

# Applying Rapid Indicator Methods and Advances in Source Tracking in California Coastal Waters

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Southern California Coastal Water Research Project

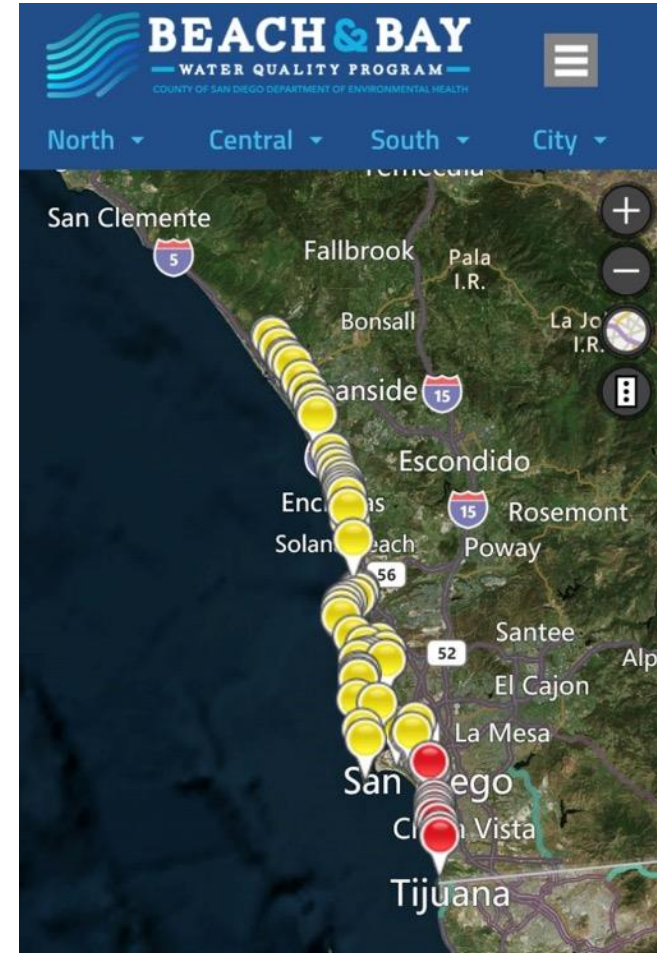
Houston Galveston Area Council

November 20, 2024



# BEACH WATER QUALITY MONITORING PROGRAM OBJECTIVES

- Measure beach water samples
- Communicate water quality



# WARNING

OCEAN WATER CONTACT MAY  
CAUSE ILLNESS



# ¡AVISO!

EL CONTACTO CON AGUA DEL OCEANO  
PUEDE CAUSAR ENFERMEDADES  
LOS NIVELES DE BACTERIAS EXCEDEN  
LOS ESTANDARES DE SALUD



ORANGE COUNTY ENVIRONMENTAL HEALTH DIVISION  
FOR FURTHER INFORMATION, CALL (714) 667-3752  
[www.ocbeachinfo.com](http://www.ocbeachinfo.com).

