Progress in Microbiology

Presentation to the SCCWRP Commission
September 11, 2015

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SCCWRP IS A WORLD LEADER IN MOLECULAR TECHNOLOGY FOR BEACH WATER QUALITY

- **Method Development**
  - Identifying and adapting the best new technology for use by member agencies
- **Method Evaluation**
  - Able to bring leading method developers together
  - Serve as an “honest broker”
- **Implementation and Technology Transfer**
  - Train member agencies and others
  - Aim is to get the best methods into the hands of member agencies

THREE MAIN AREAS OF RESEARCH

- **Rapid Methods**
- **Microbial Source Identification**
- **Epidemiology Studies and Risk Modeling**

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RAPID MICROBIAL MEASUREMENT METHODS

- **Culture methods are too slow for same day warnings**

- **SCCWRP has been instrumental in development and adoption of qPCR as a rapid method**
  - Served as EPA's West Coast partner
  - Trained 14 labs (including all of our POTW member agencies)
  - Demonstrated ability to provide information to beachgoers within 4 hours

- **Working collaboratively to evaluate qPCR across different beach types as part of the Bight program**
  - Conducted lab intercalibration
  - Serve as Help Desk for group

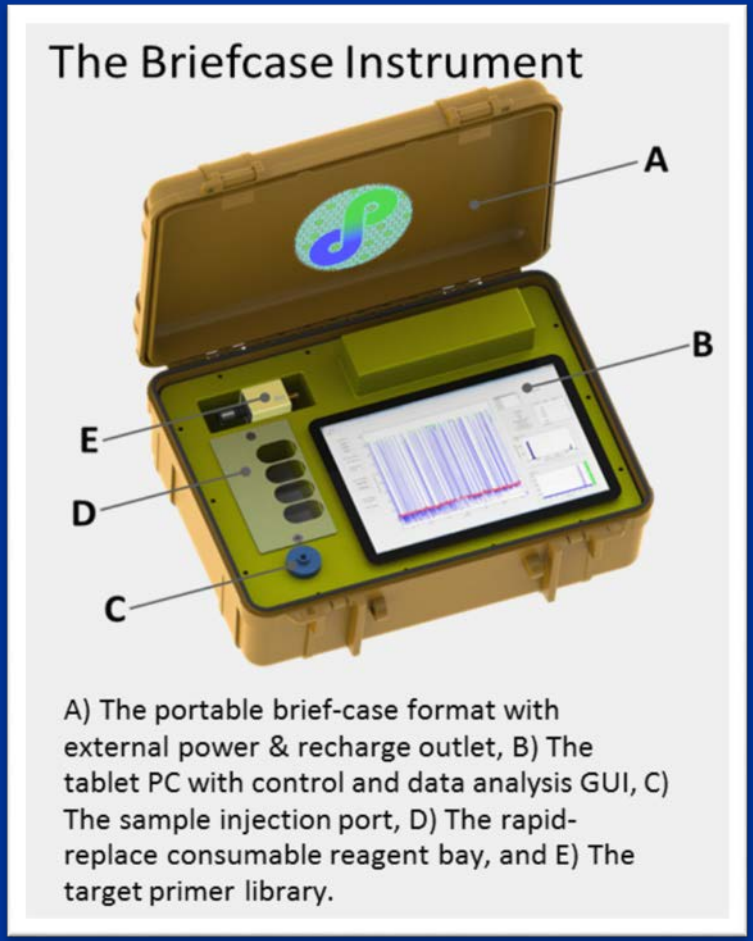
WHY IS FIELD PORTABLE SO IMPORTANT?

