On MOST visits to the beach
 RARELY when visiting the beach ___ NEVER ___

9. How frequently do you immerse your head while bathing?
 ALWAYS ___ FREQUENTLY ___ RARELY ___ NEVER ___

10. How often do you get sunburned while at the beach?
 ALWAYS ___ FREQUENTLY ___ RARELY ___ NEVER ___

Swimming Associated Illnesses

- Gastroenteritis
- Respiratory Illness
- Ear Infection
- **Skin Ailments**

Table 2

SITE-SPECIFIC RATES OF SKIN AILMENTS

SITE	RATE/100 BATHERS		RATE/100 NON-BATHERS		P
	Rate	N	Rate	N	
Site A	5.7	106	1.6	122	0.15
Site B	6.5	92	8.7	150	0.55
Site C	13.0	146	15.6	147	0.52
Site D	14.4	125	4.5	156	0.004
ALL SITES COMBINED	10.4	460	7.8	575	0.23*

*P value from the Mantel-Haenszel summary statistic.

Table 3

SKIN AILMENTS AMONG NON-BATHERS VS. BATHERS AT QUARTILES OF INDICES OF SEWAGE CONTAMINATION - ALL SITES COMBINED -

EXPOSURE STATUS	N	RATE/100	P (Trend)	P (Q1-Q4)*
Non-bathers	575	7.8	0.31	0.97
Bathers 0-14**	101	8.9		
Bathers 15-27	122	13.9		
Bathers 20-50	109	10.0		
Bathers 51-158	137	8.8		

*Tests for statistically significant differences among bathers in highest vs. lowest quartile of fecal streptococci exposure.
 **Range of indices of sewage contamination (per 100 ml of sample) comprising each quartile of exposure.

Table 4

SKIN AILMENTS AMONG NON-BATHERS VS. BATHERS AT QUARTILES OF INDICES OF SEWAGE CONTAMINATION SITE D ONLY

EXPOSURE STATUS	N	RATE/100	P (Trend)	P (Q1-Q4)*
Non-bathers	156	4.5	0.001	0.17
Bathers 0-10**	49	8.2		
Bathers 11-23	20	20.0		
Bathers 24-33	25	16.0		
Bathers 34-70	31	19.4		

Bathers only P (Trend) = 0.17***

*Tests for statistically significant differences among bathers in highest vs. lowest quartile of fecal streptococci exposure.
 **Range of indices of sewage contamination (per 100 ml of sample) comprising each quartile of exposure.
 ***Comparison between bathers exposures only.



NON-WATER-RELATED RISK FACTORS FOR SKIN AILMENTS

A SKIN RASH ANYWHERE ON THE BODY PERSISTING FOR MORE THAN 24 HOURS WITHIN 3 WEEKS PRIOR TO INITIAL INTERVIEW

A SKIN ULCER OR SORE ANYWHERE ON THE BODY PERSISTING FOR MORE THAN 24 HOURS WITHIN 3 WEEKS PRIOR TO INITIAL INTERVIEW

ITCHING AND IRRITATION ANYWHERE ON THE BODY PERSISTING FOR MORE THAN 24 HOURS WITHIN 3 WEEKS PRIOR TO INITIAL INTERVIEW


USE OF STEROIDS WITHIN 4 WEEKS PRIOR TO INITIAL INTERVIEW

USUAL FREQUENCY OF SUNBURN (ALWAYS and FREQUENTLY vs. RARELY or NEVER)

USUAL FREQUENCY OF MEDICATION IF SUNBURNED (ALWAYS and FREQUENTLY vs. RARELY or NEVER)

ILLNESS IN HOUSEHOLD WITHIN 3 WEEKS SUBSEQUENT TO TRIAL DAY

SKIN AILMENTS IN HOUSEHOLD WITHIN 3 WEEKS SUBSEQUENT TO TRIAL DAY THAT PRECEDED ILLNESS IN VOLUNTEER




SKIN AILMENTS AT SITE D

STRATIFIED BY ANY KNOWLEDGE THAT BATHING IN THE UNITED KINGDOM MIGHT PRESENT A POSSIBLE HEALTH RISK

BATHERS

Knowledge Status	N	% Reporting Skin Ailments	Odds Ratio	95% Confidence Interval
Did not have knowledge	42	4.8	1.00	---
Had knowledge*	83	19.3**	4.78	1.04 - 21.86

*Based on the study participant indicating that they had "heard" that bathing in U.K. waters might present a "health risk."
**P = 0.03



SKIN AILMENTS AT SITE D

STRATIFIED BY ANY KNOWLEDGE THAT BATHING IN THE UNITED KINGDOM MIGHT PRESENT A POSSIBLE HEALTH RISK

NON-BATHERS

Knowledge Status	N	% Reporting Skin Ailments	Odds Ratio	95% Confidence Interval
Did not have knowledge	65	7.7**	1.00	---
Had knowledge*	91	2.2	0.27	0.051 - 1.44

*Based on the study participant indicating that they had "heard" that bathing in U.K. waters might present a "health risk."
**P = 0.10




TABLE 7 SKIN AILMENTS AT SITE D


STRATIFIED BY ANY KNOWLEDGE THAT BATHING WATERS IN THE UNITED KINGDOM MIGHT PRESENT A POSSIBLE RISK TO HEALTH*

STUDY PARTICIPANTS WHO INDICATED THEY HAVE NOT HEARD ANYTHING REGARDING THE POSSIBILITY THAT BATHING IN U.K. WATERS PRESENTS A POSSIBLE RISK TO HEALTH

BATHERS VS. NON-BATHERS

Exposure Status	N	% Reporting Skin Ailments	Odds Ratio	95% Confidence Interval
Non-Bathers	65	7.7**	1.00	---
Bathers	42	4.8	0.60	0.11 - 3.24


**P = 0.71



STUDY PARTICIPANTS WHO INDICATED THEY HAVE HEARD THAT BATHING IN U.K. WATERS MIGHT PRESENT A POSSIBLE RISK TO HEALTH*

Exposure Status	N	% Reporting Skin Ailments	Odds Ratio	95% Confidence Interval
Non-Bathers	91	2.2	1.00	---
Bathers	83	19.3**	10.63	2.36 - 47.81

*Based on the study participant indicating that they had "heard" that bathing in U.K. waters might present a "health risk."
**P = 0.0002





Questions and Answers

No questions.



Criteria Development: Beach Act Requirements and Schedule

Stephen Schaub

U.S. Environmental Protection Agency

Biosketch

Dr. Stephen Schaub is a Senior Microbiologist with the U.S. Environmental Protection Agency's Office of Water. He provides scientific support to Clean Water Act and Safe Drinking Water Act programs within the Office of Science and Technology. Dr. Schaub received a B.S. Degree in Bacteriology and Public Health from Washington State University and a M.S. and Ph.D. from the University of Texas (Austin) in Microbiology (Environmental Virology). For 20 years Dr. Schaub worked as a program manager and head of the Microbiology Research for the Department of the Army's Biomedical Research and Development Laboratory at Fort Detrick. He was responsible for supporting the Military's efforts to protect soldier health against exposures to microbial pathogens in water and wastewater. Since 1992 Dr. Schaub has been a Senior Microbiologist with the USEPA's Office of Water and supported regulation development for the new family of Enhanced Surface Water Treatment Rules. He has also been involved in determining and supporting research and programmatic needs for establishment of future recreational water quality criteria to protect against gastrointestinal illnesses and determining requirements for effective approaches to reduce microbiological pathogens for safe discharge of treated wastewater. He is currently responsible for development of new recreational water quality criteria and criteria for *Cryptosporidium* in drinking source waters. Dr. Schaub is the lead for development of microbiological pathogen risk assessment protocols for water-based media and is also the lead for the establishment of Agency-wide microbiological risk assessment guidelines.

Abstract

The Year 2000 BEACH Act Amendments to the Clean Water Act requires the USEPA to prepare new or revised 304(a) Ambient Water Quality Criteria for Recreational Waters by October 2005. Over the past 4 years the Agency has conducted a series of research efforts to provide data for use in establishing the new Criteria. Principal efforts have been the following: beach sampling studies to characterize impacts of spatial and temporal, as well as environmental, factors on indicator microorganisms distributions in beach waters; new epidemiology studies to characterize the acute gastrointestinal disease incidence from swimming exposures in fresh water; and identification and evaluation of new rapid enterococci methods and other fecal indicators for recreational water monitoring and characterization of their relationship to acute disease incidence. Over the next year the Offices' of Water and Research and Development will work together to establish new or revised fresh recreational water quality criteria based upon the above studies. The Criteria will utilize the new epidemiological information on recreational exposures and acute disease risks. The Criteria will also take advantage of the rapid quantitative polymerase chain reaction (QPCR) techniques to quantify indicator levels in less than 2 hours, which will allow beach operators to know the water quality conditions before swimmers even get to the beach. Additionally, the new criteria will identify improved mathematical approaches to characterizing the indicator to disease relationships and will provide more realistic sampling protocols to monitor the dynamic water conditions typical of beach waters. During the process of development of the Criteria the Agency will seek input from the States and other stakeholders to help fine tune the criteria to meet national health protection goals for fresh water recreational activities.



