SOUTHERN CALIFORNIA COASTAL WATER RESEARCH PROJECT AUTHORITY

THEMATIC RESEARCH PLAN
FOR
MICROBIAL WATER QUALITY

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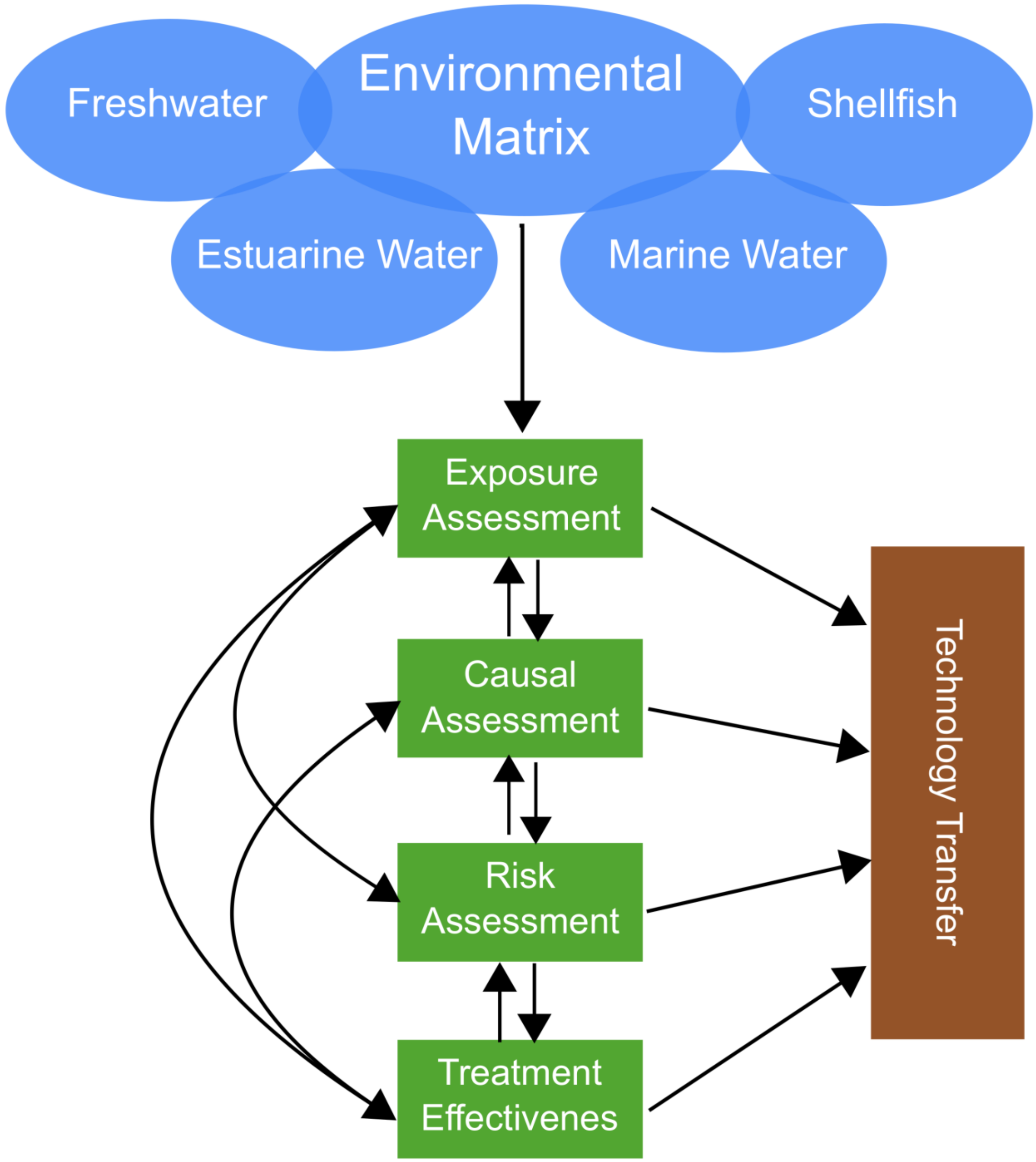
Introduction

