Assessing Enteroccoci in the Nation's Lakes, Reservoirs, Streams and Rivers: Results from the National Aquatic Resource Surveys

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Outline of Talk

- National Aquatic Resource Surveys
- The Quantitative Polymerase Chain Reaction (qPCR) Method
- Results
- Next Steps

National Aquatic Resource Surveys – A Partnership between EPA, States and Tribes


Coastal


Streams and Rivers


Wetlands


Lakes

1. Assess biological and recreational condition and changes over time of the nation's waters using indicators of condition and stress

2. Rank stressors based on the relative associations between indicators of condition and indicators of stress

3. Build/enhance state and tribal monitoring and assessment capacity

Background

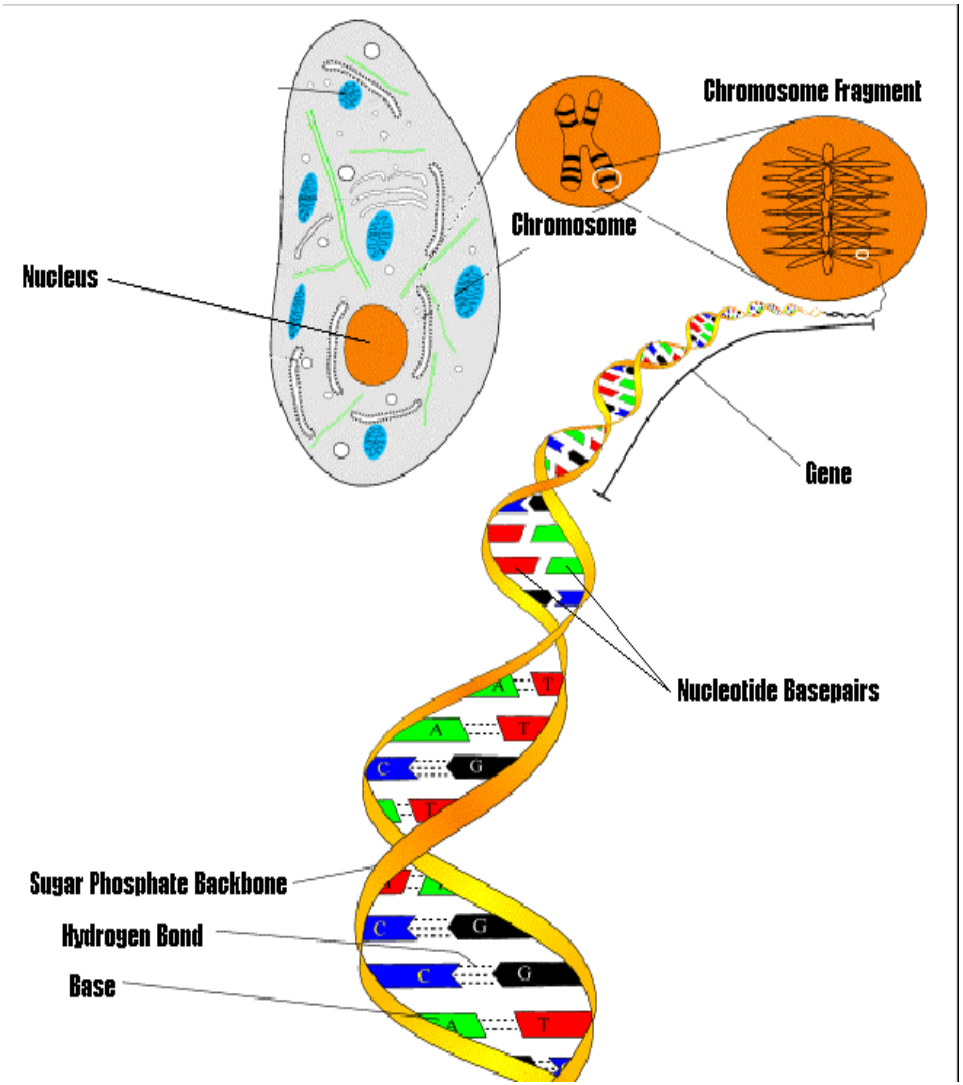
- National Lakes Assessment (NLA): First nationally consistent assessment of the nation's lakes and reservoirs
  - 1,028 unique lakes sampled, representing the condition of about 50,000 lakes nationwide
- National Rivers and Streams Assessment (NRSA): First nationally consistent assessment of the nation's rivers and streams.
  - 1,924 river/stream sites sampled, representing the condition of about 1.2 million miles of flowing water
- Consistent sampling and analysis procedures to ensure comparability of results across the country

