Implementation of ddPCR for Beach Monitoring

Presentation to the SCCWRP Commission

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What is droplet digital PCR (ddPCR)

- A method to target and quantify a specific stretch of a gene

- In this case, the target is specific to *Enterococcus* spp.
  - Same target as used in the EPA Method 1609 (qPCR)

Statement of Problem

- 2008: qPCR had solved the time lag issue associated with growth-based methods
  - SCCWRP showed water quality results could be made publicly available by noon
- 2012: EPA published a qPCR method for Enterococcus
  - Never adopted in California
- 2014: Next generation of quantitative PCR technology, digital droplet PCR (ddPCR) mature and ready for implementation
  - Lacked EPA and State approval

EPA and California Opened Door to Use of ddPCR

- EPA Technical Service Memo
  - Provides pathway to approval of alternate measurement methods
    - Method must be strongly correlated with approved method in side-by-side use

- California Senate Bill 1395
  - Allows Health Departments to replace growth-based methods required by AB411 with EPA-approved rapid method for Enterococcus for beach monitoring
  - Requires ELAP Certification of Laboratory

San Diego County Gained Approval to Use ddPCR for Enterococcus in 2022

- Impetus for study that led to approval was frequent sewage discharges from cross-border sources
  - Heath Department desired a rapid method to facilitate faster public notification of poor water quality
  - Intent was to replace growth-based methods with ddPCR for routine monitoring

- City of Los Angeles has initiated a study to gain approval for use of ddPCR at Santa Monica Bay beaches
  - Intent is to use method for characterizing the extent and dissipation of sanitary sewer spills

Study Comparison

San Diego

- 3-year study
- Initiated by Health Dept.
- Replace growth-based methods for routine monitoring
- Post and remove health advisories
- All monitoring stations (n = 51)
- Approval based on statistical parity with EPA-approved methods

Los Angeles

- 1-year study
- Initiated by POTW
- Intended for spill response - growth-based methods retained for routine monitoring
- Close and reopen beaches
- Subset of stations only (n = 15)
- Approval based on statistical parity with EPA-approved methods

Advantages of ddPCR

- Faster than growth-based methods
  - 4 vs. 24 hours to result
- Robust to inhibition
- More straight-forward than EPA-approved qPCR method
  - Absolute quantification
    - No standard curve
    - No reference material
- Sensitive
  - Lower limit of quantification can be ~20 gene copies

droplet digital PCR (ddPCR)

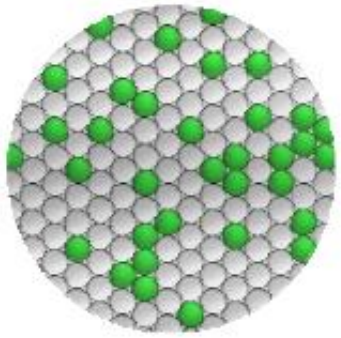
“MPN” PCR

100ml water

96 wells

20 out of 96 wells are positive
→ statistics (MPN table)
→ x *Enterococci*

25ul PCR
(tube/well)



20,000 droplets

30 out of 20,000 droplets are positive
→ statistics
→ x copies of target

Droplet digital PCR

Slide courtesy of Yiping Cao

San Diego County Study

- Compared ddPCR to two EPA-approved methods
  - Enterolert
  - qPCR

- Entire San Diego County coastline
  - 51 sample locations from diverse beaches (all routine sample locations)
  - Both wet weather and dry weather sampling
  - Over 3,000 samples collected

- Collaborative effort between SCCWRP, San Diego County Health Department and California Department of Public Health

ddPCR Met Requirements for Approval for Site Specific Use

- EPA Region 9 approved ddPCR for use at San Diego County Beaches
  - Strongly correlated with both qPCR and Enterolert
- California approved replacing 3 growth-based indicators with a single ddPCR measurement for Enterococcus at San Diego Beaches
  - ELAP Certified the County Health Department Laboratory for ddPCR
- ddPCR testing commenced in May of 2022

Unintended Consequences

- Initial public reaction generally positive
- Increased sensitivity of ddPCR led to uptick in postings and closures
  - Raised ire of businesses and politicians
- Health Department did not back down
  - Could have done a better job with outreach and frontloading stakeholders before launching the new method
  - Unusual ocean conditions exacerbated the issue

BEACH WATER MANAGEMENT TIERED SYSTEM

NEW!

1. ADVISORY

2. WARNING

3. CLOSURE

SDbeachinfo.com

Goals of the Los Angeles Beach Study

- Produce data needed to gain site-specific EPA approval for ddPCR for Enterococcus
  - Conduct analysis to determine threshold (in gene copies) equivalent to EPA-approved methods
- LASAN EMD laboratory to become proficient in ddPCR
  - SCCWRP to provide training
  - Conduct laboratory intercalibration exercise
  - SCCWRP to serve as Help Desk
- Publish study in peer-reviewed journal
  - Required for approval by EPA REGION 9

