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Molecular tools for regulating recreational water: the journey so far.....

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Outline

- History and Background
- Clean Water Act
- NEEAR studies
- qPCR
- Challenges
- Implementation and criteria
- Current status
- Future

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History and Background

Exposures to fecally-contaminated water has long been associated with illness

- Late 1800's John Snow: Cholera transmitted through fecally-contaminated drinking water
- Early 1900s: Sea bathing at sites affected by sewage outfalls linked to outbreaks of typhoid fever
- 1950s: Epidemiology studies linked an increase in reported gastrointestinal and other symptoms with fecal coliform
- 1970s: Swimming associated outbreaks linked to Shigella, and other, unknown, etiologic agents, at sites impacted by sewage

- Fecal indicator bacteria are now widely used to monitor water and manage swimming associated health risk

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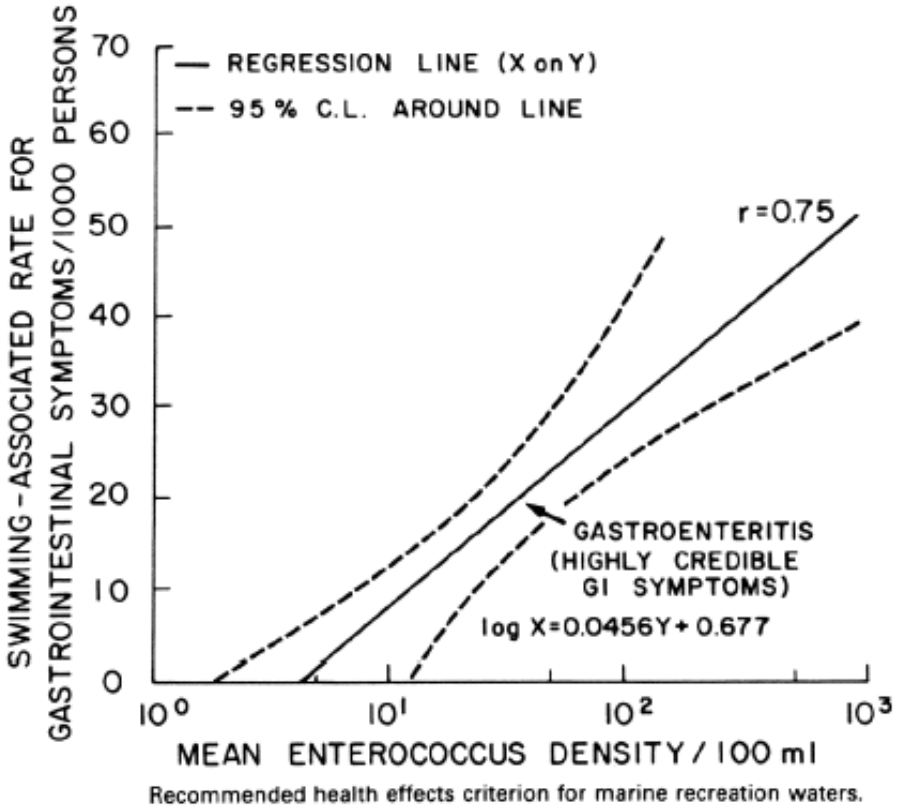
US EPA 1986 Ambient Water Quality Criteria

- POTW impacted beaches
- Reasonably sized discharging population
  - Constant ratio between indicators and pathogens
- Grouped data by day or over beach/summer
- Associations with FIB (*Enterococcus, E. coli*) and GI illness among swimmers
- Similar results obtained in randomized designs in the UK, Germany
- Demonstrated exposure response associations with FIB and swimming associated GI illness



Recommended health effects criterion for marine recreation waters.

