The National Lakes Assessment: A National Assessment of Enteroccoci Levels in Lakes Across The United States

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Outline of Talk

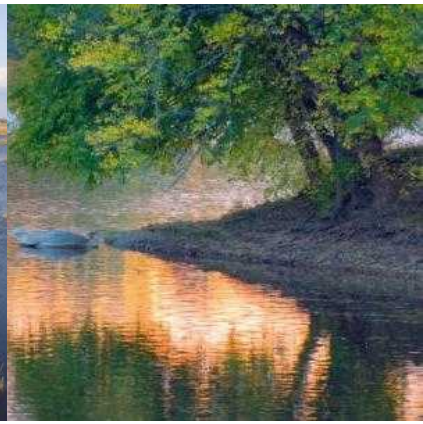
- National Aquatic Resource Surveys
- National Lakes Assessment – Background
- The Quantitative Polymerase Chain Reaction (qPCR) Method
- EPA Draft Recreational Water Quality Criteria Document
- Draft Results
- Next Steps

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

NLA Background

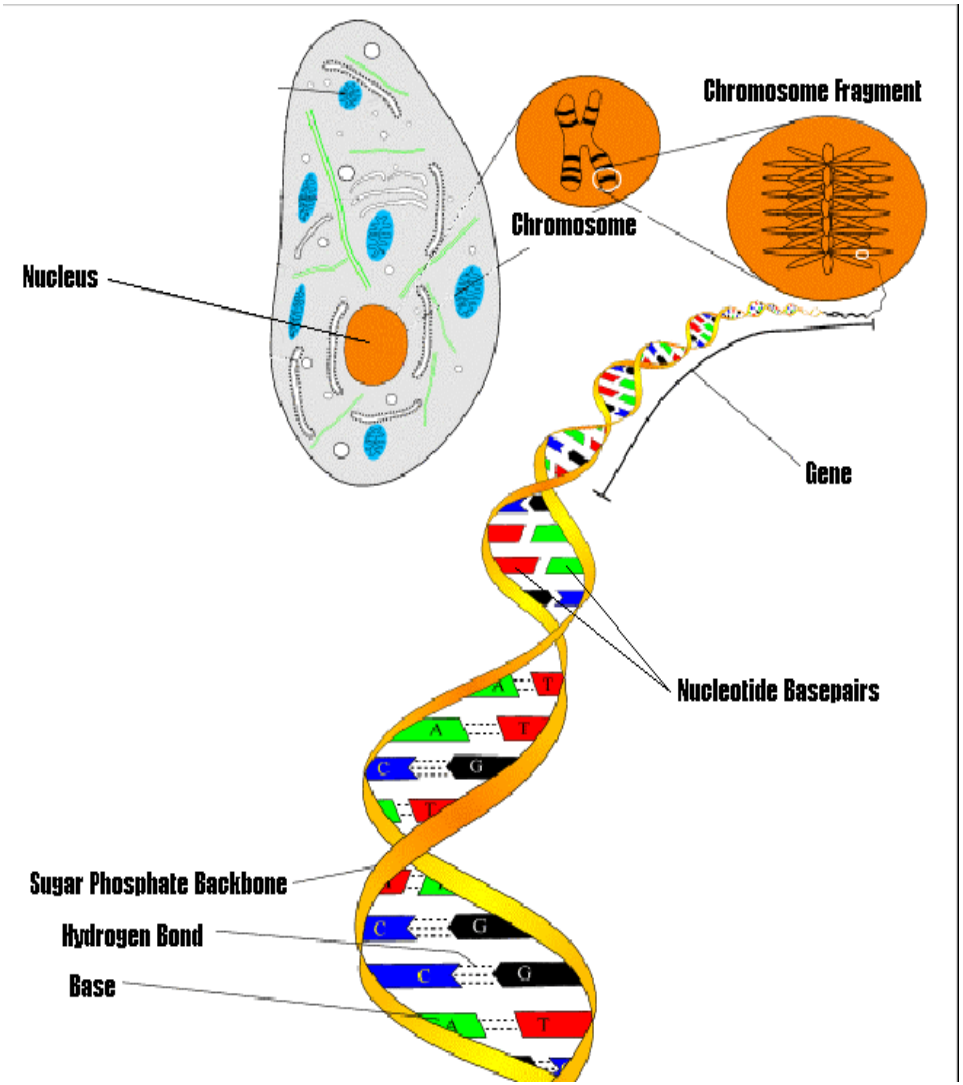
- First-ever nationally consistent assessment of the nation's lakes and reservoirs
  - 1,028 unique lakes sampled, representing the condition of about 50,000 lakes nationwide
  - Consistent sampling and analysis procedures to ensure comparability of results across the country
  - Over ½ million data points analyzed
- Groundbreaking science – first-ever national assessment of
  - Biological condition
  - Trophic status
  - Microcystin
  - Habitat condition
  - And now, the pathogen assessment based on the draft recreational water quality criteria for pathogens as predicted in 2007!