New Criteria Development: BEACH Act Requirements for 2005

Stephen Schaub, Ph.D.

USEPA, Office of Science and
Technology Health and Ecological
Criteria Division

Purpose of Briefing

- Provide background on legislative requirements of the Act and preparatory actions.
- Discuss activities and their status for application to criteria development

Legislative Background

- Overview
 - Beaches Environmental Assessment and Coastal Health Act of 2000 (BEACH Act) amended the Clean Water Act to require revised 304(a) Ambient Water Quality Criteria be prepared in 2005
 - The Amendment describes a number of activities necessary to prepare the Criteria

Legislative Background (continued)

- Section 104(v)(a) amendment requires that actions be undertaken to conduct studies within 18 months of enactment to develop information regarding:
 - An assessment of potential human health risks resulting from exposure to pathogens in coastal recreation waters, including non-gastrointestinal effects (**Epidemiology of Recreational Water**)
 - Appropriate and effective indicators for improving detection, in a timely manner in coastal waters, of the presence of pathogens harmful to human health (**Rapid Fecal Indicators**)

Legislative Background (continued)

- Appropriate, accurate, expeditious and cost-effective methods (including predictive models) for detecting in a timely manner in coastal waters, the presence of pathogens that are harmful to human health (**Water Monitoring**); and
- Guidance for State application of the criteria for pathogens and pathogen indicators to be published under section 304(a) (9) to account for the diversity of geographic and aquatic conditions.

Legislative Background (continued)

- Prepare Revised Criteria under Section 304(a):
 - Not later than October 2005, after consultation with appropriate Federal, state, tribal and local officials, the administrator shall publish new or revised water quality criteria for pathogens and pathogen indicators (including a revised list of testing methods as appropriate) based on the results of the studies conducted under section 104 (v) for the purpose of protecting human health in coastal waters



Preparatory Activities

- **Epidemiology Studies**
 - Four prospective epidemiology studies were conducted on Great Lakes beach waters during the summers of 2003-04
 - Exposed (bather) & Control (non-bather) populations were recruited from beach users & illnesses were followed for up to 12 days after beach outings.
 - *Status:* Validating and analyzing data to determine illness rates and their demographic distribution

Preparatory Activities (continued)

- **Methods Development**
 - Evaluating molecular-based rapid analytical methods
 - quantify enterococcus & *Bacteroides fragilis* fecal indicators
 - these & current cultural method for enterococcus were used to monitor beach waters to correlate fecal contamination exposure levels for bathers during the prospective epidemiology studies
 - *Status:* Best new candidate method: quantitative PCR which detects and quantifies indicators within 2 hours from start of analysis

Preparatory Activities (continued)

- **Monitoring Considerations**
 - EPA's ORD-OW study evaluated spatial and temporal factors that influence beach water quality and their implications for protective water monitoring approaches
 - *Status:* Study showed that UV, tides, wind direction, waves, sample distance from shore, & rainfall can cause water quality to change rapidly & significantly (even daily) – impacts when, where, & how to monitor beach waters
 - Independent studies confirm that these & other factors influence variability of bathing water quality

Criteria Development – Part 1

- **Develop Microbial Human Health Criteria Methodology**
- **Develop protocol for conducting microbial risk assessments to establish water criteria**
- **Conduct economic benefit analysis for criteria**
- **Develop improved approach to numerical criteria – EPA Workshop in Winter '05**
 - Topics: evaluate composite sampling, arithmetic means, re-assess single sample maximums, ranges of acceptable water quality rather than hard numbers, others

Criteria Development – Part 2


New Criteria Document

- **Many Issues to consider and resolve**
 - Studies of Great Lakes waters will be examined for applicability to all fresh waters
 - Improved correlations between gastrointestinal illness and fecal indicator levels
 - Rapid analytical methods (<2hr analysis) - know water quality before or during swimming day
 - Numerical Criteria - easy to understand & implement; improved health protection for various levels of exposure
 - Improved spatial and temporal sampling procedures and locations to characterize beach water conditions

Public and Stakeholder Review

- **Review drafts of new Human Health Assessment Tools**
- **Review drafts of new/revised Criteria document**
 - Federal, State, tribal, and stakeholder groups
 - Workgroup of EPA Regional and State representatives
 - FR notice and response to comments



 **Implications of the New 2005 Criteria and Relation to Other Beach Act Activities**

- New Criteria are only for fresh waters: statistical analysis will characterize their national application.
- Beach Act allows 3 yrs for States to incorporate the Criteria into State standards
- Marine epidemiology-indicator studies slated for the future, but new marine criteria are years away
- Federal promulgation of the Beach Rule this fall is totally separate from the new Criteria of 2005



Questions and Answers

No questions.



Evaluation of Recreational Health Risk in Coastal Waters Based on Enterococcus Densities and Bathing Patterns

David Turbow, Ph.D.

Touro University International

Biosketch

(Not submitted)

Abstract

(Not submitted)



Evaluation of Recreational Health Risk in Coastal Waters Based on Enterococcus Densities and Bathing Patterns

David Turbow, Ph.D.
Assistant Professor of Health Sciences
Touro University International

Publication

- Environmental Health Perspectives. 111(4): April 2003.
- Co-authors: Nathaniel Osgood, Sunny Jiang
- University of California, Irvine.

Motivation for Study/simulation

1. Infectious disease rates in marine bathers often unmeasured/underreported.
 2. Need to characterize behavioral swimming patterns to improve risk management practices at recreational beaches (US EPA, 1998).
 3. Epi studies for recreational water exposure are expensive to conduct. Can use existing epi knowledge to ask important questions.
- Simulation objective methodology by which to estimate illness rates under exposure-related **uncertainties** (US EPA, 1998; IEH 2000).