# WARNING

OCEAN WATER QUALITY WAS  
BAD YESTERDAY AND YOU  
SHOULD NOT HAVE SWUM



WATER QUALITY MIGHT BE OK  
TODAY

PLEASE CHECK OUR WEBSITE  
TOMORROW



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# Road to a Rapid Test

2012

2023



2012 Evaluation for qPCR

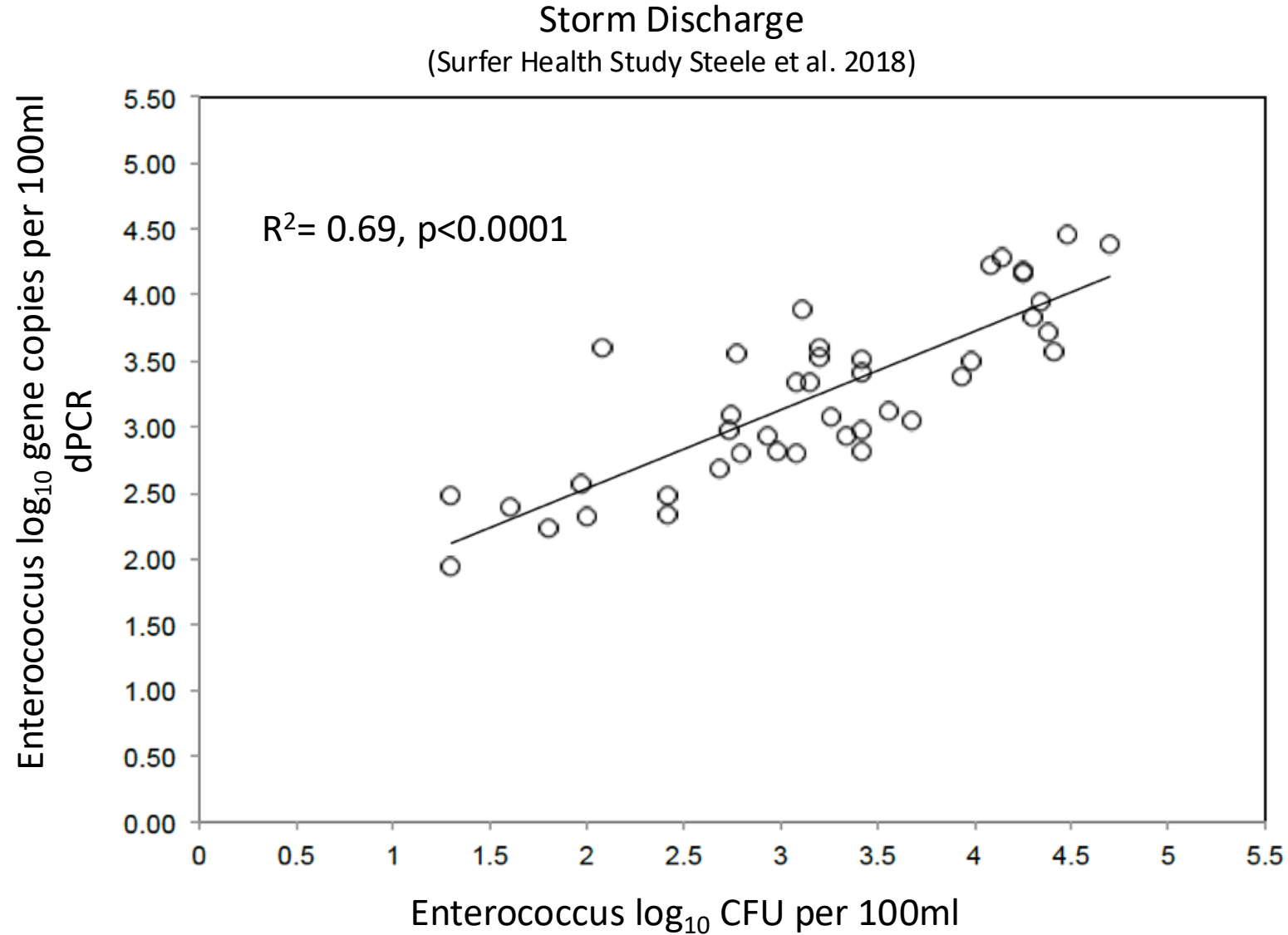
2013 small scale study

2014 SB 1395

# San Diego County Study Goals

- Identify a rapid indicator method with same day results
- Rely on single water quality indicator
- Flexibility to use rapid indicator method throughout coastline
- Implementation of rapid indicator method

# Digital PCR vs culturable Enterococcus



# 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
    - $IA \geq 0.7$
    - $R^2 \geq 0.6$

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ddPCR

2017-2019 ddPCR vs Enterolert  
Comparative Sample Study



# San Diego County Study

- Included the 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
- Nearly two-year study with sampling at least weekly