With more than 233 million visits per year, Southern California's beaches are a precious natural resource and a major economic driver for the state and region (King and Symes 2003). As such, protecting beachgoers from waterborne microbes that come from a disparate array of sources is vital to maintaining the economic benefits and perception of healthful living associated with California beach culture. Although California runs the nation's most comprehensive beach water monitoring program, the public could benefit greatly from advances in microbial water contamination detection and monitoring technologies. Existing methods to assess microbial water quality take 24 hours or more to yield results, which isn't fast enough to provide same-day warnings to beachgoers. Moreover, when environmental managers find fecal indicator bacteria that may be associated with potentially pathogenic microbes, they want to identify where the contamination is coming from and stop it at the source. Given that waterborne microbes can travel long distances, remain infectious for extended periods (as in the case of some viruses), and come from any combination of human and animal feces, the process of identifying sources of microbial water contamination can be challenging and complex – an area for which the technology is still evolving. SCCWRP's goal is to build a holistic microbial water quality program that uses cutting-edge techniques to quickly and accurately assess microbial water quality and the risk to public health at beaches and in source water across all water types in Southern California.

Conceptual Model

SCCWRP's conceptual model for its Microbial Water Quality research program is designed around improving methodologies and technologies for accurately measuring microbes in aquatic environments (**Exposure Assessment**), discovering the source(s) of microbial contamination (**Causal Assessment**), determining public health risks from microbial contamination (**Risk Assessment**), measuring outcomes of management actions (**Treatment Effectiveness**), and transitioning methodologies and technologies into adoption by the end-user management community (**Technology Transfer**). All of these elements are interconnected and work synergistically to advance the state of knowledge, ensuring environmental managers continually have access to the most relevant, timely and accurate information and protocols for managing microbial contamination. SCCWRP's approach to all of its method and technology development work is to design tools that can work across all aquatic environments, from marine shellfish industries to highly urbanized watersheds.

Microbial Water Quality Conceptual Model



Exposure Assessment

At its most fundamental level, the field of microbial water quality relies on accurate measurements of the bacteria, protists, and viruses present in aquatic environments. Although scientists have been able to detect microbial contamination for centuries, new technologies and approaches are enabling scientists to more quickly, more efficiently and more accurately focus on studying the microbial indicators, pathogens and associated markers that predict the greatest risks to humans and wildlife. SCCWRP is working to help water-quality managers better assess exposure to microbial contamination on a number of fronts:

- **Improving Existing Methods:** Emerging technologies are enabling scientists to measure microbes more quickly using more sensitive assays. These technologies offer the potential to automate the sampling process and to use molecular microbiology methods to more accurately and quickly quantify contamination.
- **New Targets:** Water-quality managers traditionally have relied on a measuring a fairly standard, limited set of fecal indicator bacteria to alert them to microbial contamination. These fecal indicator bacteria, especially Enterococcus, are always present in aquatic environments with pathogens from human sewage that can make people sick, although they may also be from other sources that are not associated with human pathogens, such as animal feces or decaying plant material. Instead of targeting fecal indicator bacteria, SCCWRP is now working to target the illness-causing microbes themselves, as well as a different set of markers that are specific to the contamination source. In this way, water-quality managers will have the ability to know which specific animals (including humans) are responsible for the observed contamination.
- **New Endpoints:** Advances in molecular microbiology technologies have enabled scientists to focus on measuring the DNA of microbes using PCR (polymerase chain reaction) -based technologies including qPCR (quantitative PCR) and dPCR (digital PCR), a far more precise way to quantify the amount of contamination present in a water sample than established culture-based methods. But these technologies are limited in that they do not determine whether the microbes are alive and/or infective. Thus, SCCWRP is pursuing development of next-generation endpoints that will enable this information to be determined accurately and rapidly.

Causal Assessment

When microbial contamination is detected, water-quality managers need to be able to identify the source(s) and cause(s) so they can implement effective remediation and risk assessment measures. SCCWRP is pursuing a multi-pronged approach to improving casual assessment strategies:

- **Microbial Source Tracking**: Microbial source tracking (MST) refers to the strategy by which water-quality managers identify sources of contamination and follow them to their point of origin. SCCWRP is continuing to refine existing methods and work toward adapting new methods and protocols used to conduct MST studies.
- **Quantitative Microbial Source Tracking Models:** MST provides a conceptual pathway to identifying the sources of contamination, but in complex regulatory environments that include TMDLs (total maximum daily loads) and the option to do QMRA (Quantitative Microbial Risk Assessment), water-quality managers also need assistance interpreting MST results in the appropriate management context. Thus, SCCWRP is working to build quantitative microbial source tracking models that are relevant to the real-world regulatory environment.
- **Case Studies**: Because source tracking involves analyzing and interpreting complex environmental field data, SCCWRP is facilitating case studies that show managers how to conduct MST studies and adapt them to specific sites.