Enterococci in the NLA and NRSA

- Included as a public health/recreational indicator
- Use of qPCR eliminated holding time issue inherent in culturable methods
- NLA 2007
  - Sampled in littoral zone at the final physical habitat station
  - Collected 0.5 m below water surface
  - Filtered and frozen within 6 hours of collection
- NRSA 08/09
  - Sampled at the “K” transect
  - Collected approximately 1 m from the bank and 1 foot below surface
  - Filtered and frozen within 6 hours of collection

What is qPCR?

- Real-Time, Quantitative Polymerase Chain Reaction (qPCR) is a gene based (Genomic) method used to identify and quantify anything with a gene sequence, including bacteria, viruses, or anything else that has some form of Nucleic Acid (DNA or RNA).



Slide materials courtesy of Jack Paar, EPA, and the EPA New England Regional Lab

Summary of qPCR Method

- qPCR AKA: *Repetitive Molecular Photocopying*
  - Analytical method that mimics the process of cellular DNA duplication
    - Method Premise - Many copies are easier to identify than fewer
    - Uses natural functions to copy, genetic material *(e.g., DNA or RNA)* to generate millions to billions of copies of target gene sequences

- Calibrator Cell Equivalents (CCE) are used as the endpoint for qPCR.
  - CCE involves determining target sequence quantities in DNA extracts from test samples relative to those in calibrator samples

Slide materials courtesy of Jack Paar, EPA, and the EPA New England Regional Lab

Some Benefits and Limitations of qPCR

- Results from method showed statistically significant correlation with GI illness among swimmers
- Method provides results the same day
- Studies in the Great Lakes and four temperate marine beaches demonstrated good performance
  - Limited information about Enterococcus qPCR method in inland and tropical marine waters

qPCR Inhibitors

- Naturally occurring environmental substances.
- May be co-extracted with sample.
- In some instances may inhibit the polymerase chain reaction.
- EPA's qPCR method includes procedures to
  - ~ measure inhibition and
  - ~ mitigate inhibition.
- Samples indicating unacceptable interference:
  - Only 1.8% of all NLA 2007 and NRSA 2008/09 inhibited.
  - Inhibited samples may still yield usable data