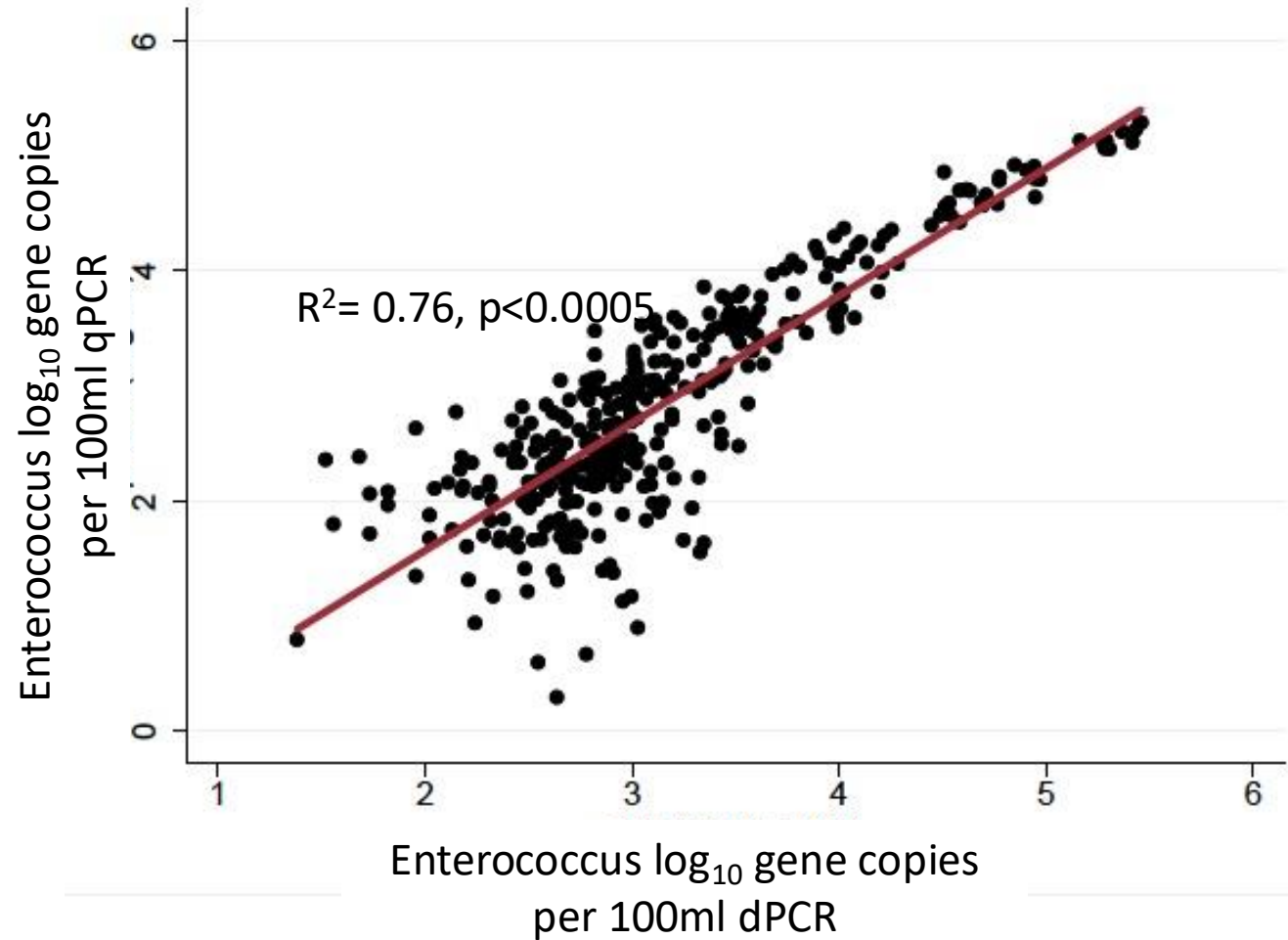


# San Diego County Study

- Samples analyzed for *Enterococcus* spp. enumeration
- Statistical comparison of
  - qPCR and ddPCR
  - Enterolert and ddPCR
- Size of study required collaborative effort
  - County of San Diego, Environmental Health – Study design, sample collection
  - County of San Diego, Public Health Laboratory – Culture analysis, PCR extraction
  - CA Dept. of Public Health – ddPCR analysis and qPCR analysis

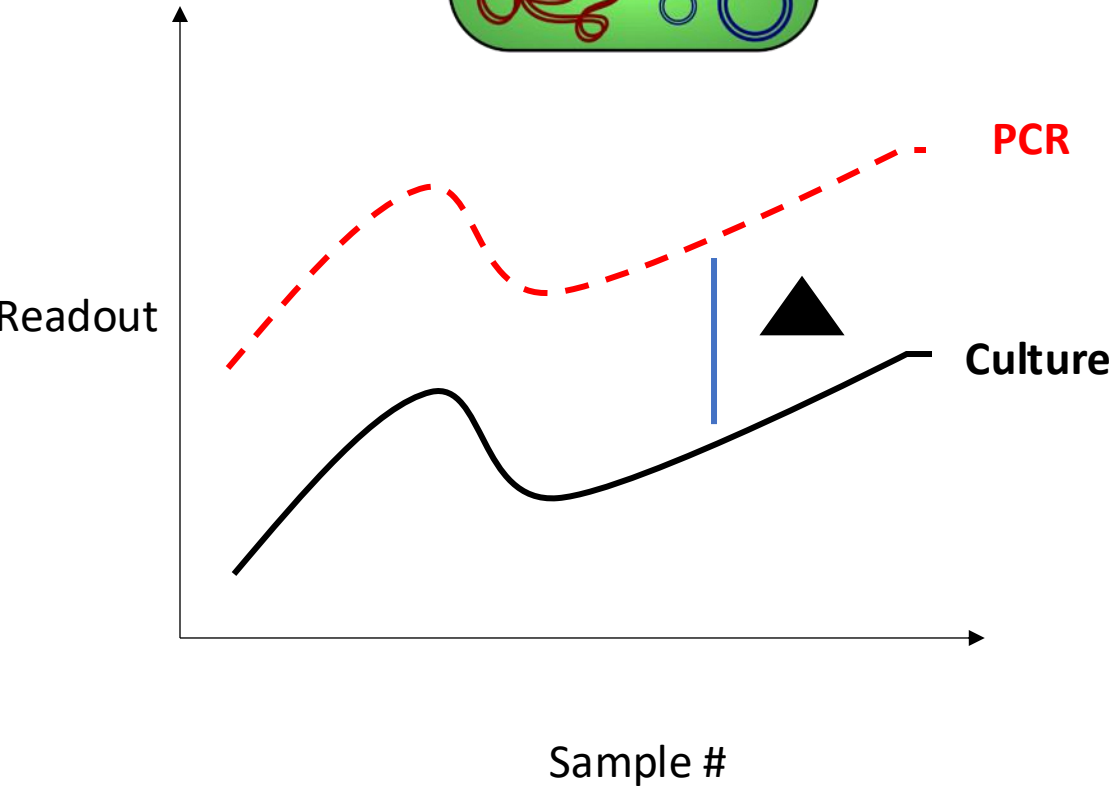
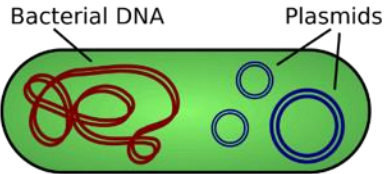
# QPCR vs Digital PCR