Map of study site

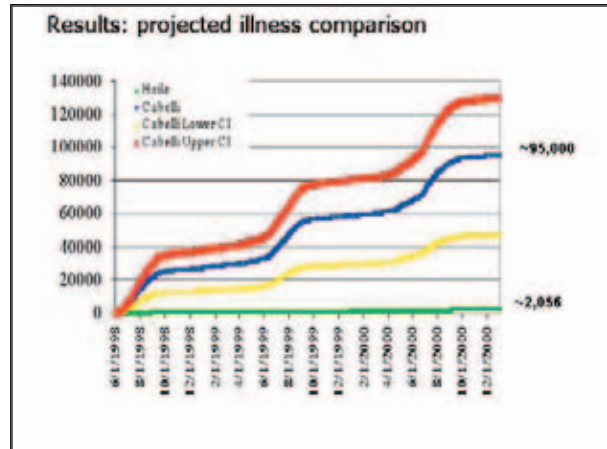
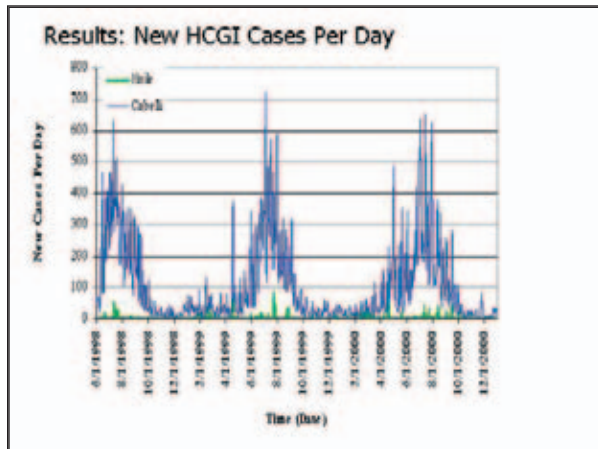
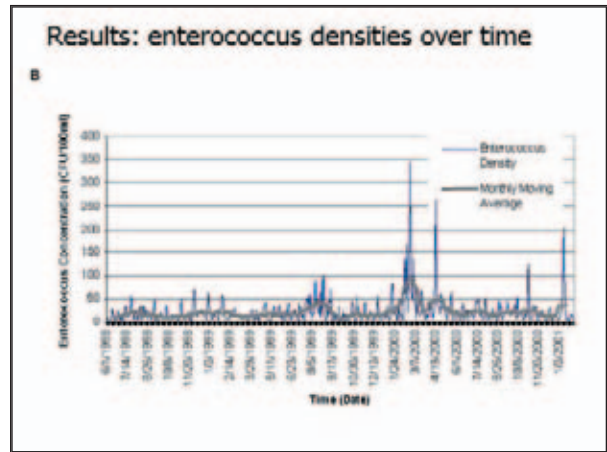
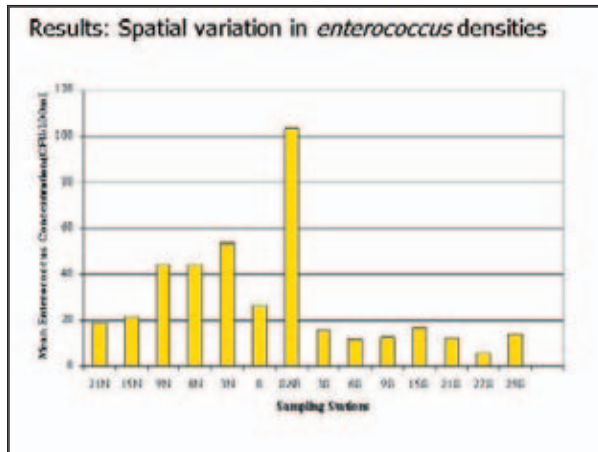
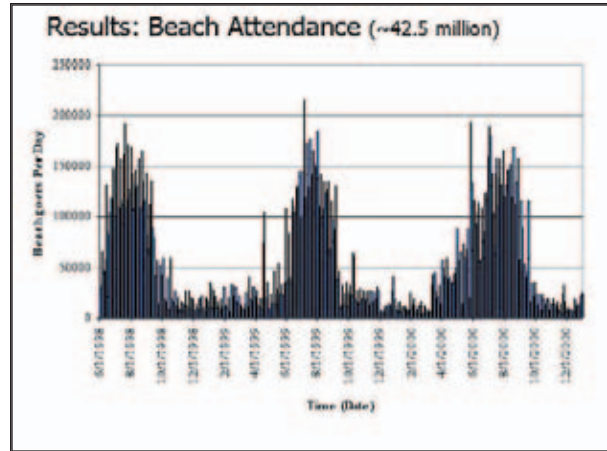
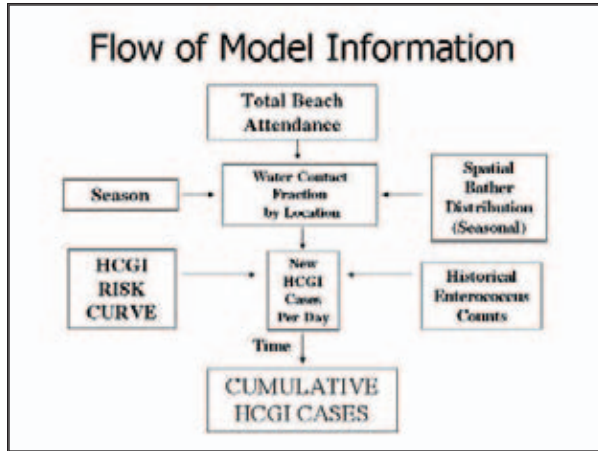


Study Goals

- Give probabilistic estimates of total bathers exposed to contamination by location.
- Estimate Highly Credible Gastrointestinal Illness (HCGI) rates in swimmers for particular beaches.
- Determine extent to which policy protects human health under current standards.

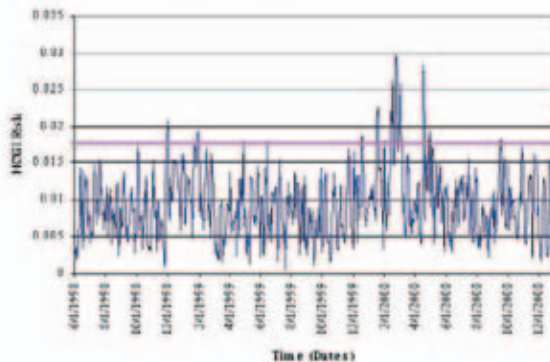
Goals of Simulation

- Calculate HCGI incidence under assumptions:
- (1) "Dose-Response" for *enterococcus* (EC) and illness risk.
 - **Sewage** (Cabelli et al., 1982)
 - **Storm Drain Runoff** (Haile et al., 1999)
 - (2) Level of swimming activity at sampling locations.
 - Uniform vs. clustered distribution
 - (3) Fraction of beachgoers who bathe during beach closures.
 - 0% vs. 10%





Results: Average daily HCGI risk



Results/Discussion

1. Thresholds for acceptable met risk when averaged over study period.
2. Total # of illnesses related to seasonal water usage.
 - Higher total # summer illnesses
 - Higher **fraction** of winter illnesses
3. Results sensitive to dose-response relationship.
4. Smaller impact of spatial distribution of bathers.
5. Results insensitive to bathing during beach closures.
→ 99% of illness cases occur when beaches open.

Conclusions

- Simulation shows that health risk is strongly related to seasonal WQ patterns, seasonal recreational water use.
- # of illnesses in model insensitive to bathing during beach closures
 - Discretion of health officials (Post or Close)
 - Enforcement of geometric mean standard
- Model is useful for identifying data needs, policy priorities.

Recommendations

1. Strengthen year-round health protection policies (e.g. sewage spills vs. wet weather sample exceedances)
2. Heighten awareness of postings, advisories.
3. Monitor bathing patterns to estimate exposure
4. Expand self-reported illness databases.
5. Conduct further epi studies (e.g. wet weather).

Limitations

- EC as an indicator
- Definition of "exposure". E.g. Single exposure, flat rates of seasonal recreation assumed
- Stationary model does not account for dynamics of disease transmission (susceptibility factors, repeat exposure, secondary transmission, pathogen shedding)

Acknowledgments

- Charles McGee (OCSD)
- Larry Honeybourne and Monica Mazur (Orange County Health Care Agency)
- Don Ito (California State Parks)
- John Blauer (City of Newport Beach Fire Department)
- Dennis Yunne (US Ocean and Safety)
- Steve Benson (City of HB Lifeguards)



Questions and Answers

No questions.

Thursday, October 14
12:00 p.m. – 1:20 p.m.

Lunch Speaker



Linking the Oceans and Human Health: Perspectives from the US Commission on Ocean Policy and the new NOAA OHH Initiative

Paul Sandifer

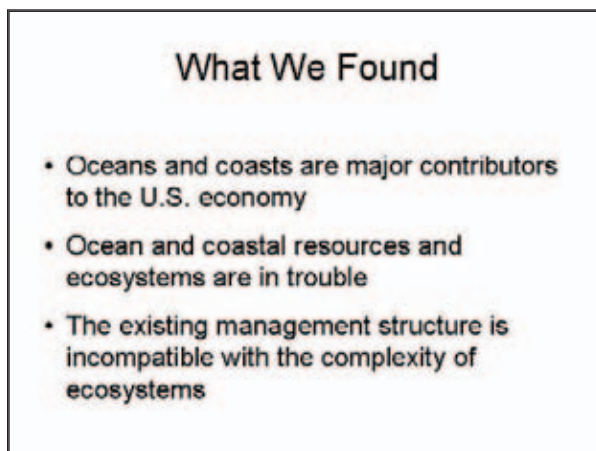
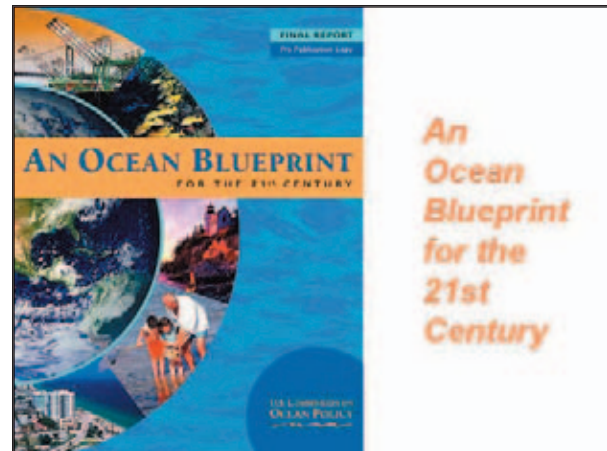
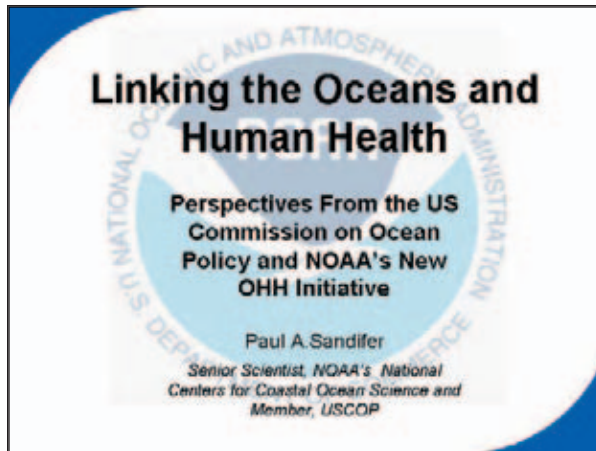
National Oceanic and Atmospheric Administration, National Center for Coastal Ocean Science, Hollings Marine Laboratory