Risk Assessment

Once water-quality managers identify the cause(s) and source(s) of contamination, they must ascertain the health risks for humans who come into contact with the contamination. Managers can perform these risk assessments through either direct measurement or modeling. SCCWRP research encompasses both approaches:

- **Epidemiology:** Epidemiological studies allow scientists to measure the empirical health risk associated with populations that come into contact with contaminated water. SCCWRP and its collaborators have performed numerous epidemiological studies at California beaches to more precisely understand water-contact illness risk.
- **Quantitative Microbial Risk Assessment (QMRA) to Establish Site-Specific Objectives:** The microbial contamination at one site does not necessarily carry the same health risks as the microbial contamination at another site, even if the measured levels of pollution are the same. This is because microbial contamination comes from a wide variety of sources, only some of which are pathogenic to humans. A Quantitative Microbial Risk Assessment (QRMA) is a strategy for predicting the risk of illness based on the number and type of microbes present in the water at a particular site. In a QMRA, the focus is on looking at multiple lines of evidence – not just the indicator bacteria that are commonly measured, but also pathogens and source-specific markers. The end goal of QMRA is to be able to establish numerical objectives for managing microbial contamination at the specific site being studied.
- **QMRA Optimization:** Microbial contamination in aquatic environments is inherently dynamic, with contaminants able to be transported at various speeds, dispersed in various ways, and remain infectious for various times. Thus, QMRA modeling strategies used to predict illness risk must be optimized to accurately estimate the fate and transport of the microbes, as well as the infective dose and resulting illnesses. The optimization of QMRA is an enormous challenge for which the science is still evolving.
- **Case Studies:** To help water-quality managers establish site-specific objectives using QMRA and to optimize QMRA's predictive power, SCCWRP initiates case studies aimed at demonstrating how to measure and model risk for populations at specific beach sites.

Treatment Effectiveness

After water-quality managers identify the risks associated with microbial contamination at a site, their final step is to implement cleanup measures. To ensure that these measures are effective, managers need tools to evaluate the successes and failures of the treatment. SCCWRP is developing and refining a number of strategies aimed at evaluating treatment effectiveness across three main aquatic systems:

- **Stormwater:** One treatment approach that managers can take is to capture stormwater runoff before it is discharged into coastal waters. SCCWRP is working to improve managers' ability to measure the FIB and pathogens in captured stormwater, ensuring that microbial contaminants do not reach the coastal zone.
- **Treated Wastewater:** When wastewater effluent is discharged into receiving waters, wastewater treatment facilities ensure that it contains only permissible levels of FIB, which are indicative of the presence of pathogens. However, while wastewater treatment processes are designed to kill FIB, some viruses and bacteria can remain viable even after the treatment process. SCCWRP is working to develop new technologies that will enable measurement of viable viruses and bacteria in treated effluent.
- **Recycled Water:** Recycled water is being used increasingly for both direct and indirect potable reuse to help solve California's chronic water shortages. Recycled water, however, presents a number of challenges for the water-quality monitoring community, including the ability to

reliably monitor microbial contamination. Hence, SCCWRP is working on strategies to more efficiently detect microbial contamination that could pose a possible health threat.

Technology Transfer

As SCCWRP develops better methodologies and technologies to assess and analyze microbial water quality, these approaches must be translated and transferred to the end-user and management communities. Thus, SCCWRP puts great emphasis on making this transfer process happen in near real time, ensuring that environmental managers, dischargers, regulators, and the scientific community can all benefit immediately. SCCWRP is pursuing a number of approaches and strategies to ensure that the best technologies find their way into widespread adoption and use:

- **Training/Workshops/Guidance Documents:** One of the most effective ways that SCCWRP transfers technology is by directly interfacing with laboratories through training and workshops, as well as publication of tailored guidance documents.
- **Intercalibration/Method Acceptance/Certification:** Because technology is only as effective as the people who are using it, SCCWRP conducts a wide range of intercalibration studies intended to gauge laboratory proficiency, facilitate method acceptance and inclusion into regulations, and help laboratories become proficient in new methods.
- **Case Studies:** SCCWRP also facilitates case studies that demonstrate the application of new methods and frameworks and set precedents for the execution of microbial water quality studies by the greater research community.
- **Informing Cost-Benefit Analyses:** For managers to identify management actions that have the largest impact for the smallest amount of money, cost-benefit analyses need to be run. Although SCCWRP does not have expertise in economics, SCCWRP can provide data on exposure, cause, risk, and effectiveness that are instrumental in running managerially relevant cost-benefit analyses.