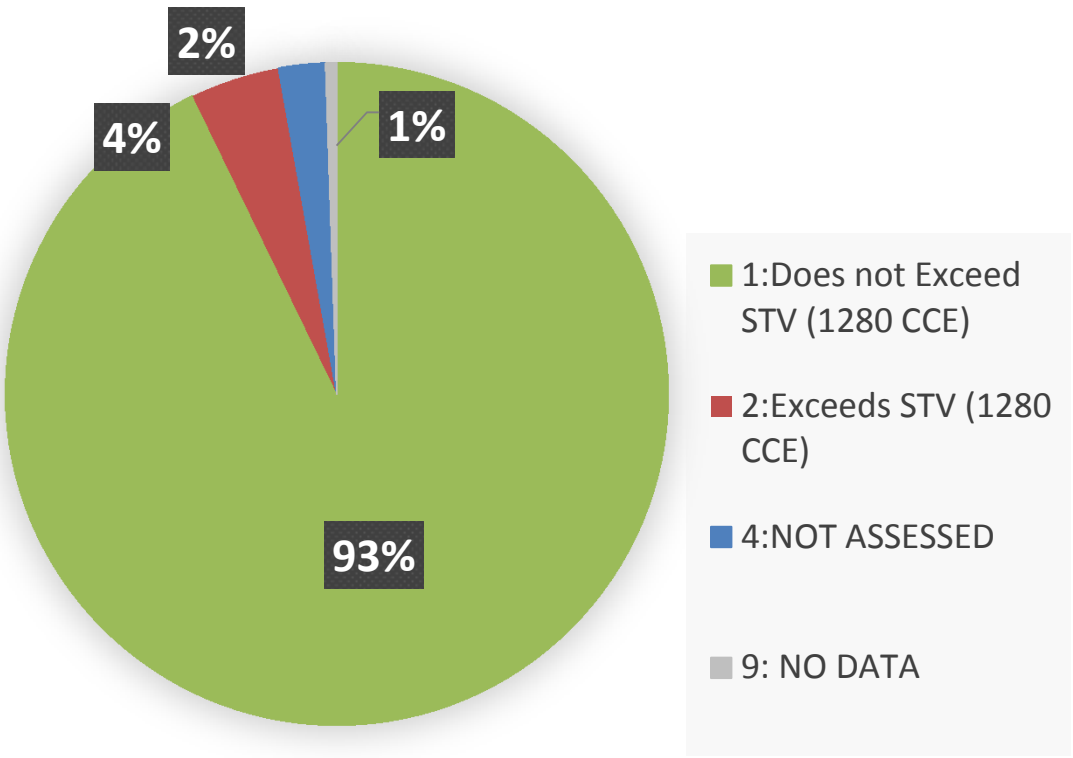
EPA Recreational Water Quality Criteria Document

- Based on two bacterial indicators of fecal contamination
  - E.coli and enterococci
- Includes criteria based on culturable and qPCR methods
  - Geometric Mean (GM)
  - Statistical Threshold Value (STV) – clarification and replacement for Single Sample Maximum
  - Also includes Beach Action Values
- **qPCR Value Used for Assessment**
  - ***STV of 1,280 CCE per 100 mL.***

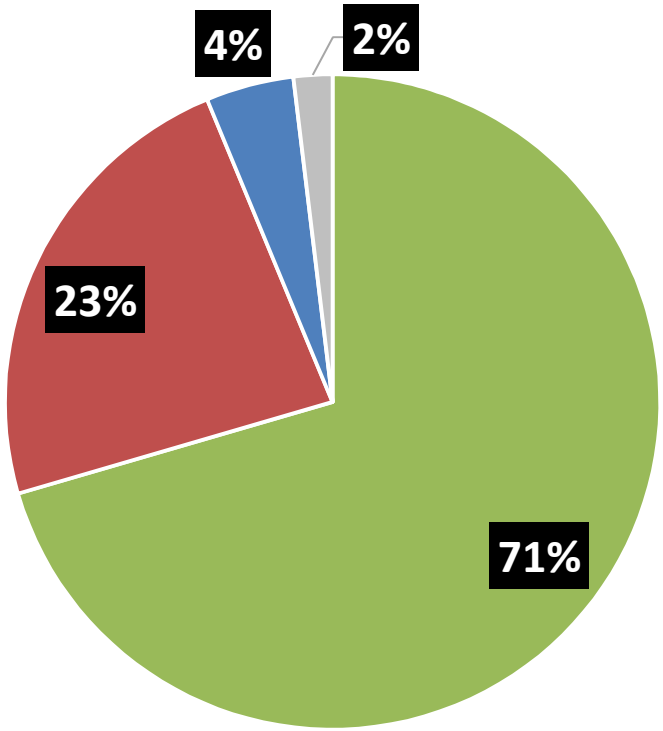
http://water.epa.gov/scitech/swguidance/standards/criteria/health/recreation/index.cfm