Biosketch

Paul Sandifer's education includes a B.S. in biology from the College of Charleston (1968) and a Ph.D. in Marine Science from the University of Virginia (1972). After completing a 31-year career with the South Carolina Department of Natural Resources, including service as agency director under three Governors, in April of 2003 he moved to NOAA where he is Senior Scientist for NOAA's National Centers for Coastal Ocean Science. He is located at the Hollings Marine Laboratory in Charleston, SC.

Throughout his career, Dr. Sandifer has been involved in marine and natural resource policy and management, mission-oriented research and graduate education. He is author or co-author of numerous publications in aquaculture, coastal ecology, and marine biology and is a member of the graduate faculties of the College of Charleston and the Medical University of SC and an adjunct faculty member at the University of SC.

Dr. Sandifer is an Honorary Life Member of the World Aquaculture Society, a Fellow of the American Association for the Advancement of Science, and a recipient of South Carolina's highest civilian honor, the Order of the Palmetto. He has served on numerous boards and committees, including the Marine Board of the National Research Council, the South Atlantic Fishery Management Council's Scientific and Statistical Committee, the Atlantic States Marine Fisheries Commission (Chairman), and the founding Board of Directors of the South Carolina Aquarium. Currently, Dr. Sandifer serves on the US National Committee for the Census of Marine Life and on the Board of Directors for the Southeast Atlantic Coastal Ocean Observing System. In July of 2001, he was appointed by President George W. Bush to the 16-member US Commission on Ocean Policy, where he chaired the Commission's Stewardship Working Group, which dealt with issues involving management of living marine resources and pollution.





Cross-cutting Themes



USCOP and Ecosystem-Based Management

- US ocean and coastal resources should be managed to reflect the relationships among all ecosystem components, including human and nonhuman species and the environments in which they live.
- Looks at the links among living and nonliving resources, rather than single issues in isolation.
- Considers human activities and their effects in the context of the biological and physical environment.

Cross-cutting Themes



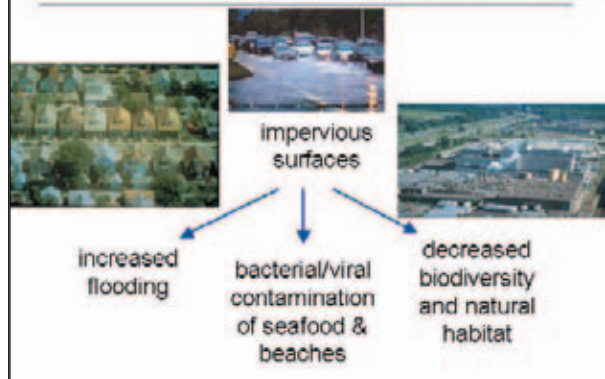
connections between ocean health and human health



Human Threats to Ocean Health

- NPS pollution, stormwater runoff
- Coastal development, wetland loss
- Overfishing, ecosystem collapse
- Ballast water, invasive species

Impacts of Coastal Development on Ocean and Human Health





Ocean Threats to Human Health

- Illnesses transmitted through contaminated seafood or contact with ocean water
 - Viruses
 - Bacteria
 - Algae
- How humans play a role:
 - disposal of waste (viruses and bacteria)
 - increased nutrient runoff (harmful algal blooms)

Ocean Threats to Human Health

- Natural disasters caused by fluctuations in climate and weather
 - hurricanes
 - flooding
 - severe winds
 - distribution of disease-causing organisms
- How humans play a role:
 - increased industrial air pollution speeds up climate change including global warming and changes in ocean temperature and currents

Ocean Benefits to Human Health

- Marine plants and animals provide beneficial medical uses
 - sources of pharmaceuticals
 - used as models in biological research that will benefit humans
 - greater biodiversity in ocean than on land
- How humans play a role:
 - increased pollution and waste decreases diversity
 - ballast water in international shipping ports brings new harmful marine species to our ocean

Based on these and other issues, the Commission recommended that the Nation:

- Increase funding for ocean and coastal research, including socioeconomic studies, at least 2-fold
- Expand investments specifically in ocean exploration and oceans and human health research
- Implement the national Integrated Ocean Observing System (IOOS)
- Expand and integrate a national monitoring network, including better coverage of coastal areas

Maintaining Coastal and Ocean Water Quality

USCOP Recommendations:

- Use ecosystem- and watershed-based management approaches
- Maintain progress in controlling point sources
- Focus greater resources on nonpoint source pollution
- Expand efforts to control vessel pollution, marine debris, and invasive species

Maintaining Coastal and Ocean Water Quality

USCOP Recommendations:

- Expand and enhance coastal monitoring programs and increase interagency cooperation at federal, state, and local levels
- Develop and deploy sensors to accurately and quickly detect pathogenic microorganisms as part of the Integrated Ocean Observing System
- Implement a strong, nationally coordinated Oceans and Human Health research program



USCOP Recommendations for Oceans and Human Health

- Establish a national OHH initiative to examine connections among ocean health, ecosystem health, and human health
- Expand efforts to discover marine bioproducts, encouraging private-sector partnerships and investments
- Develop improved methods for identifying and monitoring pathogens and toxins in ocean and coastal waters
- Fully implement all existing programs to protect human health from contaminated seafood and coastal waters

NOAA Oceans and Human Health Initiative

- Established by Congress in the FY03 Appropriations Act
- Funded at \$8 M in FY03, \$10 M in 04, and Senate committee mark for 05 is \$20 M
- Program is located in NOAA's OAR (Oceanic and Atmospheric Research) Line Office
- Has three major components:
 - NOAA Centers of Excellence in OHH
 - External grants in OHH
 - Distinguished scholars

NOAA Oceans and Human Health Initiative

- Three CoE named via competitive process:
 - Great Lakes Environmental Research Lab in Ann Arbor, MI
 - Hollings Marine Lab in Charleston, SC
 - NW Fisheries Science Center in Seattle, WA
- Specific issues being addressed by the three CoE include:
 - infectious diseases
 - chemical pollutants
 - water quality and beach safety
 - seafood quality
 - harmful algal blooms
 - sentinel species
 - data management
 - education and outreach

Hollings Marine Laboratory Charleston, SC



Hollings Marine Laboratory Charleston, SC





HML Focus Areas

- Assess the health status of key marine organisms using genomic technology
- Identify and quantify emerging chemical contaminants of concern in coastal waters and assess potential effects on marine organisms and humans
- Detect, identify and determine the sources and consequences of human pathogens in coastal waters
- Integrate results into a common ecological framework -- tidal creek ecosystem

Public Concerns/Questions

- Are the fish and shellfish safe to eat?
- Is it safe to swim in the water?
- If not, what needs to be done to make swimming and eating seafood safe?





Questions and Answers

Q: What are the chances that the recommendations for adding money for research will be acted upon favorably?

Paul Sandifer

It is hard to tell, but it is clear that the Administration and Congress are interested. The Senate Committee gives approximately \$454 million to NOAA; \$206 million of that is new over fiscal year 2004's levels. This is a significant step. Money designated for oceans and human health is increasing.

Q (Rachel Noble, University of North Carolina at Chapel Hill): What is the level of interaction between NOAA and NSF, NIHS?

Paul Sandifer

There is interaction at the investigator level. The NOAA external advisory committee includes people from NSF and NIHS. Scientists will do a better job of collaborating than administrators will.

Comment (Kelly Goodwin): Concerning the \$20 million funded by the Senate for Oceans and Health, NOAA's budget is not doing as well in the House.