Beach Samples Wet & Dry  
(Crain et al 2021)

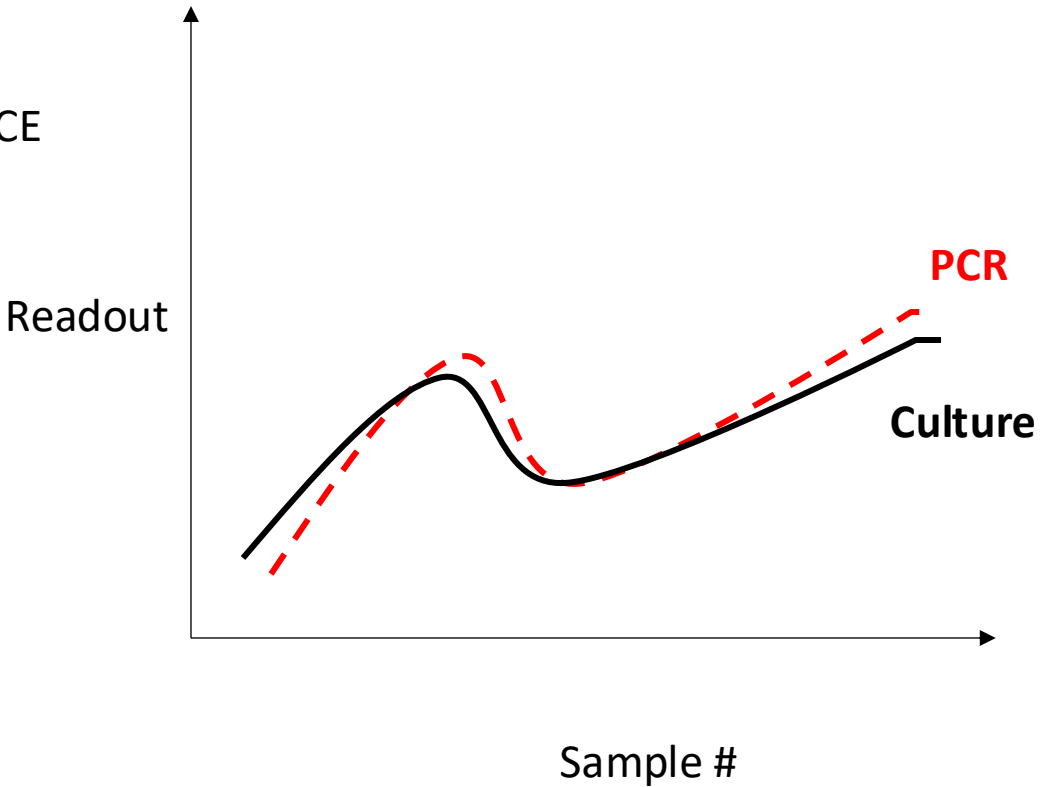
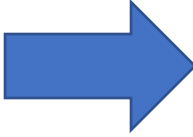


# Derivation of the intrinsic conversion equation (ICE) and data scaling

Culture and PCR methods measure same bacterial organism, but different target analytes. Analysis requires scaling to compare samples fairly