Research Priorities

Exposure Assessment

Improving Existing Methods

Accomplishments

SCCWRP is a world leader in developing and evaluating rapid molecular technologies for beach monitoring and fecal source identification and transitioning these technologies to end-users. In the beach monitoring area, SCCWRP was among the first to identify and evaluate rapid methods for measuring fecal indicator organisms (Cao et al. 2012, Converse et al. 2012, Griffith and Weisberg 2011, Noble et al. 2010, Griffith et al. 2009, Colford et al. 2007, Griffith et al. 2006). Later, SCCWRP worked closely with EPA to validate qPCR for rapid measurement of enterococci (Cao et al. 2013a, Haugland et al. 2016, Yau et al. 2014, Arnold et al. 2013, Colford et al. 2012, Shanks et al. 2011). Recently, SCCWRP has made great strides in adapting the next iteration of qPCR – digital PCR – for environmental monitoring applications (Cao et al. 2015, Cao et al. 2016a, Cao et al. 2016b, Steele et al. 2018, Wang et al. 2016). Digital PCR technology has the potential to revolutionize managers’ ability to detect and quantify microbes, including viruses and other rare targets as quickly and cheaply as qPCR.

Ongoing Research

SCCWRP is working with leading scientists across the United State and Europe, as well as equipment manufacturers, to test and apply emerging technologies that expand the type and number of microbes that scientists can detect, improve sensitivity, and provide more information about water quality at beaches, estuaries, and rivers.

> ***Project*: Incorporating digital PCR into microbial contamination monitoring protocols**
> SCCWRP is adapting assays previously developed as a qPCR format to digital PCR, the latest advance in quantitative PCR (Cao et al. 2015). Digital PCR offers advantages in sensitivity (i.e., it can detect targets at very low concentration), in resilience to inhibition, and in comparability across laboratories because it does not require a standard curve. Most qPCR assays can be readily adapted to digital PCR, and duplex assays increase the efficiency of measuring pathogens or indicators by allowing two targets to be measured simultaneously.

Priorities for Future Research

While SCCWRP has made significant progress adapting molecular methods to microbial water quality research, there are still opportunities for technological improvements. Further development of digital PCR, automated sampling, sample processing, and quantification will add to the ease of monitoring and enable higher-resolution data on microbes in marine, estuarine, and freshwater environments.

> ***Future Focus Area*: Automated Sample Processing and Digital PCR**
> SCCWRP and MBARI have proposed developing a backpack-sized sampling instrument that could interface directly with a sample acquisition module and field-portable digital PCR machine. The portable unit would allow collection of many water samples without the need to return to the field lab to offload heavy water samples, and also enable semi-permanent installations (at lifeguard stations, for example), where water samples could be processed by a lifeguard. The speed and versatility of this instrument would not only produce more rapid results, but also would enable higher-resolution data in real time for areas where problems have been identified.

New Targets

SCCWRP is continuing to develop assays to directly measure source-specific markers, pathogenic bacteria, viruses, and protists in environmental waters.

Accomplishments

SCCWRP has worked with the EPA and other partners to develop rapid measurement methods for fecal indicator bacteria and new source-specific markers. By targeting the types of bacteria that are more commonly found in the guts of most animals, instead of the FIB that are traditionally measured, SCCWRP has improved managers' ability to identify specific sources of fecal contamination (Boehm et al. 2013, Cao et al. 2013b, Cao et al. 2013c, Ebentier et al. 2013, Harwood et al. 2013, Layton et al. 2013, Raith et al. 2013, Schriewer et al. 2013, Stewart et al. 2013, Ryu et al. 2012, Converse et al. 2009, Field et al. 2003, Griffith et al. 2003). Using the sensitivity of digital PCR, SCCWRP and its collaborators also have developed and adapted molecular assays to detect and quantify the primary pathogens found in recreational waters: DNA and RNA viruses (e.g., human Norovirus, Enterovirus, and Adenovirus), and bacteria (e.g., *Campylobacter* and *Salmonella* species) and protistan parasites (e.g., Giardia and Cryptosporidium species; Steele et al 2018). Although the primary focus has been quantification of pathogens, digital PCR also offers an ability to target genes that impart toxicity or pathogenicity, a capability that will be further enhanced by assays targeting new endpoints (see next section: New Endpoints).