Thursday, October 14
3:20 p.m. – 5:00 p.m.

**Session Eleven:
Plenary Panel Discussion**



Introduction

Session Moderator: Steve Weisberg

Southern California Coastal Water Resources Project

Biosketch

Dr. Stephen Weisberg is Executive Director of the Southern California Coastal Water Research Project (SCCWRP) where he specializes in the design and implementation of environmental monitoring programs. He serves as chair of the Southern California Bight Regional Monitoring Steering Committee, which is responsible for developing integrated regional coastal monitoring for the Southern California Bight. He also serves on the Steering Committee for the US Global Ocean Observing System (GOOS), the National Oceanographic Partnership Program's Ocean Research Advisory Panel, the Alliance for Coastal Technology Stakeholder's Council, the State of California's Clean Beaches Task Force, the National Research Council Committee on Waterborne Pathogens and on Technical Advisory Committees for the University of Southern California Sea Grant Program and the Southern California Wetlands Recovery Program. Dr. Weisberg received his undergraduate degree from the University of Michigan and his Ph.D. from the University of Delaware.

Panel Members

Denise Keehner

U.S. Environmental Protection Agency, Office of Science and Technology

Biosketch

Denise Keehner is the Director of the Standards and Health Protection Division in the Office of Science and Technology in the Office of Water. Her Division is the Headquarters Office responsible for the Water Quality Standards Program, the Beach Program, and, the Fish Advisory Program. Denise has been in this position since May 2003.

Abstract

Current methods for enumerating indicator bacteria require an incubation period of 18 to 96 hours, during which time contaminated beaches remain open. Several technologies that have the potential to produce results in less than four hours are under development. Here we evaluated four of those technologies, including immunomagnetic capture with ATP quantification, flow cytometry, dual wavelength fluorimetry, and quantitative PCR (Q-PCR). Fifty-four blind samples encompassing a range of bacterial concentrations and matrix complexity were processed and compared to values obtained by standard culture-based methods performed at six reference laboratories. Each method was evaluated for speed, accuracy, sensitivity, precision, robustness across different matrices, as well as ease of use.

Prior to her joining the Office of Water, Denise was the Director of the Biological and Economic Analysis Division (BEAD) in the Office of Pesticide Programs (OPP) and the acting Director of the Environmental Fate and Effects Division in OPP. She has been with USEPA at Headquarters for 26 years and has served in management positions since 1985.



Shannon Briggs

Michigan Department of Environmental Quality

Biosketch

Shannon Briggs has a B.S. in Animal Science, Ph.D. in Pharmacology & Toxicology—all at Michigan State University. She started working with beach monitoring programs in 1999. She is currently the President of the Great Lakes Beach Association, which is an informal group of people from local, state, and federal agencies that conduct research or beach monitoring programs

within the Great Lakes Region. They network daily with each other via a beachnet listserv. The web address for the Great Lakes Beach Association is <http://www.great-lakes.net/glba/index.html>. She currently manages over 30 individual beach monitoring grants with health departments and non-profit groups in Michigan. Beach monitoring grants in Michigan receive state funding from the Clean Michigan

Rachel Noble

University of North Carolina at Chapel Hill, Institute of Marine Sciences

Biosketch

Dr. Rachel Noble is an Assistant Professor at the University of North Carolina at Chapel Hill, Institute of Marine Sciences in Morehead City, North Carolina. She previously held a joint appointment between the University of Southern California's Wrigley Institute for Environmental Studies and the Southern California Coastal Water Research Project and focused her work there on regional assessment of water quality along the Southern California shoreline, and detection of enteroviruses in stormwater impacted areas of the coast. In July of 2001, she moved from the West Coast to the East Coast, and there has focused upon the use of molecular techniques, such as Quantitative Polymerase Chain Reaction (Q-PCR) for identification of sources of fecal material in estuarine, coastal, and freshwater environments,

for use in assessment of microbiological water quality. Dr. Noble's research currently focuses on the quantification of enteric human pathogens in a variety of environments, including recreational areas, shellfish beds, and commercial fishing areas. She is interested in relating the presence of known human pathogens such as enteroviruses, *Vibrio vulnificus*, and *Salmonella sp.*, to levels of fecal coliforms, *E. coli*, and enterococci in recreational waters in order to better protect human health. Other current research foci are basin-scale determinations of pathogen persistence, fate and transport in estuaries, and the impacts of nutrient loading and eutrophication on pathogen survival and ecosystem health. Dr. Noble has also recently been involved in the development of real-time detection of both pathogens and indicators as tools for creating accurate hydrologic and probability-based models of estuarine and coastal systems.



Mark Gold, D.Env.

Heal the Bay

Biosketch

Mark Gold, D.Env., is Heal the Bay's Executive Director. Heal the Bay is an environmental group dedicated to making Santa Monica Bay and Southern California coastal waters safe and healthy for people and marine life. Dr. Gold's extensive work with water quality and coastal natural resource topics ranges from sewage treatment, contaminated sediments, legislative and environmental education issues to urban runoff, contaminated fish and wetland restorations. In 1996, working in conjunction with the Santa Monica Bay Restoration Project and the USC Medical Center, he was a co-author of the first epidemiological

study of swimmers in runoff-polluted water. He also has co-authored several stormwater, contaminated fish and beach water quality bills and ordinances, and he created Heal the Bay's Beach Report Card®. He is a vice-chair of the Santa Monica Bay Restoration Commission, sits on the State Water Board's Clean Beach Advisory Group and served on the EPA's Urban Wet Weather Federal Advisory Committee. Dr. Gold also was appointed to the California Ocean Trust. Dr. Gold has bachelor's and master's degrees in biology from UCLA, and he received his doctorate from UCLA in environmental science and engineering in 1994.

Monica Mazur

Orange County Environmental Health

Biosketch

Monica Mazur is the Supervising Environmental Health Specialist for the County of Orange Health Care Agency's Ocean Water Protection Program. She has over 30 years experience protecting public health in this area. She oversees the day-to-day program operations including ocean water closure decisions. Ms. Mazur currently serves on

numerous technical and advisory committees including the State Water Resources Control Board's Clean Beach Task Force and Beach Water Quality Working Group. Ms. Mazur has a bachelor's degree in Social Ecology from the University of California at Irvine. She is also a California State Department of Health Services Registered Environmental Health Specialist.



Question 1: After everything that you have heard here, what aspects of beach programs need the largest improvement given existing technologies? How can federal, state, and local programs work together most effectively?

Panelists' Responses

Denise Keehner

A year and a half into the beaches program as well as other programs, I can see interesting things that people just in the beaches program may not see. Also, by listening to these speakers here, I've helped form ideas on what EPA needs to do. When you ask what direction we need to head in, we need to ask ourselves what is the destination here. We need to collectively have the same sense of what the destination is. If we don't have a sense of that it is difficult to prioritize things. Its not about having affective advisories and closings, its to reach a point where we don't need advisories and closures because things are improved enough that its rare we need those things. Source tracking, making available better science, tracking where contamination is coming from and what can be done. I think about how things need to be integrated between programs. Are local departments engaged as much as they should be.

We need to invest in source tracking—improve science so that we can identify sources of fecal contamination and figure out what can be done to eliminate the source. We need to better integrate the beach program with water quality standards and Clean Water Act programs. We need to ask if state and local government as engaged as they should be. EPA needs to do more to identify the governments that are working well in an integrated way, to share experience of what works for success, such as how they handle closures and postings. The people closest to those issues need to share their experiences of how to integrate programs, what made it happen and what were the critical factors. EPA needs to do more to help those agencies be effective. EPA workshops are important because they help us see what really makes a difference in the environment. EPA should take the role of sponsoring workshops and other opportunities to get people talking.

But there is still value in getting better indicators and more rapid methods, and better linkages with indicator and human health risk. But, over the next several years, EPA needs to shift some resources to other areas that result in improved water quality over the near term.

Shannon Briggs

I sent an email regarding this question to the Great Lakes Association members. From their responses, I realized that we already have an email listserv locally. Richard Whitman suggested that we start utilizing this listserv, so we found someone to host it. It is called the great lakes



information network, and it has been a wonderful tool. It's a great way to share information. For example, someone had seagulls on the beach. They noticed one day that the seagulls all were drunk. Then, the next day, all the seagulls were all dead. He asked what happened, so everyone saw the email and could learn about this together. It was a good way for people to learn. With an email listserv, everybody has a chance to input ideas. The Great Lakes listserv is open to everyone. Everyone can learn at the same pace.