- **Beach water quality**
  - Need an answer by noon
  - Not realistic to send individual samplers to every beach

- **Microbial source tracking**
  - Sources of contamination are often ephemeral
  - Need to be able to track contamination back to source

WE HAVE A PROTOTYPE INSTRUMENT

- **Field Portable**
- **Same basic technology as old method**
- **Could be mounted in a land or automated underwater vehicle or operated by a lifeguard**



DIGITAL PCR

Old Method

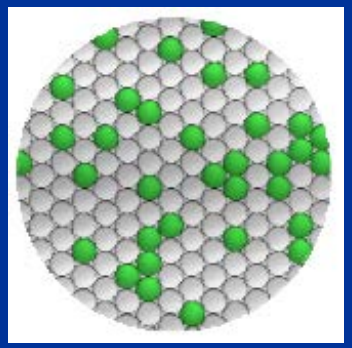
20µl PCR reaction

Unknowns plus standards

Compare to Standard Curve

Digital PCR

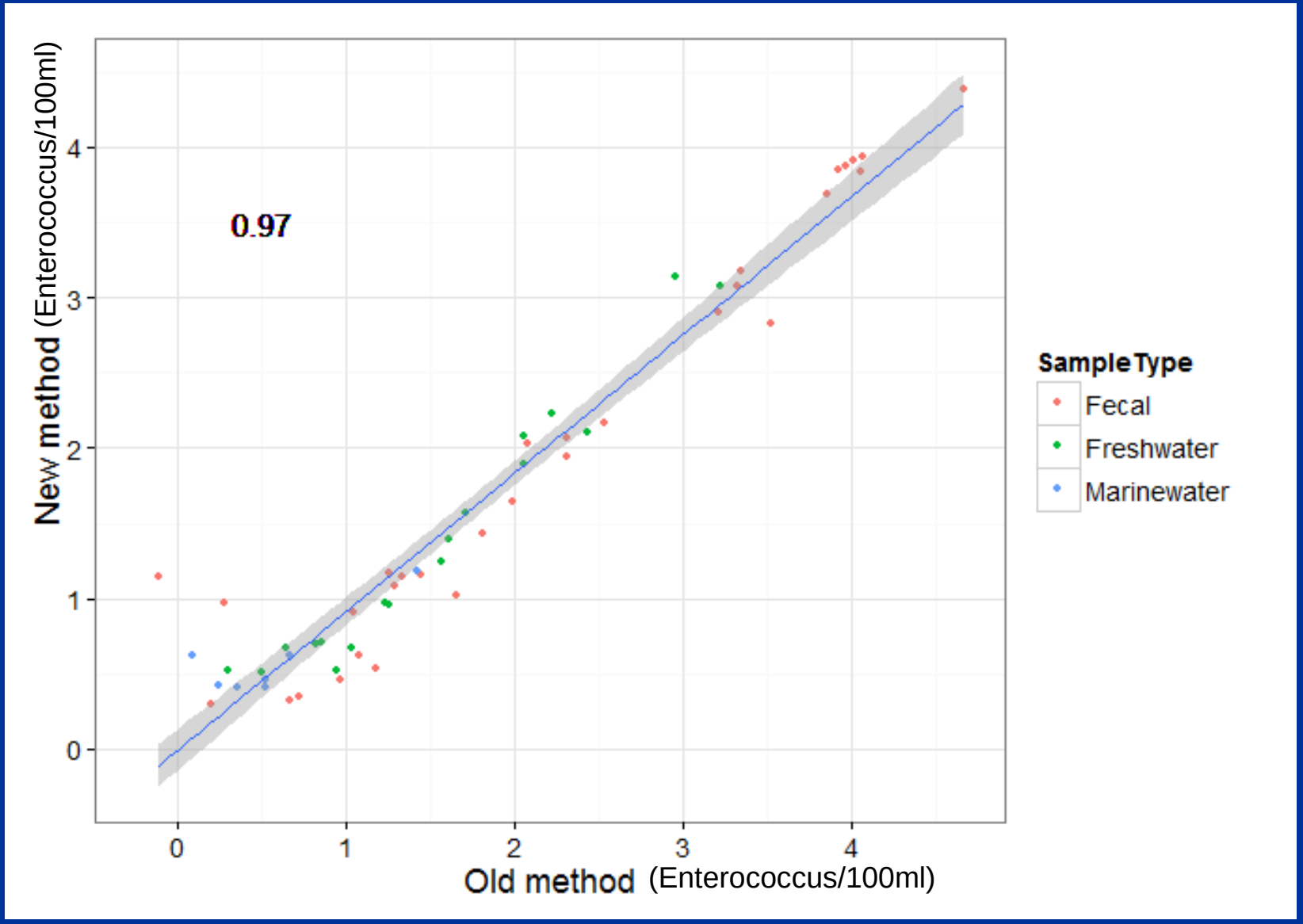
20µl PCR reaction



10000 - 20000 droplets or chambers

Direct quantification using statistics

HIGHLY CORRELATED WITH CURRENT METHOD



ADDITIONAL ADVANTAGES

- **Solves many of the inhibition issues associated with qPCR**
- **Greatly reduces the potential for false negative results**

RESISTANT TO INHIBITION

Humic acid concentration (ng/ul)	Old Method (gene copies)	New Method (gene copies)
0	1810	1810
1	1165	1680
2.5	184	1700
5	0	1870

NEXT STEPS

- **Presently testing instrument in the lab**