Enterococci and NLA 2007

- Included as a public health/recreational indicator
- Eliminated holding time issue inherent in culturable methods
- EPA did not yet have, but was planning to issue, criteria for enterococci using qPCR method
  - WQ Criteria not ready in time for release of NLA 2007 Report will be published in October 2012.

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

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qPCR Inhibitors

- Naturally occurring environmental substances
  - May be co-extracted with the sample
  - May inhibit the polymerase chain reaction
- EPA's method includes procedures to
  - Measure inhibition
  - Mitigate inhibition (inhibited samples may still yield useable data)
- Only 1.8% of all NLA 2007 samples inhibited (n=26)

Some Benefits and Limitations of qPCR

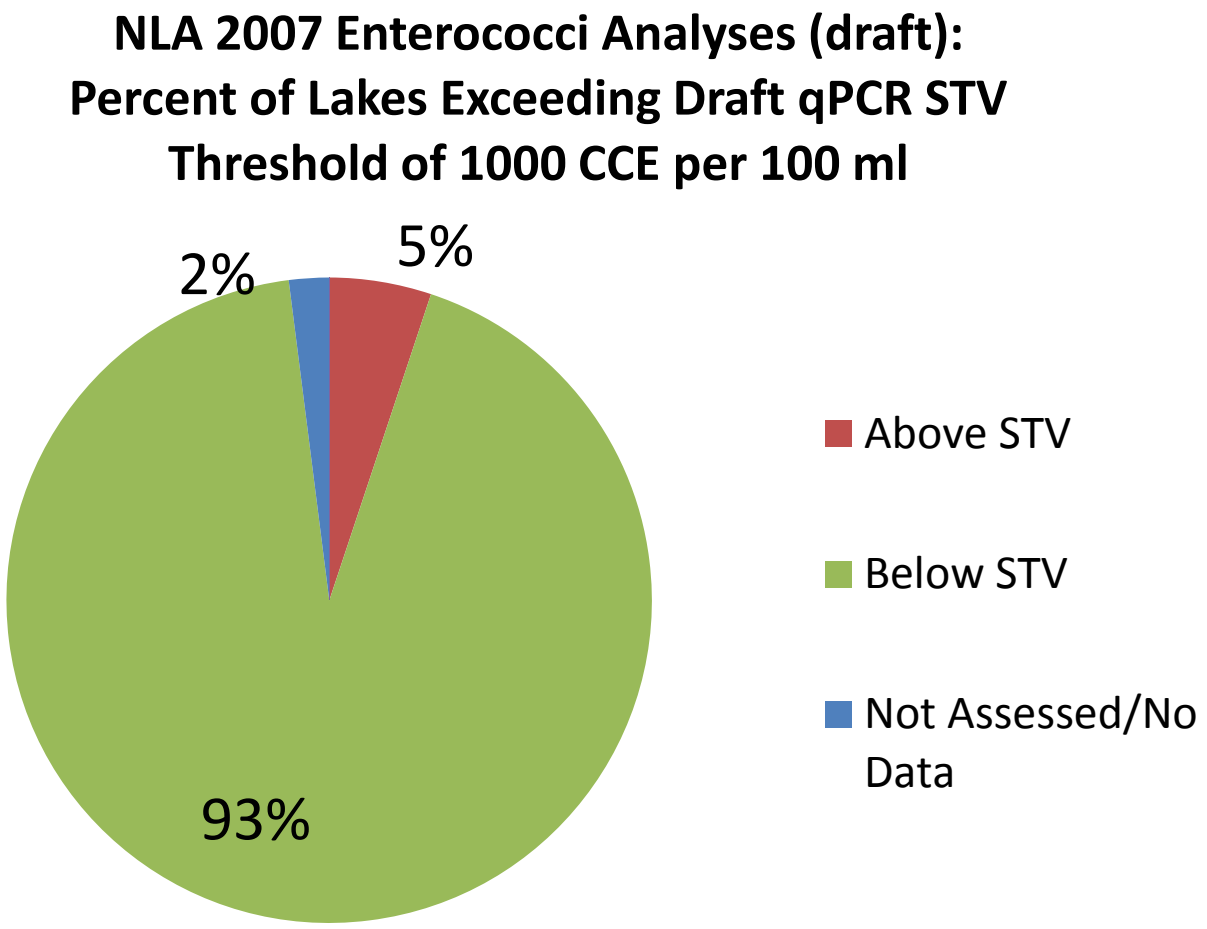
- Results from method showed statistically significant correlation with GI illness among swimmers
- Method provides results the same day
- Studies in the Great Lakes and four temperate marine beaches demonstrated good performance
  - Limited information about Enterococcus qPCR method in inland and tropical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