Scaling with ICE

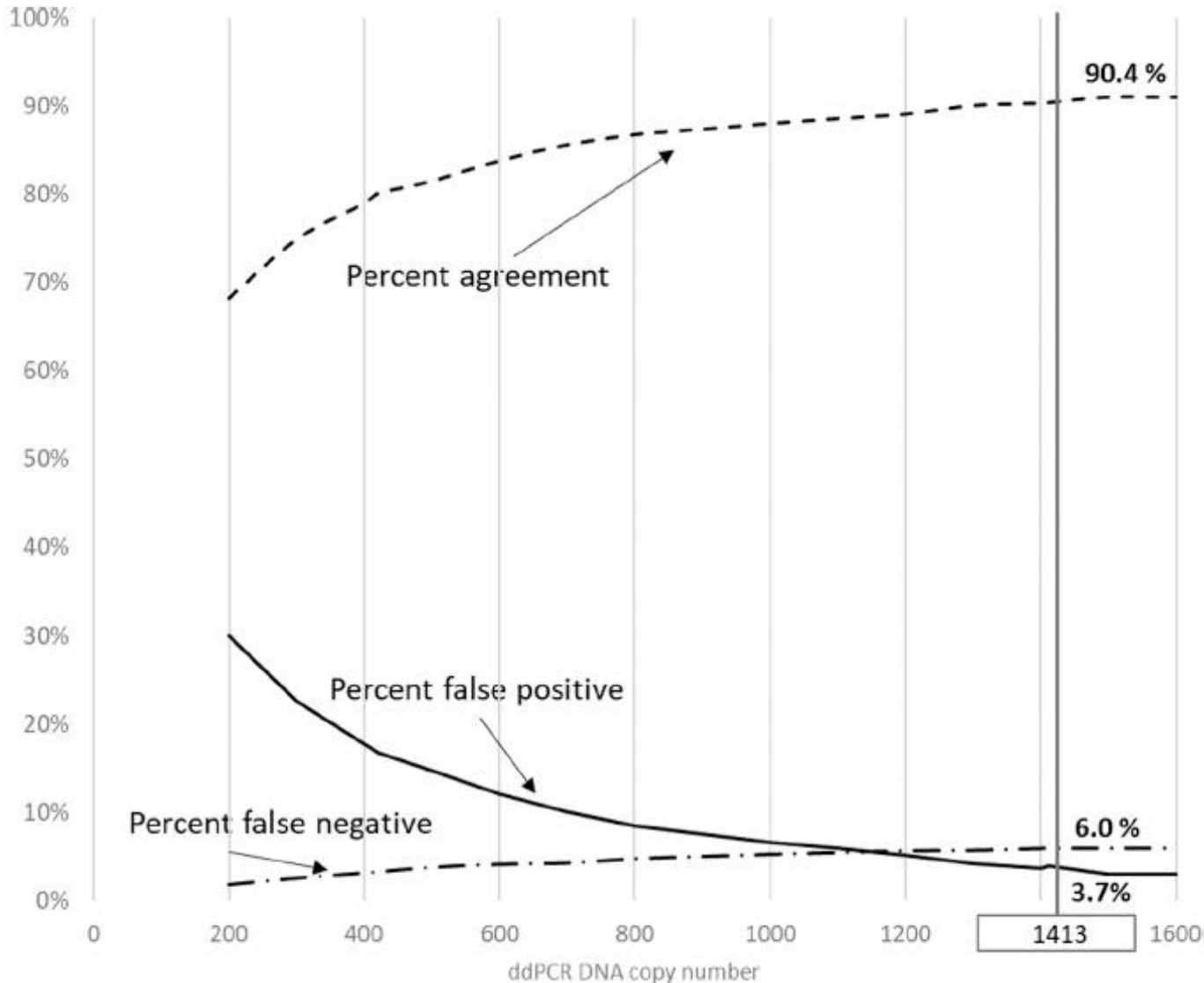


# Index of Agreement (IA) values and Pearson's correlation coefficient ( $r$ ) between Enterolert and ddPCR for all sites combined

Site Category (#)	Number of Samples	IA SCALED Log transformed (mean)	IA SCALED (mean)	IA SCALED Log transformed (median)	IA SCALED (median)	Pearson's $r$ SCALED log transformed	Pearson's $r$ SCALED
<b>Combined</b>	1086	0.64	<b>0.72</b>	<b>0.72</b>	<b>0.74</b>	0.44	0.60

\* Values in bold represent groupings that exhibited minimum threshold for IA (0.7)

# Choosing a county-wide threshold



- Looked for a threshold which would provide most similar outcomes
- Found multiple statistical cut-points and compared agreement with *Enterococcus* standard
- 
- 1413 copies per 100ml had:
  - the highest percent agreement
  - the lowest false positive
  - the lowest false negative