- **Will start field trials next Spring**

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MICROBIAL SOURCE IDENTIFICATION

- **When you have a problem, you want to know the source**

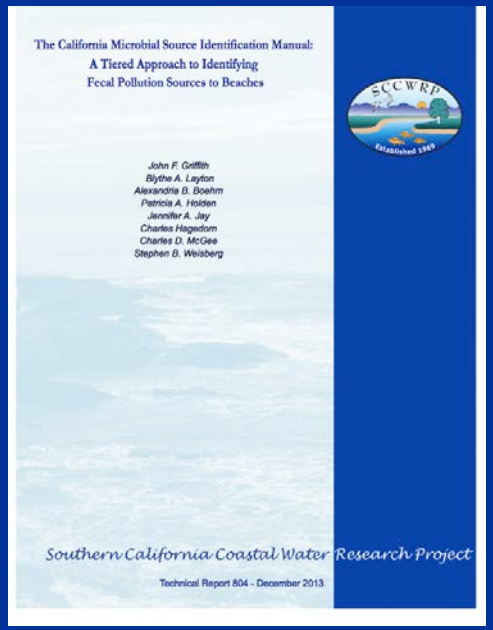
- **Need to be able identify sources of bacteria and extent of contamination**
  - Can expend a lot of resources without fixing the problem

MICROBIAL SOURCE ID RESEARCH

- **SCCWRP a national leader in microbial source identification**

- **Conducted largest Microbial Source Identification Method Evaluation Study**
  - Included all the top researchers in the US and Europe
  - Dedicated issue of *Water Research*
  - Achieved broad scientific consensus regarding best methods

- **Microbial Source Identification Manual**
  - Bible for source ID work
  - State has adopted for all Bond funded work



REMAINING CHALLENGES

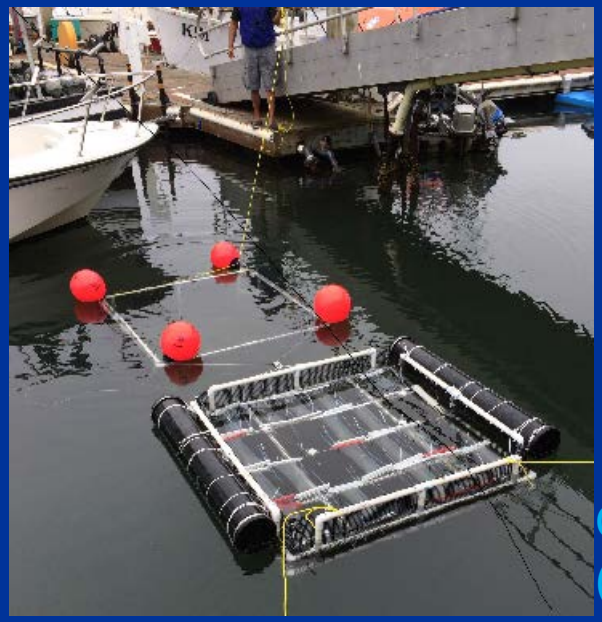
- **Studying relative degradation of microbes**
  - Source associated markers
  - Fecal indicator bacteria
  - Pathogens
- **Need understand how markers behave in the environment**
  - All previous work has been done in fresh water
- **Developing a model to help interpret results**