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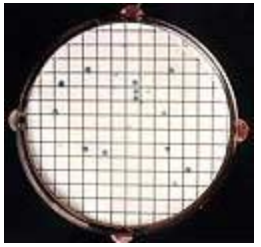
Fecal Indicator Bacteria

- Limitations of current methods:
  - Culturable methods; require 24-48 hours for results
  - Variability in fecal indicator bacteria density over the course of the day, time and location of sampling
  - Can result in inaccurate beach closings and notifications
- Development of methods to more rapidly detect environmental microorganisms



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Legal/Regulatory Framework: Clean Water Act

- EPA, under the CWA develops guidelines for beach notification and protection of designated uses of ambient/bathing waters
  - States must adopt into regulation
  - Beach notification: Local enforcement
  - Protects “designated use” (e.g. swimming, fishing)
    - Permits required for discharges
    - “Impaired” designation results in TMDLs
- EPA develops guidelines which states adopt

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BEACH Act Amendments

- Beaches Environmental Assessment and Coastal Health Act of 2000 (PDF): Amendments to the Clean Water Act
  - "appropriate and effective indicators"
  - "timely detection"
  - "assessment of human health risks"
- Required EPA to publish new or revised criteria based on these studies within 5 years and every 5 years thereafter
- EPA failed to publish new criteria and was successfully sued by the NRDC and Los Angeles County

The NEEAR Water Study

Research question:

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

Figure 1: Plume from Tijuana Estuary flowing south to Border Field State Park and Playas De Tijuana in Baja California, Mexico. Photo: Ocean Imaging, Inc.

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Study Design

- Summer weekends
- Prospective cohort
  - Target population: all beach goers
  - Household sample
  - Three interviews:
    - Enrollment
    - Beach interview
    - Telephone interview 10-12 days later
- Site selection
  - POTW impacted, range of FIB, sufficient population

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Measures of Water Quality

- Criteria for consideration:
  - Quantitative results could be obtained within a few hours;
  - Frequent detection
- RAPTOR Fiber optic Biosensor
  - Portable, fluoresence based immunoassay antibody based detection
- Luminex
  - Flow cytometry based immunoassay
- EPA Method 1600
  - Standard reference for *Enterococcus*
- CLAT
  - 5 hour colipahge method
- qPCR
  - Note: *E.coli* method not available in 2000
- Several other technologies evaluated in EPA labs but not included in studies

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Health Outcomes

- Gastrointestinal illness (GI)
  - Diarrhea (3 or more loose stools in a 24 hour period); Vomiting; Nausea and stomach ache; Nausea or stomach ache and impact on activity
  - Note difference from Dufour, Cabelli
- Upper respiratory illness (URI)
  - Any two: sore throat, cough, runny nose, cold, fever
- Skin rash
- Eye irritations (watery eye or eye infection)
- Earache

NEEAR Water Study: Results

Marine Beaches (Wade, Env. Health 2010)