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Comparative Sample Study

2020 Provisional Acceptance





ELSEVIER

Contents lists available at ScienceDirect

## Journal of Microbiological Methods

journal homepage: [www.elsevier.com/locate/jmicmeth](http://www.elsevier.com/locate/jmicmeth)



### Application of ddPCR for detection of *Enterococcus* spp. in coastal water quality monitoring

Chad Crain<sup>a,\*</sup>, Keith Kezer<sup>b</sup>, Syreeta Steele<sup>c</sup>, Judith Owiti<sup>a</sup>, Sphoorthy Rao<sup>a</sup>, Maria Victorio<sup>c</sup>, Brett Austin<sup>c</sup>, Alon Volner<sup>a</sup>, William Draper<sup>a</sup>, John Griffith<sup>d</sup>, Joshua Steele<sup>d</sup>, Marva Seifert<sup>e</sup>

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2020 Provisional Acceptance

2021 Journal Publication

2021 Approval for use in SD County

2022 Use in San Diego Beaches



# Implementation

The San Diego Union-Tribune

PADRES AZTECS THINGS TO DO CROSSWORD SIGN UP FOR NEWS ALERTS

ENVIRONMENT

## Swim at your own risk: How safe are Coronado and Imperial Beach waters?

The San Diego Union-Tribune

PADRES AZTECS THINGS TO DO CROSSWORD SIGN UP FOR NEWS ALERTS

ENVIRONMENT

## Coronado beaches could see new 'warning' signs for Tijuana sewage over Fourth of July weekend

The San Diego Union-Tribune

PADRES AZTECS THINGS TO DO CROSSWORD SIGN UP FOR NEWS ALERTS

ENVIRONMENT

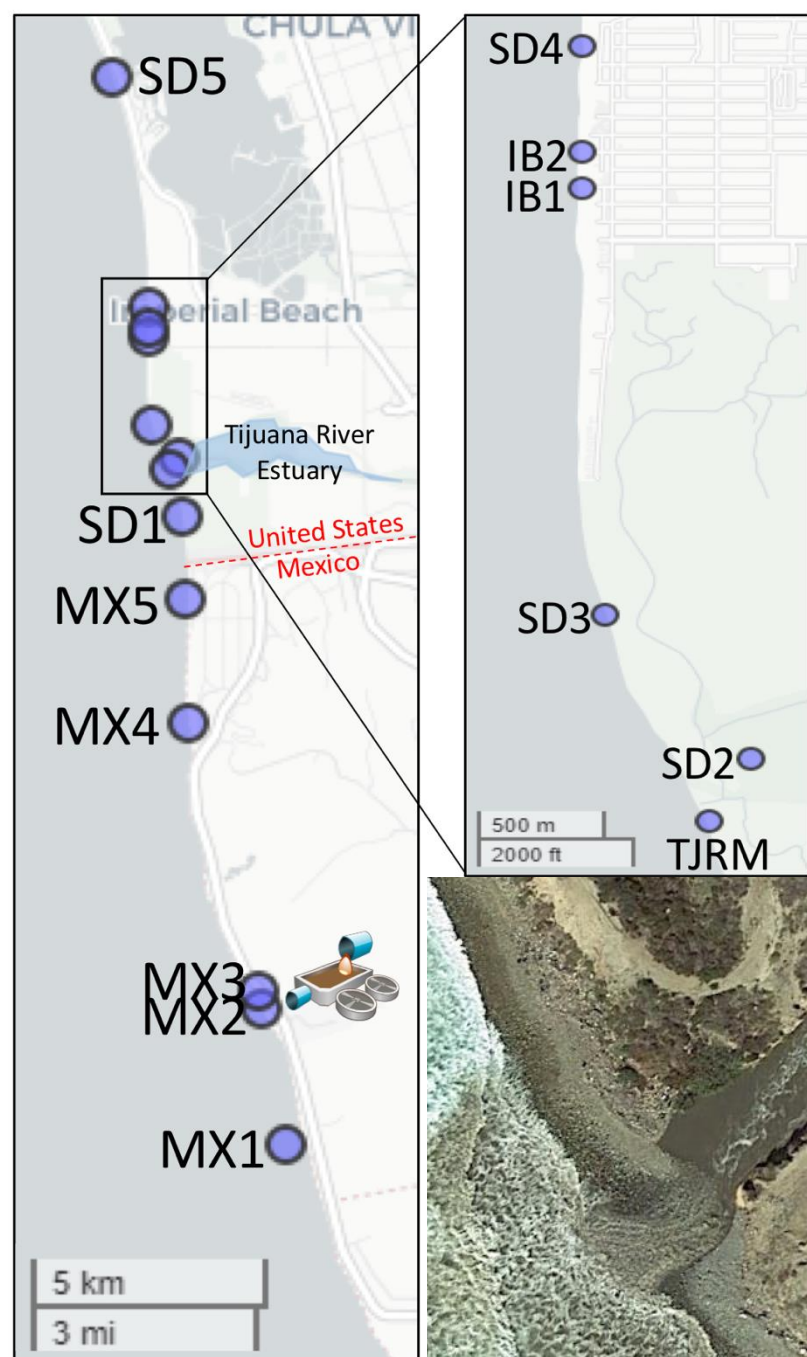
## Will Coronado, Imperial Beach shorelines be closed all summer? New testing reveals rampant Tijuana sewage