Dialysis bag containing ambient water and 5% v/v sewage

Shade cloth covering half of experiment

Freshwater (Irvine)

Brackish water (Santa Barbara)



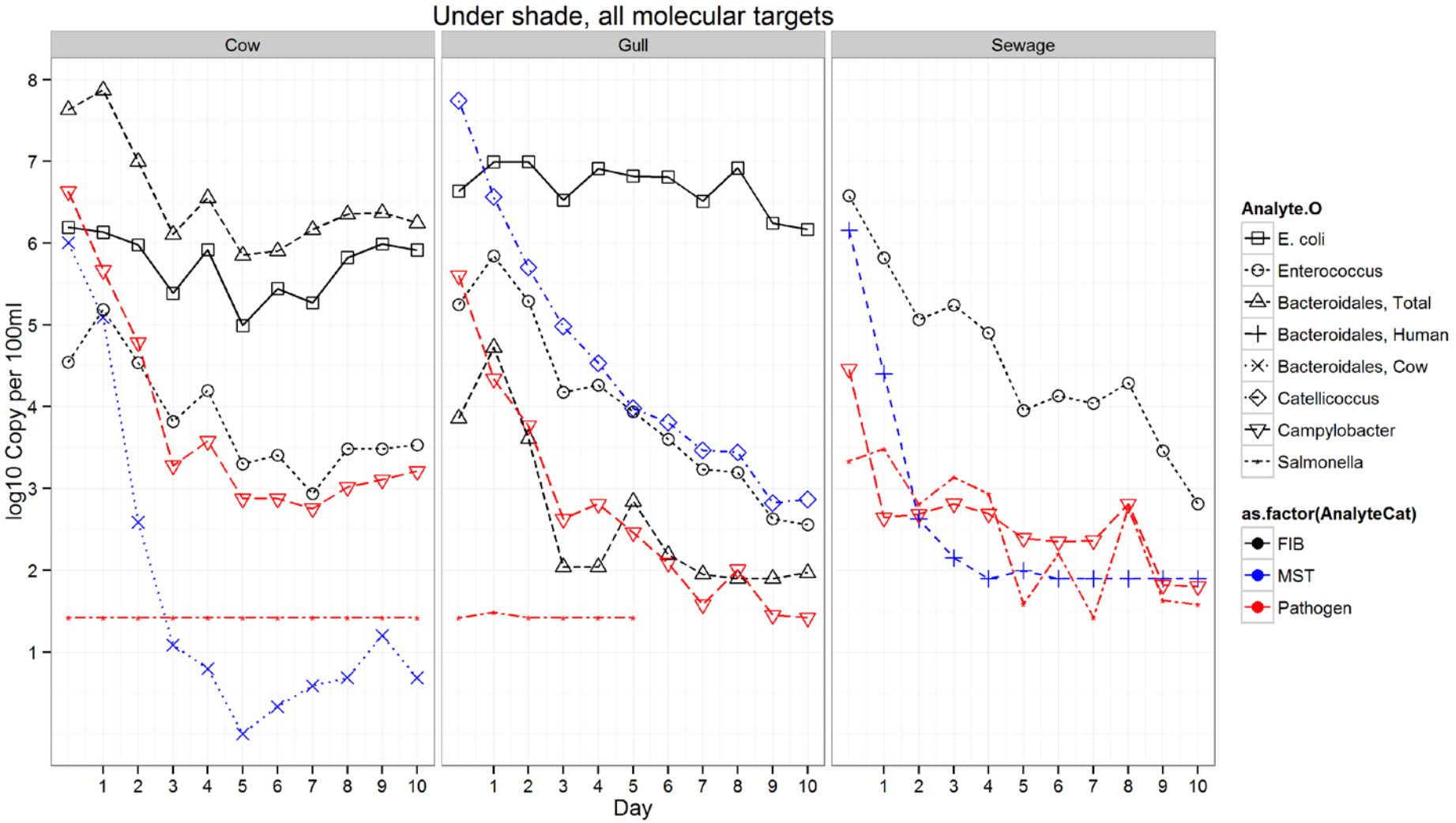
Ocean water
(Pillar Point Harbor)