Ongoing Research

SCCWRP is developing and adapting molecular assays to measure waterborne pathogens, toxic cyanobacteria, and microbes of emerging concern. These assays are bringing increasing clarity to microbial water-quality data obtained from a variety of aquatic environments, including beaches, rivers, estuaries, stormwater, and treated wastewater.

***Project*: Adapting phage as a viral fecal indicator**
Coliphage, a bacteriophage that infects E. coli, is being considered for approval by EPA as a viral fecal indicator (US EPA 2015, Griffith et al. 2016). EPA recently released a draft method for enumeration of coliphage in recreational waters (Method 1642, US EPA 2018). SCCWRP is providing guidance for application of the new culture-based EPA method for application to routine beach monitoring, including evaluating coliphage levels compared to existing *Enterococcus* levels as part of the Bight '18 microbiology sampling efforts. In addition, SCCWRP is laying the groundwork, with multiple partners, to develop assays that rapidly quantify coliphage and distinguish genotypes used as source ID with molecular methods.

***Project*: Alternative indicators for recreational shellfish harvesting waters**
The current water column-based fecal coliform (FC) water quality objective (WQO) for SHEL is a monthly median of <14 MPN FC/100 mL, with no more than 10% of any single sample exceeding 43 MPN FC/100 mL. This standard applies to almost all marine and estuarine areas in California regardless of whether shellfish are presently harvested for commercial or recreational purposes. However, it may not be relevant to ensure that shellfish that are recreationally harvested are safe to consume. SCCWRP is collecting matched shellfish tissue and water column samples, and using molecular assays and cultivation techniques to compare and evaluate levels of alternative indicators and pathogens in the paired samples.

***Project*: Tracking antibiotic-resistance genes in aquatic systems**
SCCWRP and its four wastewater treatment member agencies are examining whether viable antibiotic-resistant bacteria – and the genes that code for antibiotic resistance – are being discharged into the environment following the wastewater treatment process. The study's goal is to develop a baseline understanding of how prevalent antibiotic resistance genes are in wastewater effluent at Southern California's treatment facilities. The study also will examine whether differences in wastewater treatment regimens and effluent discharge practices across Southern California affect the viability of antibiotic-resistant bacteria and genes. SCCWRP will next use new tools and techniques, such as next-generation sequencing and bioinformatics, to characterize and track the prevalence and type of antibiotic resistance genes in stormwater, wastewater, and recreational water.

***Project*: Tracking Vibrio in Southern California marine waters and shellfish**
SCCWRP is using molecular assays and cultivation techniques to assess the abundance and pathogenicity of two Vibrio species – *V. parahaemolyticus* and *V.vulnificus* – in estuarine waters across Southern California. Recent increases in surface ocean temperature may have generated conditions that have allowed the

growth and persistence of pathogenic Vibrio species in southern California marine waters. Vibrio, which can cause wound infections in surfers and ocean swimmers, historically did not grow in Southern California's coastal zone because of inhospitably cold temperatures. However, brackish and freshwater estuaries in the coastal zone may now be serving as reservoirs for these bacteria before they are flushed into marine recreational waters during storms. The long-term increase in ocean water temperatures due to climate change could increase the frequency of pathogenic Vibrio species in the coastal zone, including in Southern California coastal waters and shellfish. SCCWRP is also exploring potential environmental drivers of Vibrio and pathogenic Vibrio subpopulations, including associations with particular algal species.