National Assessment: Enterococci*

NLA 2007 Enterococci Analyses
% of lakes



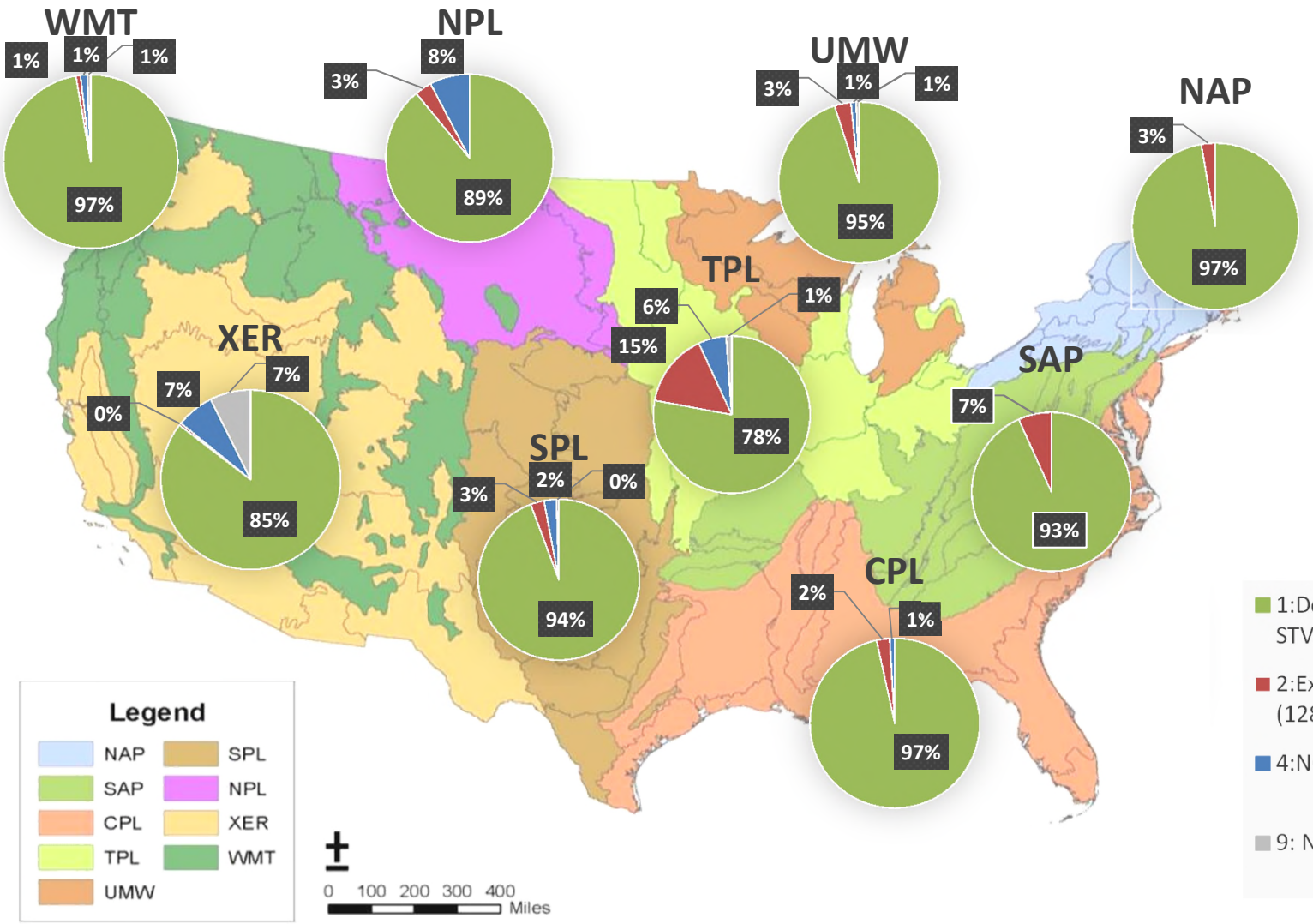
Draft NRSA 2008/09 Enterococci Analyses
% of rivers/streams