- **10 day deployments**
- **Summer and winter**
- **Full sun or shade**

WHAT WE ARE MEASURING

Category	Analysis	Sewage	Cattle	Gull
FIB (by culture or PCR)	Enterococcus (culture)	X	X	X
	E. coli (culture)	X	X	X
	Enterococcus (PCR)	X	X	X
	General Bacteroidales (PCR)	X	X	X
MST markers (by PCR)	HF183 (human)	X		
	HumM2 (human)	X		
	BacHum (human)	X		
	CowM2		X	
	Catellicoccus (gull)			X
Pathogens (by culture and PCR)	Norovirus (RT-PCR)	X		
	Campylobacter (culture)	X	X	X
	Salmonella (culture)	X	X	X
	Campylobacter (PCR)	X	X	X
	Salmonella (PCR)	X	X	X
Community analysis	PhyloChip (microarray)	X	X	X
	Illumina (sequencing)	X	X	X

PRELIMINARY RESULTS



NEXT STEPS

- **Field studies complete**
- **Conducting data analysis**
  - Manuscripts for CTAG in 6 months
- **Model available**
  - Summer 2016

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EPIDEMIOLOGY STUDIES

- **Need to have thresholds in order to interpret results from new markers**
  - What level is important for health risk?
- **Epidemiology studies determine thresholds**
- **SCCWRP has been a national leader in epidemiology studies**

BASICS OF EPIDEMIOLOGY

- **Recruit swimmers and non-swimmers to participate in study**
- **Take concurrent water quality measurements**
  - Need to know levels of indicators/markers at time of exposure
- **Compare illness rates between exposed and unexposed participants**
  - Difference (if any) attributed to water exposure
- **Determine if there is a relationship between illness and measured levels of indicators/markers**

CHALLENGES OF A WET WEATHER STUDY

- **Not as many folks swimming in the winter**
  - Usually recruit at beach and follow up by phone
  - Must use exposure days to make up for small sample size
- **Makes field operations more difficult**
  - Rain
  - Big surf
  - Fast moving water