***Project*: Developing an early warning system for cyanobacterial blooms**
SCCWRP is developing digital PCR assays targeting DNA and RNA of cyanobacteria, with a goal to detect potential cyanotoxin production prior to large biomass and/or toxin buildups. Early warning of cyanobacterial blooms in surface water and drinking water reservoirs could help alleviate the threats that the toxins produced by cyanobacteria, such as microcystins, can pose to drinking water supplies and wildlife, pets and livestock when ingested. Although many species of cyanobacteria are capable of producing toxins, not all of them do, which means that digital PCR assay could help managers potentially predict when it may be necessary to change drinking water treatment regimes to remove toxins before they enter the water distribution system.

Priorities for Future Research

***Future focus area*: Enabling real-time CyanoHABs monitoring**
As managers move increasingly toward routine monitoring of cyanobacterial harmful algal blooms (CyanoHABs), faster, cheaper and better monitoring tools will be needed. Building upon its experience in automating digital PCR for field-portable applications and in providing real-time monitoring for fecal contamination, SCCWRP intends to develop similar platforms for CyanoHAB monitoring. Existing monitoring tools often do not detect blooms until there has been substantial biomass accumulation; they also can be time-consuming and costly.

***Future focus area*: Developing assays for microbes of emerging concern**
SCCWRP will continue to adapt and develop assays for pathogenic microbes as they become relevant to southern California waters. As the climate warms, there will likely be an increase in pathogens and parasites previously only found in more southerly latitudes. In freshwater, the "brain-eating amoeba" Naegleria is likely to become an emerging pathogen of concern; assays that can detect it with great sensitivity could help protect public health.

***Future focus area*: Using next-generation sequencing to analyze environmental samples**
Advances in technology have sharply reduced the cost of DNA sequencing, while increasing the size of an aquatic community that can be sequenced. This opens up the possibility of using sequencing directly on environmental samples to measure rare targets (e.g., pathogens), to provide holistic information about which microbes are present in environmental samples, and potentially to identify unexpected pathogens or indicator microbes (Cao et al. 2013b).

***Future focus area*: Adapting metagenomics to study environmental microbiomes**
Existing quantitative molecular assays rely on targeting a specific gene and quantifying that gene using PCR. This technique, while powerful, is limited in that it requires specifying the target in advance. SCCWRP intends to apply advances in gene sequencing of whole bacterial and viral communities, as well as advances in bioinformatics, to analyze all of the microbes in a sample at once. SCCWRP expects this approach to become particularly powerful in the viral communities, where there is a strong likelihood that unknown waterborne viruses will cause illness.

New Endpoints

SCCWRP has long recognized that existing molecular microbial detection methods necessarily pick up dead bacteria and inactive viruses when they quantify microbial contamination. SCCWRP is focused on developing sensitive, accurate molecular methods that can detect only the bacteria that are alive and active, as well as the viruses that remain infective, in environmental waters even after going through wastewater treatment processes.

Ongoing Research

SCCWRP is working to bring molecular assays more closely into alignment with culture-based techniques. One strategy that SCCWRP is pursuing is to target single-copy genes in bacterial genomes, such as for Campylobacter species (see next section: Risk Assessment), instead of genes present in multiple copies (Steele et al. 2018).

Priorities for Future Research

Treatment and natural degradation can inactivate and kill bacterial and viral pathogens and indicators at variable rates, which can confound measurements intended to ascertain risks from contamination sources. New RNA-based techniques, however, can be combined with rapid, sensitive molecular methods to enumerate microbes while simultaneously measuring viability or infectivity. This research has the potential to inform other aspects of SCCWRP's microbial water quality research, especially treatment effectiveness, risk assessment, and causal assessment.

***Future focus area*: Using PCR to measure viable bacteria**
qPCR and digital PCR provide a sensitive, robust way not only to count bacterial gene copies in water with higher efficiency than culture methods, but also to detect and measure the genes of dead or inactive bacteria (either indicators or pathogens). SCCWRP is interested in using sensitive molecular techniques to measure only the active genes in bacteria (i.e., only the genes that are active when the bacteria are alive and metabolizing); this approach would be both quantitative and restricted to active, living bacteria.

***Future focus area*: Using PCR to measure viral infectivity**
qPCR and digital PCR measures all viruses, including those that may be inactive and not infective. For some viruses, it may be possible to cultivate and measure just the active genes of the virus using molecular techniques; SCCWRP is interested in using this technique to provide rapid (<6 hours) measures of infectivity for some viruses. For other viruses, assays will need to be developed that combine cultivation (where possible) with cutting-edge molecular or proteomic methods.