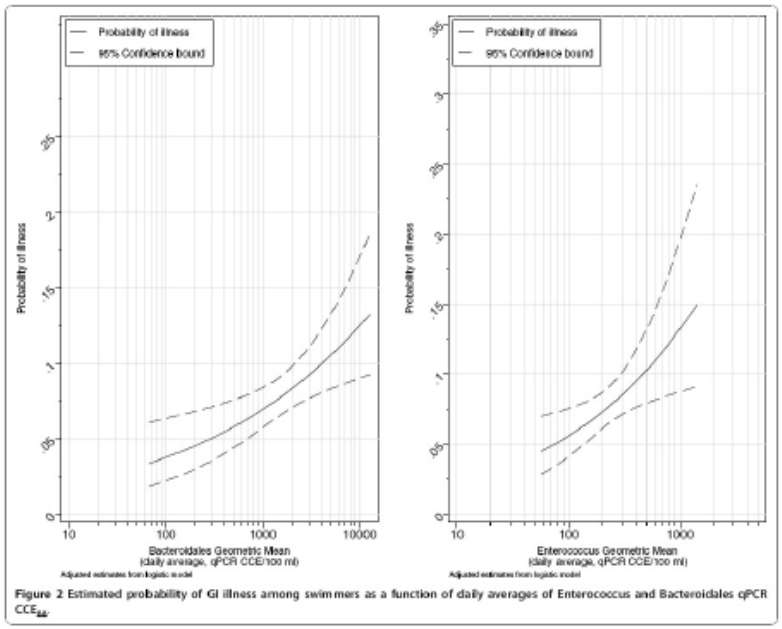
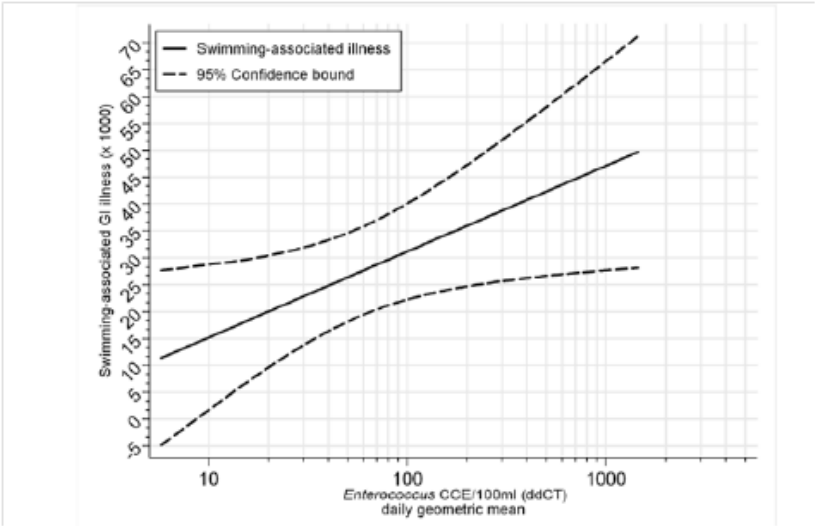


Figure 2 Estimated probability of GI illness among swimmers as a function of daily averages of Enterococcus and Bacteroidales qPCR CCE$_{AA}$

Freshwater Beaches (Wade, Epidemiology 2008)



- Nearly 30,000 enrolled and 3,000 water samples tested
- GI illness associated with fecal indicator bacteria measured by qPCR

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NEEAR Studies: Key Results

- Molecular-measures of fecal indicator bacteria were associated with GI illness among swimmers.
  - Dose dependent associations established
- Associations with GI illness were stronger and more consistent than for culture-based measures of *Enterococcus*
  - *Freshwater AOR: 1.36 (p=0.0006)*
  - *Marine AOR: 2.56 (p=0.007)*
- Morning measured by qPCR also associated with GI illness

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Tropical Beaches and Beaches Impacted by Urban Runoff

- Questions about applicability of indicator/health associations to:
  - "Runoff" beaches
  - Tropical beaches
- Airlie Conference recommendations
- Consent Decree and Settlement Agreement requirements

Consent Decree

5. Pursuant to CWA § 104(v), no later than December 15, 2010, EPA shall complete the following studies:

(a) an epidemiological study in marine waters impacted by urban runoff in temperate region, and

(b) an epidemiological study in a tropical region.

"Marine waters impacted by urban runoff" means marine waters impacted by urban sources of pollution, including possible discharges of storm water or storm water runoff. Marine waters impacted by urban runoff do not include marine waters that are known to EPA, at the time EPA makes the final selection of the site for the study, to be impacted by (1) discharges from publicly owned treatment works or combined sewer overflows or (2) identified discharges of untreated human waste from sanitary sewer systems that would interfere with the ability to accurately determine the relationship between human illness and fecal indicators that originate from urban runoff.

Surfside Beach, SC

- Surfside Beach selected from initial list of 179
- 89% urbanized
- Received non point contamination through swashes
- Sufficient rainfall
- Highly visited
- 45% of samples exceeded state standards
- Water quality was linked to rainfall
  - Dry weather: *Enterococcus* CFU/100ml: 23-27
  - Wet weather *Enterococcus* CFU/100ml: 285-346

Boquerón Beach

- Historical data: range 0->600 *Enterococcus* CFU per 100 ml
- WWTP: 0.25 MGD, often overloaded, serves 13,200 population
- Two smaller privately owned plants, discharge to "'‘mangrove"' swamp