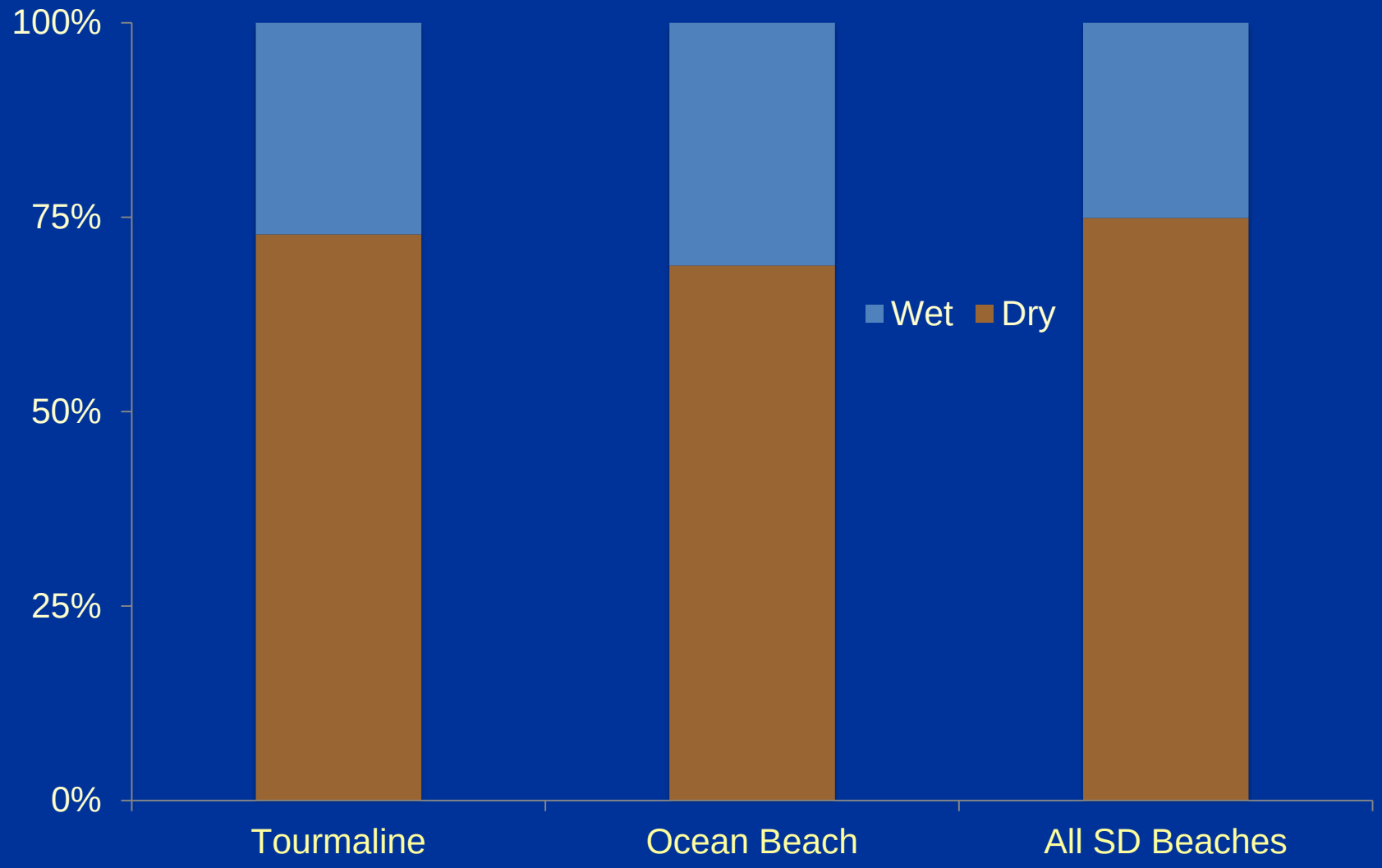
STRATEGY

- **Targeted 2 popular surfing beaches in San Diego**
  - Tourmaline Surfing Park
  - Ocean Beach
- **Followed health throughout study (16 weeks) in wet and dry weather**
  - Developed phone and web apps
  - Provided incentives for staying in study
- **Daily beach water quality measurements for duration of study**

EXPOSURE DAYS

	Subjects Enrolled	Total Days of Follow-up	Ocean Exposure Events
Surfer Health Study	**654**	**33,377**	**10,081**
Boqueron, PR	15,726	172,986	12,111
Surfside, SC	11,159	122,749	8,073
Silver, MI	10,921	120,131	5,651
Mission Bay, CA	**12,469**	**137,159**	**4,524**
Doheny, CA	9,525	104,775	4,335
Avalon, CA	6,165	67,815	3,891
Malibu, CA	5,674	62,414	2,559
Washington Park, IN	4,377	48,147	2,360
West, IN	2,877	31,647	1,668
Goddard, RI	2,977	32,747	1,080
Fairhope, MS	2,022	22,242	823
Huntington, OH	2,840	31,240	757
Edgewater, AL	1,351	14,861	741