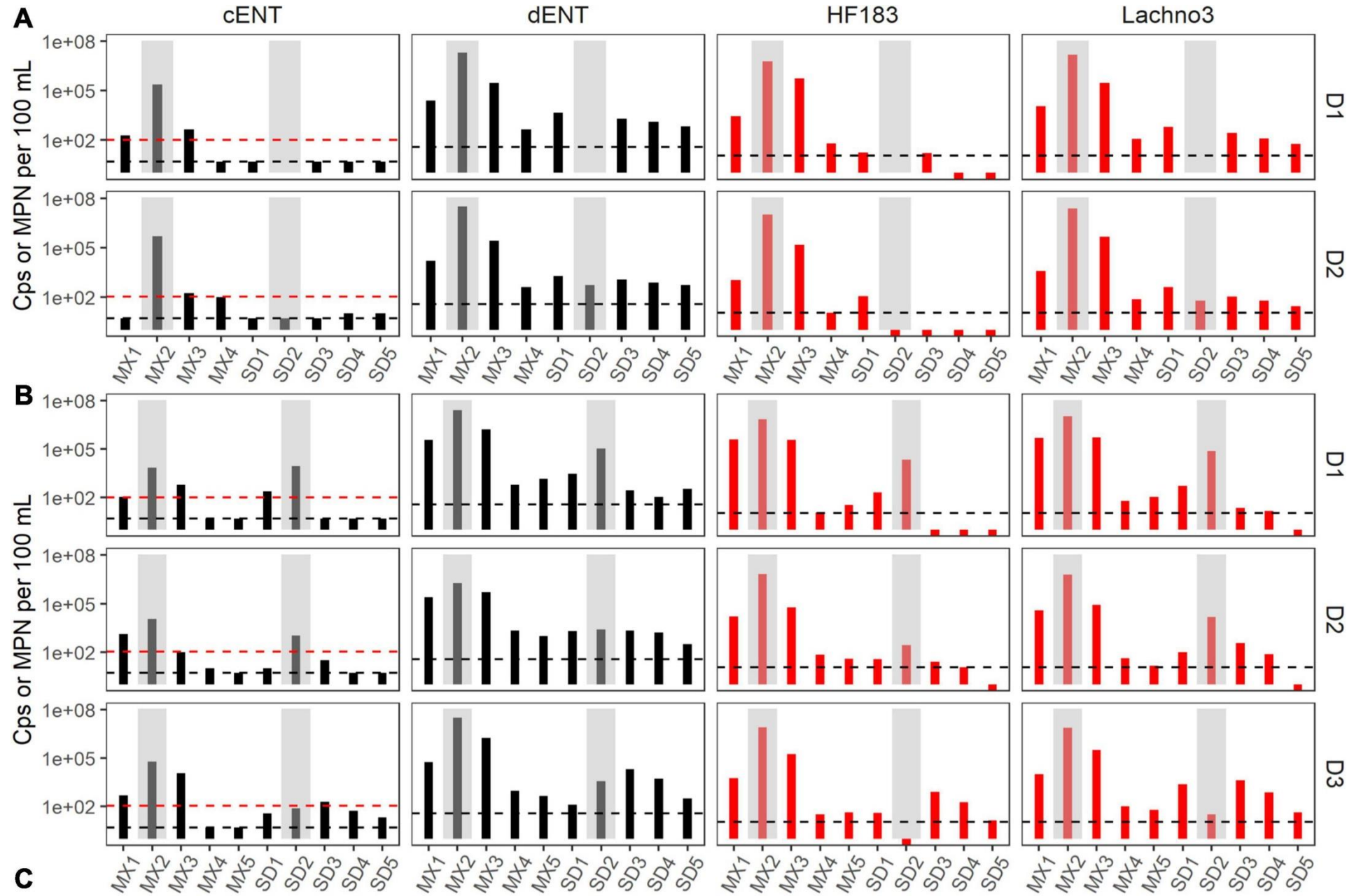
Photo: Nelvir C. Cepeda, San Diego Union - Tribune