Advisory Committee

Mas Dojiri - City of Los Angeles

Lusi Mkhitaryan - County of Los Angeles, Department of Public Health

Eric Trevena - California Department of Public Health

Eric Dubinsky - US Environmental Protection Agency, Region 9

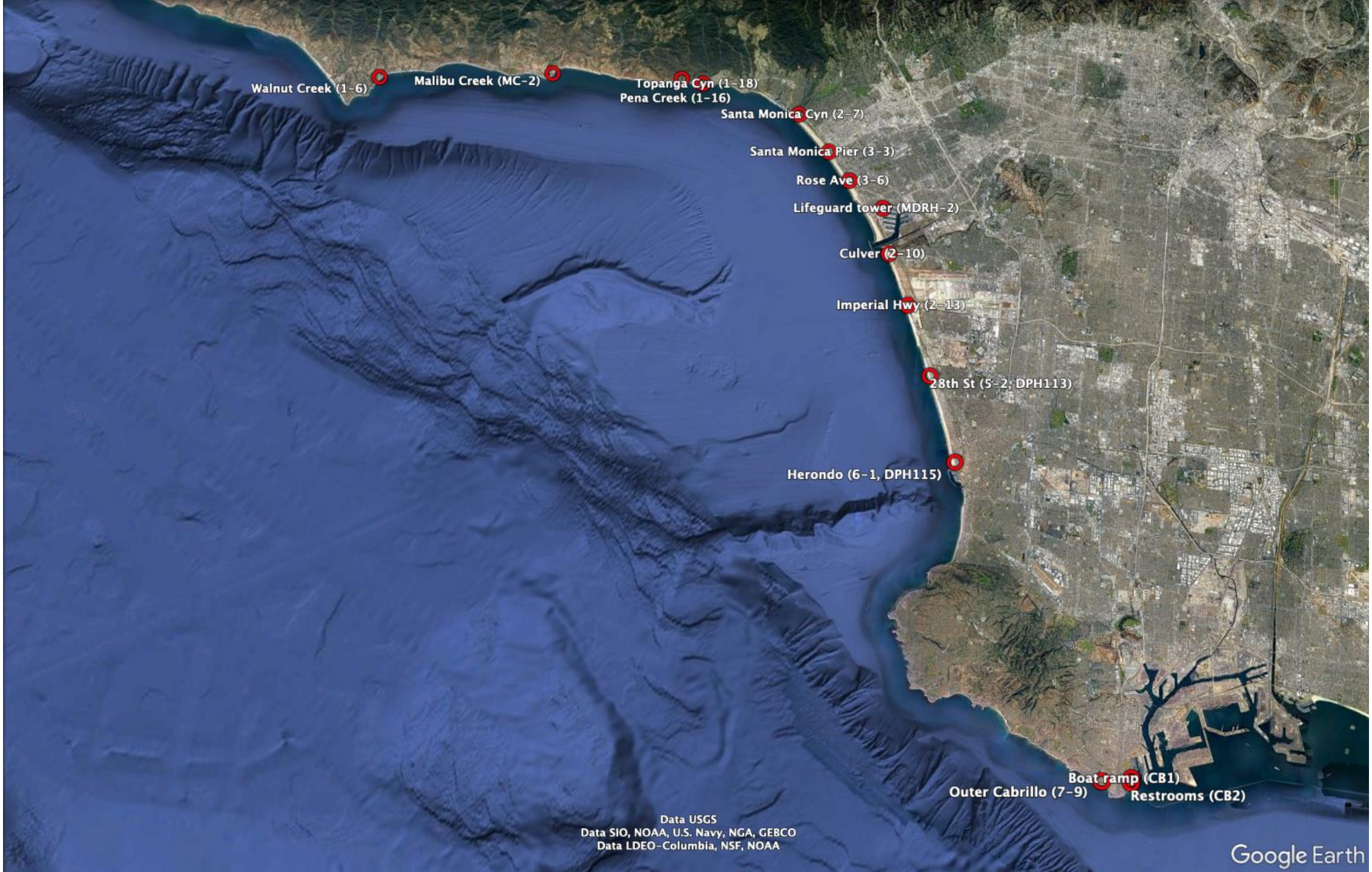
Alison Wu - Heal the Bay

Shelley Luce - UCE Consulting

Approach

- 15 sites
  - Minimum 30 samples each in dry and wet weather
  - Adequate range of quantifiable measurements

- Enterococcus measured by 3 methods
  - IDEXX (EMD Lab)
  - EPA qPCR and ddPCR (SCCWRP)


Walnut Creek (1-6)
Malibu Creek (MC-2)
Topanga Cyn (1-18)
Pena Creek (1-16)
Santa Monica Cyn (2-7)
Santa Monica Pier (3-3)
Rose Ave (3-6)
Lifeguard tower (MDRH-2)
Culver (2-10)
Imperial Hwy (2-13)
28th St (5-2, DPH113)
Herondo (6-1, DPH115)
Boat ramp (CB1)
Outer Cabrillo (7-9)
Restrooms (CB2)
Data USGS
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
Data LDEO-Columbia, NSF, NOAA
Google Earth

Requirements for Site-specific Alternative Method Approval

- California Requires
  - EPA approved method or equivalent alternative method published by EPA

- EPA Requires
  - Comparison with approved method at sites with sufficient number of samples
  - Sufficient range of samples to cover variation
  - Strong correlation with approved method
    - IA ≥ 0.7
    - $R^2 \geq 0.6$

Progress to Date

- LASAN EMD began dry weather sample collection in August
  - Filters archived for batch analysis