WET VS. DRY EXPOSURE



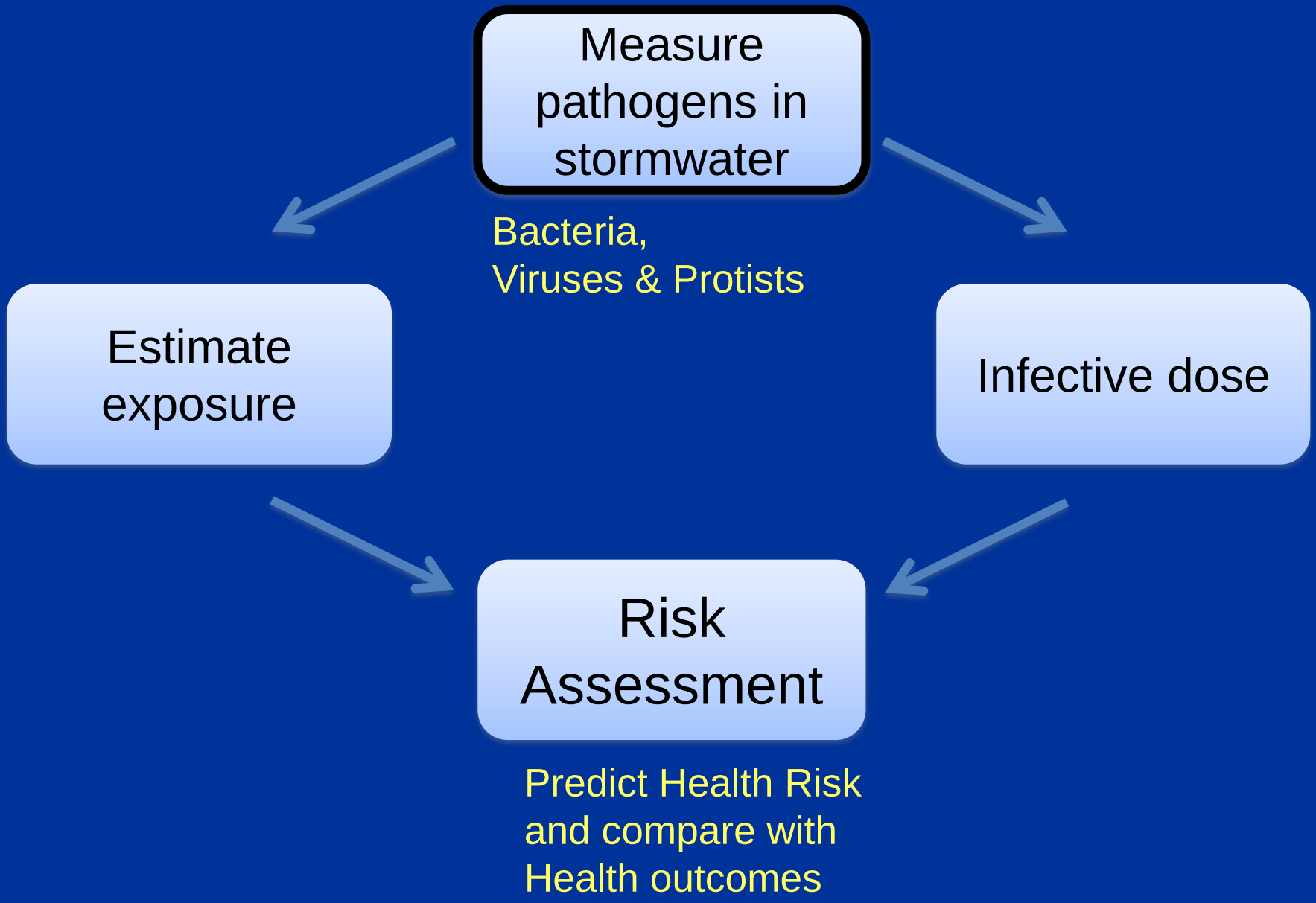
NEXT STEPS

- **Field work complete**
- **Data analysis underway**
- **Expect a report in early 2016**

QUANTITATIVE MICROBIAL RISK ASSESSMENT

- **Less expensive alternative to epidemiology study**
- **EPA has established method**
  - 2012 Recreational Water Criteria
- **We are conducting a prototype**

WET WEATHER QMRA STRATEGY



WHAT WE ARE MEASURING

Measurement	Method
Enterococcus	Culture and PCR
Coliphage	Culture
Campylobacter	PCR
Salmonella	PCR
Norovirus	PCR
Adenovirus	PCR
Enterovirus	PCR
Giardia	PCR
Cryptosporidium	PCR
Human Marker	PCR
Dog Marker	PCR
Gull Marker	PCR

NEXT STEPS

- Field work complete
- Data analysis underway
- Epidemiology will show if QMRA is working
- Embarking on a dry weather QMRA study at Cabrillo Beach

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