Summary

- "Runoff beach"
  - Health risks were low and water quality was good at a beach impacted primarily by runoff
  - Lack of association may be attributable to good water quality and lack of human fecal input
  - Observed higher risks and better illness-water quality at beaches located near treated sewage discharges
- "Tropical" beach
  - Water quality was good despite proximity to sewage discharge
  - May not have impacted beach site
  - Unable to draw firm conclusions regarding qPCR-measured indicators and illness associations at tropical site
  - Only site where GI illness was not elevated among swimmers, lower background rate than other sites.
  - Questions about pre-existing immunity of population

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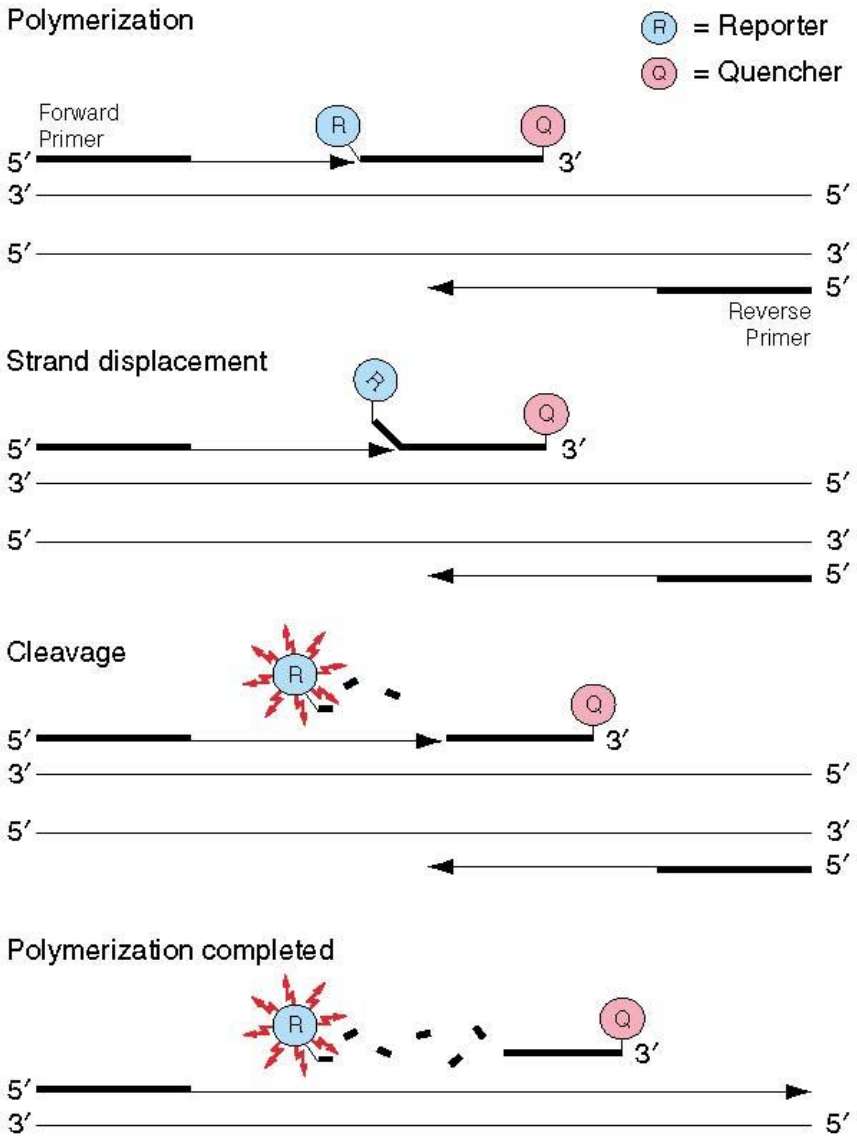
Important Attributes of qPCR

- Uses fluorescent tag on the DNA amplification process to quantify the amount of amplification.
- The quantity of the amplified DNA is measured in real time
- Measures both live and dead cells
- Same day results
- Use enumerated cell suspensions of a representative target species as calibration standards.
  - Calibrator samples are extracted in same manner as test samples.
  - Within each PCR run, results from whole cell calibrator samples adjust for variations in DNA extraction recovery from cells as well as PCR conditions.