EPA Draft Recreational Water Quality Criteria Document

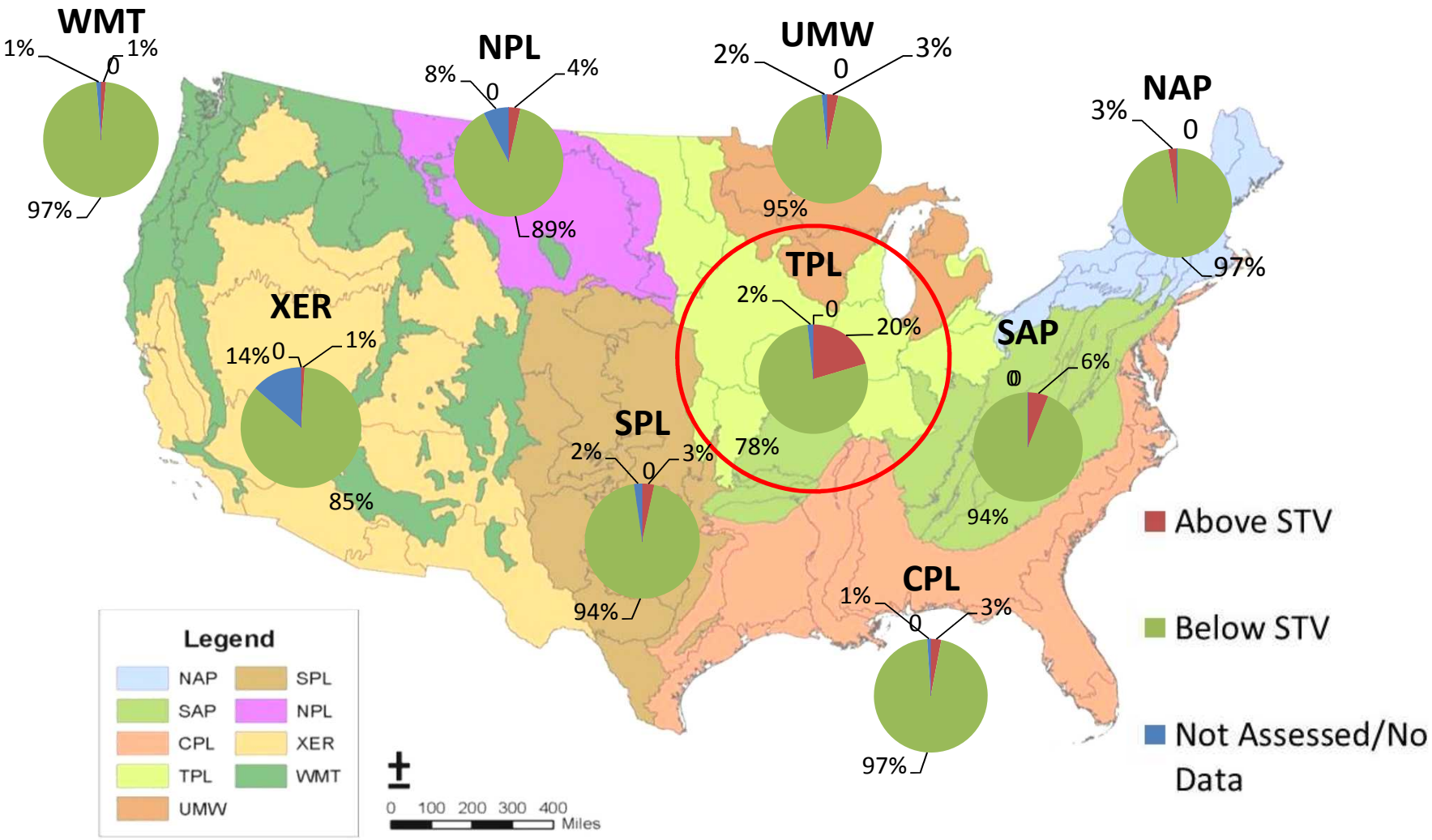
- Based on two bacterial indicators of fecal contamination
  - E.coli and enterococci
- Includes criteria based on culturable and qPCR methods
  - Geometric Mean (GM)
  - Statistical Threshold Value (STV) – clarification and replacement for Single Sample Maximum
- Final Criteria Expected Fall 2012
- qPCR Thresholds
  - Geometric Mean of 475 CCE per 100 mL
  - ***STV of 1,000 CCE per 100 mL.***

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

5% of the Nation's Lakes are above the Draft qPCR-based Enterococci STV Thresholds: 2007



Ecoregional Results: The percent of lakes above the draft qPCR-based enterococci threshold range from <1% to 6% except for Temperate Plains at 20%



Next Steps

- Development of supplemental report on the enterococci indicator
- Potential for other publications by EPA and other scientists
- Other questions to be considered:
  - Are there differences in enterococci levels considering
    - Land use
    - Lake size, type, etc.
  - Analysis of reference, or least disturbed sites, in comparison to other lakes

NLA 2012

- Will not include a bacteria indicator
  - qPCR : Concerns
    - Field time
    - Threshold
  - Culturable methods
    - Holding time issue is a barrier for probability sampling with national labs

Acknowledgements and Thanks!

- NLA field crews
- EPA Staff (among many others)
  - Amina Pollard
  - Jack Paar
  - Dave Peck
  - Kevin Oshima
  - Rich Haugland
  - Robin Oshiro
  - Shari Barash

- For more information on the NLA:
  - http://water.epa.gov/type/lakes/lakessurvey_index.cfm