I know that Charles Kovatch has a listserv for EPA beaches. How open is that to everyone? We have local health departments, USGS, and people from Canada using ours. I received comments from federal, nonprofit, state and local agencies, as well as from agencies in Canada. It's a great way to get info out.

I'm looking for existing technology to help standardize sampling. We should agree on the right way to sample. For example, some health departments use sampling rods when they sample, but do you stir the water up, or keep it still, sample upstream or downstream, sample in the morning or afternoon? How do you standardize these things? The sampling methods can affect whether beach will be open or not. I also think we should look at ankle deep water more—the swash zone. More people go into ankle-deep water than in chest-deep waters, so should we be collecting our sample at ankle-depth?

Also, we need to get better grip on the data. We need to figure out what to do with it and how to analyze it. How do we organize our schema so they make sense to the government and to the modelers, its nice to have the data on a website, but then what do we do with it? We need to get a better strategy for organization and use of the data.

Mark Gold

I helped to write California's Beach Initiative and Beach Water Quality Act (AB 411). In California, we like waves and surf, and we like our wildlife alive. We have to have greater national consistency in our programs. For example, we will see a talk tomorrow asking why California and Hawaii do not count as far as having good beaches because we monitor and post more frequently. People in California monitor and post and close beaches more often than beaches in other states. A posting in California should mean the same as a posting in Florida. People that go to different states need to know what the postings mean.

You've seen the epidemiological studies. We need to put everything together to target the most at risk, the most exposed individuals. The children who swim or play in ankle-deep water are the most exposed. Those are the same populations that swim at creek mouths. The use of other multiple indicator criteria is important. We need clear definitions of high, medium and low risk. There needs to be at least weekly monitoring for low risk beaches, or why bother monitoring at all? And, there should be daily monitoring for high-risk beaches. Closing beaches after sewage spills needs to be mandated, not just recommended.

Posting exceedances of standards is a right to know issue, even if you don't know the source of bacterial contamination. When the source is unknown, posting an advisory is still the best thing you can do. If the source is unknown, closing may be a waste of time and effort.

Money is needed for all the research that needs to be done. There is a need for more research for epidemiological studies in Southern California. Would it ever hurt for EPA to do an epidemiological study on the west coast? The second major round of EPA epidemiological work does not include California. It needs to happen.

In addition, chronic exposure issues needs to be addressed, such as the surfer populations that are out there surfing every day all year long. The surfing population should be targeted for health risks and chronic exposures.

Rachel Noble

Data management issues are the high priority that agencies such as EPA and NOAA face. It needs to be addressed top down, and it needs to be handled quickly. The funding is important.



Researchers are constantly trying to come up with ways to come up with new ways to conduct research, and are constantly leveraging money from other projects for basic research that should be supported because we need to answer research questions. Funding is a big issue, especially to study real world problems. From the scientific perspective, the European Union (EU) and World Health Organization (WHO) have recently moved forward with the idea of testing for the specific species, *E. coli* and *E. faecalis* as indicators of fecal contamination, rather than relying on detection of the entire Enterococcus group. This move needs to be addressed in the United States. Communication between the United States, and EU and WHO needs to improve, there are redundant research studies being conducted that would benefit from the knowledge gained by others on the other continent. We can improve the way that we manage water quality, especially to help much of the undeveloped world in the area of public health. Urban runoff in relation to health risk is an important area. I live in an area where dual beneficial uses reign (areas where shellfish harvesting and recreational waters are side by side), and the idea that NOAA, EPA and the National Shellfish Sanitation Council don't communicate as far as their standards go (fecal coliforms for shellfish and *E. coli* for recreational waters), is a problem. There is little movement of them coming to a compromise. Communication between these organizations would help us improve things.

I also examine the process of managing recreational water quality monitoring programs and programs for TMDL development, and have found that the two groups don't communicate. TMDLs implemented upstream of the coastline are being run by agency representatives that don't communicate with the people managing the coastline. It's a matter of the number of hours in the day. These agencies are severely hampered by resources. I am also interested in seeing in situ monitoring stations, the use of remote sensing, and the use of predictive models for improving our management of coastal water quality. We (people in the water quality field) can link up to people who understand hydrology, land use, physical oceanography, and we can make use of predictive models for assessing water quality. The wind model, for example, could be utilized.

Monica Mazur

We find that we need more risk assessment and epidemiological studies on the west coast because it is uncertain if one study (the Santa Monica Bay Restoration Project's "A Health Effects Study of Swimmers in Santa Monica Bay") is transferable to other locations. However, there is a large need for more funding because our local programs (state, counties and cities) don't have enough money to do these studies. There is a net cost to the counties to administer the ocean and bay water quality programs and they don't have the money in some cases to do the routine year round monitoring, even with the state AB 411 monies and the EPA Beach Grant monies. NRDC reported in 2004 that California spent 3 million dollars last year on monitoring. In Orange County, we spent \$3 million alone on monitoring. We need more funding for our NPDES and storm water programs, as well as for data management. The \$3 million did not even include the cost for special watershed characterization studies. There are huge costs to monitor and sample watersheds. It can cost millions of dollars to do watershed studies and remediation for small areas. \$10 to 15 million was spent to conduct the special studies and some remediation just in the Huntington Beach area.

There are equity issues when comparing state-to-state programs. We have so many postings in California, but is it because we are doing a better job of monitoring and posting and have stricter standards? We don't compare well to other states, many which aren't monitoring and posting for as long a coastline or for year round programs (back East, state monitoring programs may be for three months). We almost need a batting average approach that we can use to compare accurately and an even playing field for standardizing sampling and posting programs. But, we shouldn't apply same bacterial standards for different types of beaches. We have found that one size does not fit all. You have different risk levels and different contamination and use factors involved at different beaches. In California if you have good samples for a certain period of time (e.g., 2 years) you can stop sampling at that location. But, that isn't right either. Underground infrastructure ages and leaks may occur at any time, so sampling vigilance is necessary.



Data management and evaluation are other issues that we need to improve. We can't just collect the data; we need to do something with it. We have to ask, what does a sample represent, what time of day do we sample, how many samples do we collect per location, how far apart to we take the samples, where do we post the notices to the public, etc. There are a lot of issues based on those concerns that need to be standardized. As a priority, we should standardize bacteriological criteria and what the samples represent.

Audience Discussion

John Norton (California Water Resources Control Board)

Concerning monitoring programs, right now the way EPA is handling them is a disincentive for states to invest more in monitoring programs. States like California have very thorough monitoring programs. As an incentive I'd like EPA to lay out grading criteria for monitoring programs because many other states don't post advisories because they don't monitor very often. I'd like beach-mile-day to be the measuring unit used when EPA and others look at the number of closures and postings each state has, so that things are more comparable nationally. All areas need to be treated equally because the current method is not sufficient.

Mark Gold

EPA could consider funding only the programs that meet model criteria that everyone agrees upon.

Denise Keehner

We had intentions to make the data available this year but ran into some Internet technology (IT) issues with getting state data easily migrated into EPA's system.

Muriel Cole (Ocean.US)

We are a national office sponsored by nine agencies. Our purpose is to promote an integrated ocean and coastal observation system. I'd like to reiterate something Rachel mentioned, which is the need for cooperation and coordination among governments, agencies, nongovernmental organizations (NGOs), and academia. That is a priority.

David Rockwell (Great Lakes National Program Office)

We've been looking at data from the Department of Natural Resources (NRDC) web site. EPA should make data available. In one incident, Milwaukee, Wisconsin discharged water to Illinois beaches due to a heavy rainfall, then Illinois accused Milwaukee for closing Illinois' beaches. We should quantify a city's contribution to *E. coli* concentrations.

Steve Weisberg (Southern California Coastal Water Resources Project (SCCWRP))

This conference has brought together a wide array of people from different sectors. An impression is also made about who is missing: There is nobody here from the European Union (EU), Center for Disease Control (CDC), or shellfish organizations. We should look for other groups such as these to reach out to for guidance and for money.

Charles McGee (Orange County Sanitation District)

The Mission Bay study shows that one size does not fit all. Maybe standards don't mean the same thing in every location. We should use the Annapolis Protocol where people look at the situation, the beach, the inputs, and the fate and transport, and then design the monitoring program around that information instead of just trying to make the shoe fit. We need to look at each situation as situation-specific.

**Mark Gold**

The policy may apply and change depending on the risk level. If you have a highly populated beach, you might not close it after one high sample. There are flexibilities depending on the level of risk. The policy should be developed in a way that eliminates these conflicts.

Steve Weisberg

From what I have heard everyone say so far today, we want consistency, but we also want flexibility.