*Threshold of 1280 CCE/100 ml

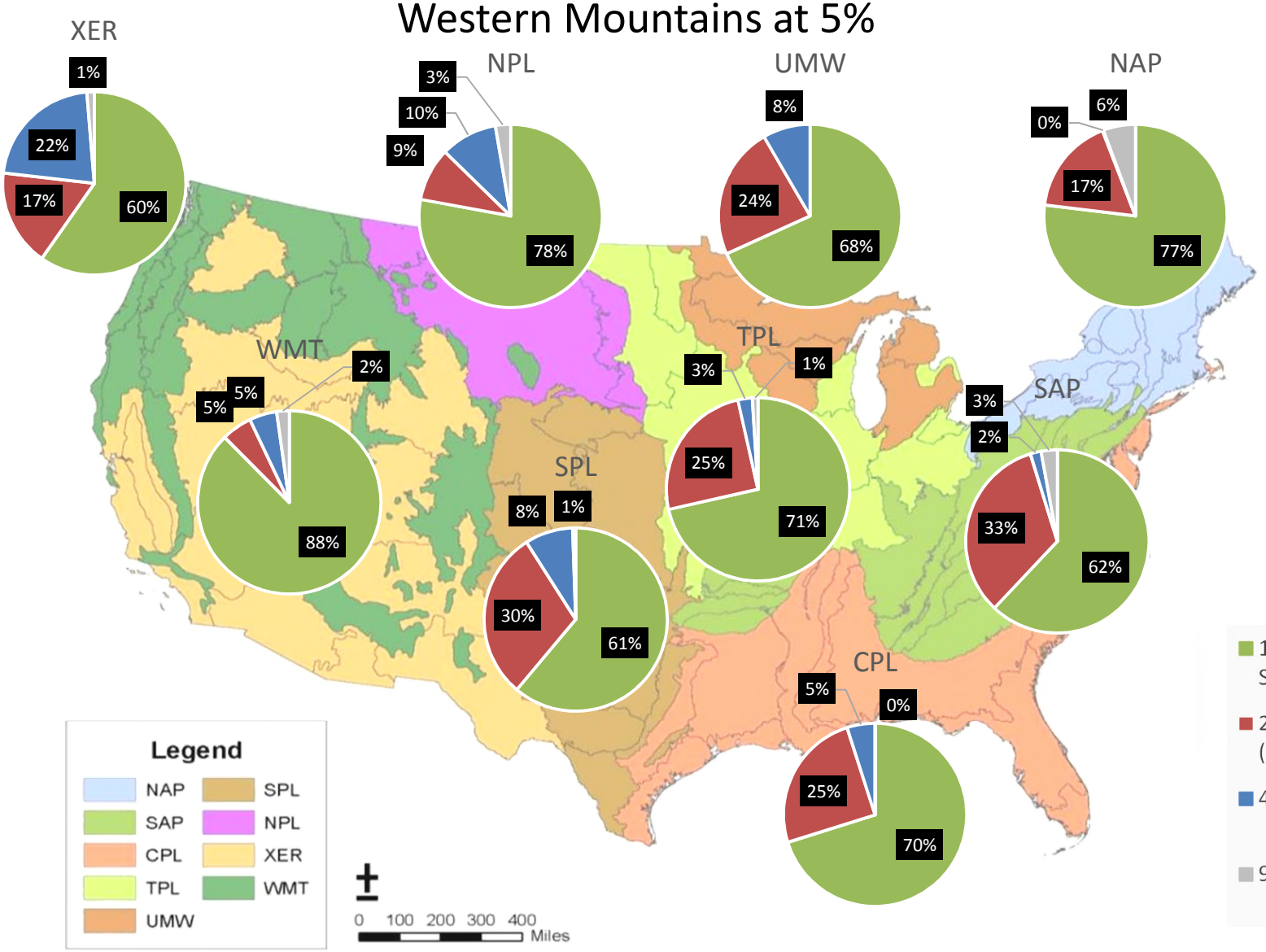
Regional Results - Assessment of the Nation's Lakes: Enterococci

Ecoregions range from <1% to 3% except for Temperate Plains at 15% and Southern Appalachians at 7%



Regional Results – NRSA - Enterococci

Ecoregions range from 17% to 33% except for Northern Plains at 9% and Western Mountains at 5%



Next Steps

- Finalize NRSA report for publication
- Potential for other publications by EPA and other scientists
- Other questions to be considered:
  - Are there differences in enterococci levels considering
    - Land use
    - Waterbody type/size
  - Others
- qPCR Analysis included in NRSA 13/14 and upcoming NCCA 2015

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