# Study Approach

- Sampled coastline between Tijuana WTP outfall and Silver Beach, CA
- Targeted consecutive days during south swell conditions during dry weather
- Sampled 4 events over two summers
- Enterococcus measured by culture and digital PCR
- Human markers measured by digital PCR
- 16S rRNA community analysis

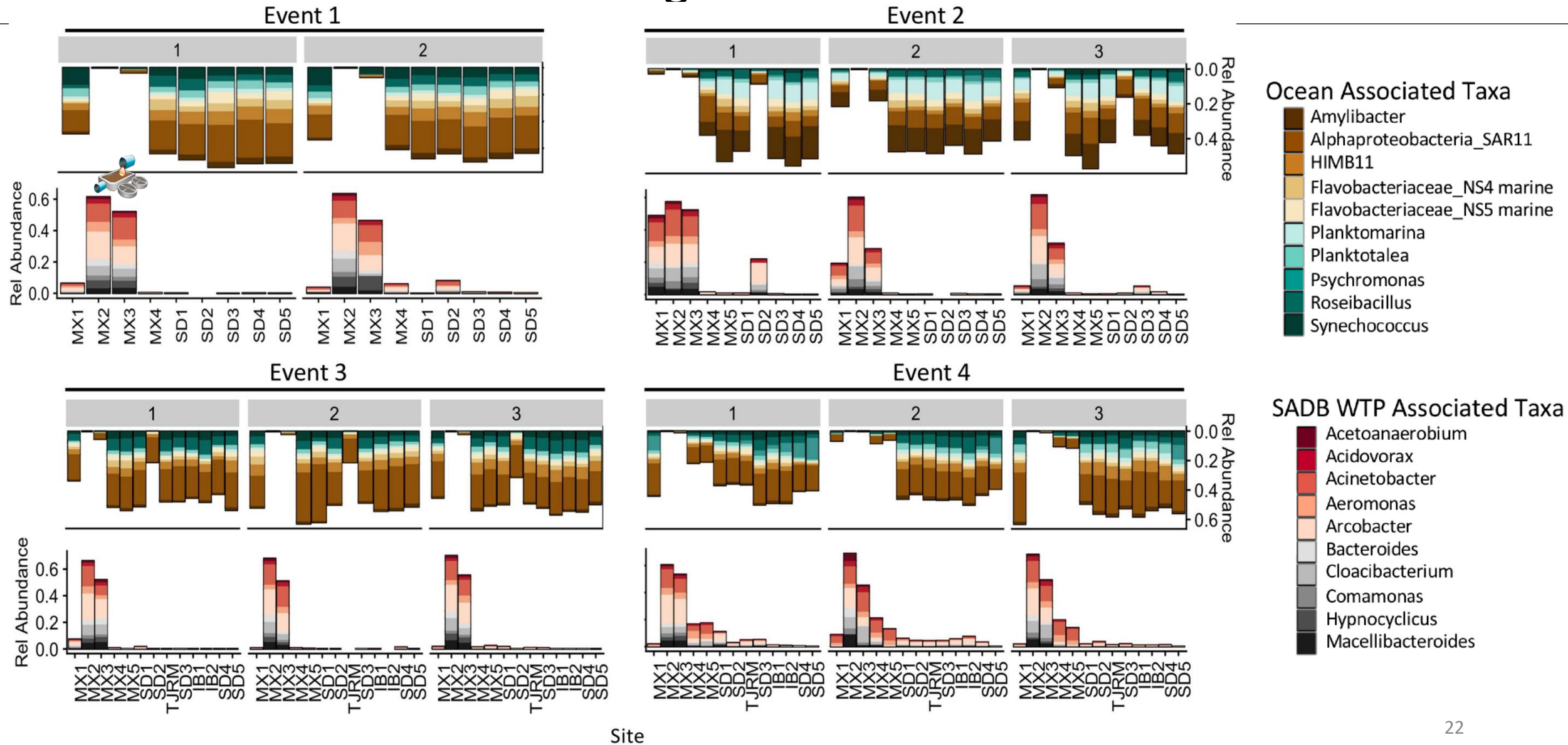


# South Swell FIB & Human Marker Comparison



Zimmer-Faust et al. 2021 *Frontiers in Microbiology*

# Microbial community sequencing results during a strong south swell



# Implications and Next Steps

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- Controversy over beach closures with the application of the new, rapid methods
- Heavy rains and infrastructure failures have made water quality worse
  - Southern San Diego Beaches have been closed for more than 1,000 days
- These studies have led to increased international collaboration by US-Mexico
- Increased calls for study linking swimmer illness risk to molecular methods
- Interest in automation to improve time to result
- Need for more tools distinguishing between human sources



Questions?

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Photo: Joshua Emerson Smith, San Diego Union - Tribune