Toni Glymph (Wisconsin Department of Natural Resources)

Wisconsin didn't know that it was optional that we didn't have to monitor if there was a sewage outfall. For us, one of our frustrations is that since we are working with the local health departments and there are many different fiscal years, the money is needed and given at different times. We get the money from EPA in June, but we start monitoring in May and we can't charge back. So it would be nice if that could be corrected because the money is needed ahead of time when monitoring and work actually begin. Because of our small budgets and the limited availability of our Internet technology (IT) staff, money is tight. We give our staff a budget to work with, but we often have to change what we need them to do, requiring additional work, which is frustrating, because it wastes time and resources.

Roger Fugioka (University of Hawaii)

For over 20 years it has been reported that all streams in Hawaii have exceeded standards. It's difficult to understand why a state would accept a standard that it can't meet. Epidemiological studies do not apply everywhere, but the criteria are derived from those studies, therefore that is what states are supposed to use for their standards, regardless of whether the pollution is from point source or non-point sources. EPA has stated that 40 percent of coastal pollution is from non-point sources. Hawaii will use the EPA criteria, but why can't EPA consider the source of bacteria. This is similar to what was found during the Mission Bay study, where the pollution was from non-point sources. Hawaii says it will accept the EPA standards and wait to hear about new indicator standards, but I heard that the new indicator standards will not be out for a while.

Denise Keehner

Existing epidemiological studies are looking at the indicator organisms that seem most appropriate, and it can preclude us, but if a study is not done in the correct way, the studies are not consistent and it is difficult to use them to develop criteria. I'd like to look into the extent those epidemiological studies could be used. We can ask Steve Schaub about this.

Gregg Pettit (Oregon Department of Environmental Quality)

I understand that there is a desire for consistency among programs, such as for 303(d) listings. But one size does not fit all. In one year we looked at our data and dropped monitoring at some beaches in Oregon because those beaches met standards. Also, there were not many people in the water because the temperature is only approximately 55 degrees all year long. There are kayakers, but it's not the same magnitude as the number of beach users in California. Therefore, the appropriate program for one place may not be the same as for somewhere else. We need a program to continue monitoring so we can try to identify beaches with chronic problems.

Paul Sandifer (National Oceanic Atmospheric Administration (NOAA))

Communication should be broadened. One way to increase communication is to invite more people who are dealing with harmful algal blooms to the conference. They are a big problem in certain areas, like in reservoirs and in Florida. Some of the researchers are working along paral-



lel tracks as the researchers working with human pathogens. Inviting more people to this type of conference may help to eliminate the redundant work that is done. That would provide benefits, and may help solve some problems and bring in a new perspective.

Rachel Noble

In labs in North Carolina, they are finding that the pathogens are attached to the algal blooms. This is a good reason to add those people.

Clay Clifton (County of San Diego, Department of Environmental Health)

I agree with the Hawaii comment, that we should “strike while the iron is hot.” This week we’ve exponentially increased our collective knowledge on monitoring and indicators, but EPA is telling us that they are still several years away from modifying standards and changing criteria. It is frustrating and not inspiring. Maybe it is not the time to use a new indicator, but it is the time for EPA to make more specific recommendations on use of beach types and sample design and needs to make decisions on what should be mandatory and what should be discretionary.

Denise Keehner

The work that ORD is doing, with frequency and location of monitoring, will be put in a final report and a guidance document we are producing on monitoring. That is different from the new indicator ideas. New criteria involve a more standardized process. You may need to talk to someone else to find out if there are studies that have been done that will develop into marine criteria. Ask Steve or Rebecca if there are coastal studies on new indicators.

Shannon Briggs

In the Great Lakes, we often don’t have the money to do what we want to do. Even though EPA may not be doing something, you should still bring the ideas to EPA and try to collaborate with them so they can work with you and you can share some of the money. They don’t have the money to do everything, but we can get research together by patching together grants from different places to get the work done. For example, I take tests from the area and send them to Al Dufour so he knows what is going on. Working alone will not get as much done.

Clay Clifton

We have done that. We sent comments on the implementation guidance, but we don’t know what our impact was.

Rebecca Calderon (USEPA, National Health and Environmental Effects Research Laboratory)

To respond to Steve’s comments on the National Institute of Health (NIH) and CDC—CDC was invited to this conference, but they opted not to come. However, they are engaged with EPA. We have worked with both organizations, and NIH feels that unless you are doing something that deals with homeland security or bioterrorism, they are too busy to work with us. This isn’t their priority. This program is an unfunded mandate. There is no great flowing of money to handle the Beaches program. The program is the result of money being brought together. The studies that are being done in our research and development office are scraped together with the funds we have. Even though the state people look at us as having lots of resources, it’s difficult for us to get things done with the limited funding. If the Beaches Act does not get renewed, the program will go away because there are other pressing issues too. It is congress that makes appropriation decisions so we need to be sure that they have accurate information on the benefits to human health of the Beach program. In addition, EPA plans to do epidemiological studies in California in the next couple of years.



Sonia Nasser (County of Orange)

The Army Corps of Engineers (USACE) should be here too. We are doing massive watershed studies, but Orange County often has a problem because the Corps is not authorized to study water quality and so they are not engaged with EPA. They have money to spend on the studies, but can't do water quality. A joint USACE and EPA water quality study would be helpful.

Steve Weisberg

I know that some of the other agencies aren't here because they have other priorities, but I'm glad you did try to contact CDC (to Rebecca Calderon).

Denise Keehner

Responding to the comment on the USACE—USACE is working in other states with other groups. There are areas where there is collaborative work going on with EPA and the Corps in the area of water quality. Whether you can get the Corps involved depends on the project. It is good if you can form that collaborative effort around it because the Corps has a lot of funding to bring to the table.



Question 2: We've heard new technological developments: what is the role of EPA in the development of these technologies and where should their priorities be placed?

Panelists Responses

Rachel Noble

There are so many promising things out there, we should look at them all and not close our eyes yet to new ideas. We can identify new successes for the future. At this point, from a research perspective, there are a lot of people working in different environments, such as the food industry and bioterrorism, that have a lot to offer, and we should cross those boundaries and really examine the available technologies. We haven't gone far enough from an academic perspective. For EPA, we need to make some basic decisions on 3 different levels: near term (now-2 years), medium (2-5 yrs), and long term (10-15 yrs) so that we can look at specific technologies as being promising within the right time frame. There are things out there applicable for use the near term, I won't just advocate quantitative PCR for enterococci determination, which I think is useful, because its not as low cost as some of the other technologies. There are molecular methods that are useful. The fluorescence-based measurements like the Idexx adapted technology, dual wavelength fluorimetry. We need to look at new applications of some of the available methods. For medium and long term, we should look at electrochemical applications for sensitive detection of microbes—there are several means of using electrochemical attributes of bacterial cells to concentrate and detect cells and this should be further examined. It is used in other fields such as space science and may have applications in water quality.

Monica Mazur

We look to EPA and the federal government for the big picture items we can't do locally. Concerning the money issue, to pay for all of the new technological developments, I think you need to bring all the researchers you can together and find a big sponsor, which in this case would be the EPA, to develop rapid indicator and source tracking techniques which are key. But, once you have the rapid indicators or other technologies, what do you do then? Will we just be more confused faster? The expectation of faster methods may lead the public to want everything done faster—collection, analyses, notifications and postings. The public may want more samples collected—temporal and spatial. What does this mean to us? Logistically, it still takes a while to make a sampling run. We collect 20–35 samples along one stretch of beach before going to the lab for analysis.

Are we analyzing for the right things and what do we do to solve the source identification problem? Or if it's a natural source, what do we do to "fix" the input, for example the bird



sources? But first, we need the methods to determine for certain if it is a bird source at a particular beach.

You need the new methods to work with, but have to get to a point where they are used routinely. Methods acceptance by state and federal agencies are going so slowly now, and new indicators will add even more years to the process.

Shannon Briggs

Rapid methods are key. We get faster results and a new toy, but we can't look at it as the solution. Communities get excited about a new toy. Once they are more acceptable, usable, and cost effective, communities will be more interested in using them. Lots of private lake associations in our area want us to monitor local lakes. Funding is an issue, and we look at other sources of funding from anyone who is interested, including the army corps. The Department of Defense had a contract to look at nanotechnology, and they got people together and tried to get some money for that.

Health departments know they need change to keep up. An issue we face is if we are able to get rid of human sources of pollution, how do we get rid of other sources (i.e., sea gulls)? We could find other places for gulls to go, but we still have to deal with what they left behind—what is the risk assessment for that? What about other animal sources? What are the risks of those? And, how do we use the data?