How qPCR works



R-----Q is the probe

Forward & reverse primer & probe w/ attached R & Q attach to one or the other of the two separated DNA strands.
Primers are extended by Taq creating complementary strands.
Probe just sits there.

One of the new complementary strands bumps into the probe as it is extended.
Other complementary strand continues forward.

R is released from the probe by the Taq and because it's no longer suppressed by the Q, it fluoresces.

One fluorescent molecule released for each new strand made. Fluorescence is measured each PCR cycle.
Extension of both strands (polymerization) continues until both new strands are complete (lower strand not shown here).

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Other Studies

- Southern California Coastal water research project and UC Berkeley
  - Incorporated qPCR in their epidemiology studies
  - Recently published results support an association (with caveats)- issues with source of contamination
- Ohio State
  - Study in inland lake: observed association with E. coli, not qPCR
- CHEERS study (secondary contact)
  - Successfully applied qPCR
  - Concluded:

    "The use of qPCR monitoring in our setting would generate more timely results without compromising the ability to predict parasite presence or density"

    Dorevitch Journal Environmental Monitoring, 2011

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Challenges

- Use of FIB as a measure of wastewater treatment efficacy (chlorination)
  - Culture-based measures very sensitive to chlorination
  - Reduction in these organisms form the basis of permitting requirements
  - qPCR based measures are not as sensitive to chlorination and persist following treatment
  - Concern how qPCR can be “implemented” for permitting and discharge maximum levels
- Cost
- Training
- Equipment
- Inhibition

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EPA Draft Criteria

- Released 2011—Out for Public Comment

http://water.epa.gov/scitech/swguidance/standards/criteria/health/recreation/upload/recreation_document_draft.pdf

- Policy makers had strong desire to maintain culture as primary recommended method
- qPCR provided as an option, and guideline values provided
- States could allow *both* qPCR and culture
  - Culture for permitting, qPCR for beach notification
  - Not clear of qPCR would be allowed for permitting

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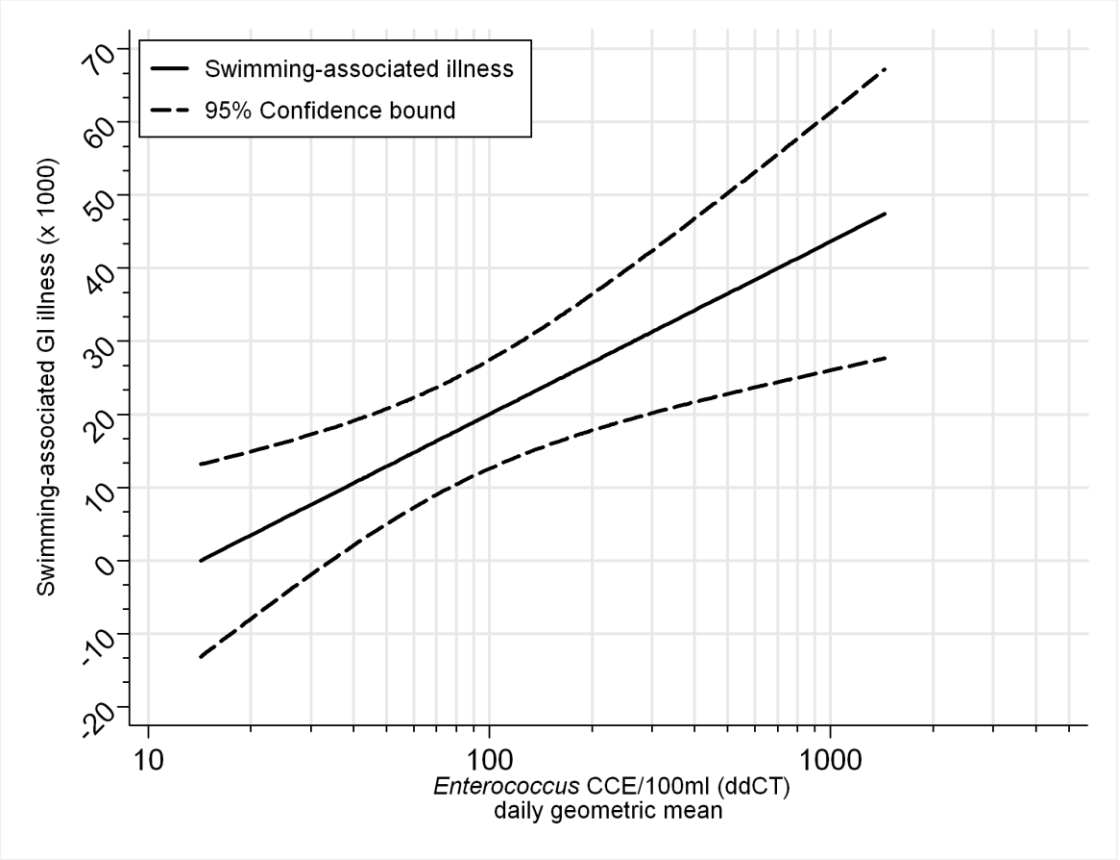
Guideline values for *Enterococcus* qPCR CCE

- Combined marine and freshwater
- Geometric mean of 475 CCE per 100 ml
- Statistical threshold value: 1000 CCE per 100ml

Swimming-Associated GI illness and Daily Average Enterococcus qPCR CCE. All Subjects, marine and freshwater beaches combined (Intercept= -0.02730280, Slope= 0.02364574)



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Current Applications

- California
  - Committed to application of rapid methods
  - May rely on their own epidemiological studies
  - Differ in interpretation of qPCR justification, enumeration.
- Great Lakes
  - Racine, Julie Kinzelman uses qPCR regularly
  - Great Lakes Restoration Initiative: Funded several products to provide training and equipment for molecular methods
- New Jersey

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California Demonstration Project

- SCCWRP: Steve Weisberg
- Goal: Determine feasibility of QPCR as a beach water quality monitoring method
- Participating labs received:
  - All necessary equipment
  - 6 days of hands-on training
  - 4 weeks to practice QPCR
- After training period, beach management decisions made based on QPCR results

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California Demonstration Project

- Successful technology transfer
  - Consistently high amplification efficiencies
  - High repeatability of results
- Same-day health warnings posted by 11 AM
- Concerns:
  - Up to $100K in equipment costs
  - $25/sample for QPCR and $10/sample for culture methods in supplies
  - Increased costs associated with more frequent monitoring

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Future

- *Enterococcus* qPCR not only rapid: better indicator of swimming associated illness
- Further characterization of qPCR assays
  - Multi-lab studies
  - Comparison with pathogen occurrence
- Uncertain how many states will use given budget problems
- Source tracking to determine human sources
  - Critical factor: presence of human contamination
  - Molecular tools will allow characterization of these risks
- Issue with water treatment/disconnect still remains

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Key Questions

- How can qPCR/molecular measures be used for beach monitoring, permitting and compliance when they are insensitive to chlorination?
- Are qPCR measures of FIB better indicators of swimmer-health risk? If so, why?
- How can quantification accurate enough for risk characterization be obtained for inhibited samples, complex matrices?
- qPCR and culture-based measures show variable correlation and measure fundamentally different states of FIB. How can these differences be resolved if both methods are to be used?
- How can molecular measures of source tracking/source apportionment be incorporated into risk management?

- Other questions:
  - How can swimmer risk be characterized in a few samples?
  - How can a meaningful monitoring program be implemented with limited resources and budgets?

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- Coauthors/collaborators:
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Selected Publications

Wymer, L.J., Dufour, A.P., Brenner, K.P., Martinson, J., Stutts, W. & Schaub, S.A. *The EMPACT Beaches Project: Results From a Study on Microbiological Monitoring in Recreational Waters. EPA 600/R-04/023* U. S. Environmental Protection Agency (MCEARD), **2004**(EPA 600/R-04/023)

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