Also, how do we use the data that we collect? And, we can't ignore the swash zone and the wet sand. That is where things wash up and the bacteria live. And everyone walks through it, and kids play in it. We need to focus on that.

Steve Weisberg

Can you clarify the issue of who is going to be the first kid on the block, who will be the kid with the new toy? Are you willing to do that and not wait for it to be verified and accepted? If the technology exits before EPA endorses it, will you use it?

Shannon Briggs

We are already doing that, such as with rapid tests.

Denise Keehner

In terms of the emerging technology, the rapid tests have real significance of implementation in our program. It will be interesting in how they play out. The more you look the more you find. If the rapid tests are affordable, there will be increased pressure to use them, and there will be more pressure for more testing, with more finding of impaired areas, and more issues with management. We will have more pressure to do source tracking, control releases, prevent overflows, and manage runoff. If we haven't done the research to understand what will mitigate those risks, we will be in trouble. It will trickle into lots of areas.

Concerning issue of differentiating between animal and human sources and which results in human health impacts, EPA should look at this. People will be asking questions on how fecal from animals compares to human impacts. It's a big question that needs more money to research. But, once we have some answers, EPA can then take on bigger issues with that.

Audience Discussion

Blake Traudt (Texas General Land Office)

Texas is in a unique position. My agency has no authority to implement the Beach Act. Our problem is we have a city that doesn't want to know what is in their water (our city doesn't want



to know after 24 hours have passed). The rapid indicators would really be beneficial for that reason. A lot of local governments will want to know once those indicators are being used.

Shannon Briggs

In Michigan, I'm in a similar situation. I can't really test the beaches, or open or close them. I have to go to the health departments because they are the only ones with the authority to close or monitor the beaches. I try to highlight the health departments with the best programs so that the other health departments are envious and want to show that they have good programs as well. That way they all participate. Our senator got it passed that if you have a public beach you have to post a sign saying whether your beach is monitored or not. I pitted the mayors against one another. Now, all the health departments respond.

Toni Glymph

Denise made a comment that we'd be in a worse situation if our technology supersedes our guidance. Not only do we have to regulate beaches, but we also have to regulate the wastewater coming into it. This causes a problem for regulations because wastewater and beach water do not use the same indicator. We are shifting from fecal coliform to *E. coli* at our beaches. They are using new technologies that we can't regulate. It is not consistent with wastewater. Things are all over the board. We are forced to move forward, but we can't control things. How do we defend ourselves? What do we tell the public? We need guidance for wastewater effluents. How do they defend themselves and say they have to do something with no reason? We need more guidance and clearer rules.

Denise Keehner

That method has been validated by interlaboratory methods. The effluent wastewater has been validated scientifically, even though it has not been officially released yet or published.

Toni Glymph

They are going to use the Idexx ones because they are simple.

Charlie McGee (Orange County Sanitation District)

We should focus attention on rapid detection technologies. Jay Fleisher pointed out that no one at the beach was ever exposed to the limits that were set. We were looking at getting the information on water quality at the beach in the morning, and comparing it to the illness rate. Concerning methods, Rachel talked about three terms of approach. If we want to analyze a sample in a controlled stream we are required to use EPA methods. I hope we can improve on the already approved methods and start using those right away. Using the Connecticut Procedure approved, right away, for enterococci. Mark Gold had to leave, but he wanted to share that same idea.

Matt Liebman (USEPA Region 1)

Until yesterday, I was on the rapid indicator bandwagon. But then we will have a rapid method to get us confused more quickly. Stanly Grant talked about a plume of bacteria in Huntington that lasts for about 2 minutes and then goes away. We need to think about exposure. If we have a rapid method ocean observance system and can get 20 to 30 measurements per day, we would have a good sense of what the exposure is—what the water quality is and the potential illnesses. Would that result in an increase in postings and advisories?

Monica Mazur

This brings up the question of how often to sample and what standard do you use? It's important to understand what currents do with bacteria levels. With the ocean observing system used with the bacteria levels, you have a better idea of what is going on out there. But this can add to



the confusion. If we simplify the method, who else will use it? Will locals and lifeguards monitor as well? This will bring about questions of who has the jurisdiction to put signs up. But, we still need the methods.

Steve Weisberg

One size doesn't fit all. Rapid indicators will push us to believe that even further. Once we have the rapid indicators, you still have different types of beaches. Some beaches have chronic sources, which the rapid methods won't help. With a chronic source, water quality may still change because of which way the wind is blowing. In cases like that, models will help determine where the pollution will be. Rapid indicators will help more with an unexpected problem and lead to quicker reaction, for example, by identifying a spill you didn't know about.

Rachel Noble

One thing to consider with rapid indicators is to demonstrate the relationship to pathogens quantitatively. Another thing to consider doing is to conduct an epidemiological study that involves humans, where people provide stool and blood samples, to see the actual pathogen, indicator, and disease relationship. Many epidemiologists can't believe this hasn't been done yet. It's a huge undertaking, though, but needs to be done.

Carl Berg (Hanalei Watershed Hui)

One problem is that the rapid test for enteric viruses may be worthless in tropical environments. One thing I see is a lack of consideration of pathogens associated with urine, not feces. There are many very serious diseases that come from wildlife, like *Giardia*, *Cryptosporidium*, and *Leptospira*, which have made people sick and/or have been fatal. We're not just dealing with skin rashes. In Samoa, we had an outbreak of leptosporosis, and we did blood testing of animals and people to find a better idea of the source. So, there are models here that can be used. We should ask EPA to pay more attention to other pathogens that aren't feces-related, but are potentially more deadly.

Rachel Noble

In North Carolina we are working on detection of other pathogens that are not routinely monitored but are becoming a problem due to changing climate and global warming issues. They deserve more attention.

Shannon Briggs

The issues that Carl Berg pointed out are an example of why we need to be connected by email, so we find out about these things right now and not every few years at a conference. You are limited with staff, resources, and time, and if we have an email system, that would help us communicate.

John Norton (California Water Resources Control Board)

I ask for old technology such as keeping sewage off the beach and in the pipe. EPA needs to make sure we have good reporting on sewage closures at beaches. Good sewage reporting provides the backbone of fixing the problem.

Shawn Ultican (Washington State County Health District, Kitsap County)

From all of the uncertainty that exists with the tools we are using, it seems misleading that we tell the public that we are keeping them from getting sick. We can't get there from here. We need to do what we can to correct long-term chronic sources, and then go in and do the surveys and determine the sources. We can't do that with the tools we have available now. In working at the county health district, the greatest asset is public trust. If I lose my credibility, then the money



and science doesn't matter because the public will ignore the health advisories that I give them. I worry about making false claims. I am concerned that my credibility is being lessened by posting advisories and telling the public they will get sick if they swim, but if they continue to swim and they don't get sick, then they will stop listening to the advisories. Until we can accurately assess health risk, we should be concerned about taking those claims and putting advisories out there that we can't necessarily support. Is there credibility in what we do?

Steve Weisberg

Is there credibility? There are two parts to that. Our measurement systems are imperfect, so what is our responsibility to warn the public when there is a possible risk versus when we know that our science is right. We have had many comments made here today, but the most common ones I am hearing are (1) develop a better epidemiological relationship, whether it's looking at the number of beaches or the kind of beaches we are sampling—otherwise it's hard to make the statements that people will get sick if they get into the water; (2) standardization is important; (3) coordination is important; (4) rapid indicators are important; and (5) we need to make sure as we are developing this technology that we have some certainty and we develop guidance. In addition, we need more money, which might take coordination between other agencies.

Denise Keehner

One final thing I'd like to convey is to use common sense around communities where there is a chronic source of pollution and balance whether it makes sense spending time to precisely quantify the human health risk from that before taking some action. I wonder if that is the best use of that money, compared to going back and figuring out what we can do for something like fecal contamination. There are ways we can move in the direction of fixing the problem rather than spending millions precisely quantifying the risk. We can instead say we have an issue (human fecal contamination) and take some action to understand the source and mitigate it. Concerning public health, think of the old days when waste was dumped out of the window and into the streets. We didn't have a quantitative risk assessment back then, we had major health problems associated with dumping human waste and we did something about it. It's not a big leap to think that what we are doing in our coastal areas is essentially the same, but into our waters instead of into the streets. We have many people moving to coastal areas, and we are developing those areas. Be careful about spending too much money trying to precisely quantify risk. Instead, let's use some of that money to take action to actually solve problems.