Causal Assessment

Microbial Source Tracking

Accomplishments

SCCWRP was instrumental in establishing consensus on the most effective microbial source tracking (MST) methods for various fecal sources and in developing protocols for conducting field studies. SCCWRP conducted the largest, most comprehensive international method evaluation study ever undertaken. The study involved 27 laboratories in five countries testing a total of 41 methods, the outcomes of which were published as 14 articles in a dedicated special issue of the journal *Water Research* (Boehm et al. 2013, Cao et al. 2013b, Cao et al. 2013c, Ebentier et al. 2013, Harwood et al. 2013, Layton et al. 2013, Raith et al. 2013, Schriewer et al. 2013, Stewart et al. 2013). As the leader of the state-funded Source Identification Protocol Project, SCCWRP also developed the California Microbial Source Identification Manual: A Tiered Approach to Identifying Fecal Pollution Sources to Beaches (Griffith et al. 2013). This manual, adopted by the State Water Board, became the template for MST studies around California and the nation.

Through both field and laboratory studies, SCCWRP has measured relative degradation rates that are essential in interpreting MST marker results in the context of compliance monitoring (based on FIB) and risk assessment (based on pathogens). For example, non-detection of a human marker that decays faster than FIB can lead to the false conclusion that there is little human fecal contribution to FIB exceedances. Conversely, non-detection of a human marker that decays faster than human pathogens can lead to a false conclusion that risk of illness from human pathogens in low (Mattioli et al. 2017; Maraccinia et al. 2016; Zimmer-Faust et al. 2017).

Ongoing Research

***Project*: Bacterial community sequencing and non-targeted chemical analysis to detect and differentiate between human fecal sources**

SCCWRP is working to increase the utility of microbial community analysis in MST, especially as costs go down and as next-generation sequencing technology paves the way for advancements in bioinformatics (Cao et al. 2013b). SCCWRP will use community analysis to identify new MST markers for fecal sources that do not currently have source-specific markers, as well as identify new markers that mimic decay behaviors of FIB or pathogens. In addition, SCCWRP hopes to be able to distinguish between closely related sources (such as raw sewage, effluent discharge, and septic systems), as well as to identify non-fecal sources of bacteria, such as sand and beach wrack, that may not have a source-specific single marker.

***Project*: Quantifying exfiltration from sanitary sewer lines**

SCCWRP is working with member agency collaborators to measure exfiltration rates from sanitary sewer lines. This project will include both bench scale and *in situ* testing of sanitary sewers, and will examine differences in exfiltration based on pipe material, age, and substrate. For *in situ* measurements, an inert tracer such as KBR will be used to determine if water from intact sewer pipes can be detected in nearby stormwater conveyances.

Priorities for Future Research

***Future focus area*: Factors affecting sanitary sewer exfiltration and overflow**

Exfiltration from sanitary sewer collection pipes, along with sanitary sewer overflow, and exfiltration from septic systems and private laterals has the potential to contribute to human fecal contamination in separate storm drain systems. SCCWRP intends to apply tracer experiments on sewer infrastructure to determine leakage, inspection of pipe integrity, new microbial source tracking tools, and hydrologic models to try to understand the contributions of sewer infrastructure to microbial water quality in storm drain systems.

***Future focus area*: Potential for false detections of human fecal markers in irrigation with reclaimed water**
As recycled water irrigation increases, there is growing concern over the potential for false positives of human fecal markers. SCCWRP intends to evaluate background levels of human fecal markers in treated wastewater to better understand potential false positive detections of human fecal marker. This will include evaluating human fecal markers and pathogens in surface waters impacted by runoff from areas where reclaimed water irrigation is applied.

***Future focus area*: Evaluation of alternative human markers to HF183**
While the HF183 human fecal marker has been used successfully to identify and remediate sources of human contamination, it is sometimes found in low levels in human companion animals and wildlife. Thus, there is a need for one or more additional human markers that are sensitive and specific to human fecal contamination.

Quantitative Microbial Source Tracking Models

Identifying the sources of contamination is an important first step towards remediation and assessing potential public health risk. However, quantitative interpretation of MST results is needed to determine potential load reductions in the context of TMDLs, and to determine extent of human fecal pollution at a site when assessing eligibility for site-specific objectives via QMRA.

Accomplishments

The Source Identification Protocol Project led by SCCWRP included the development of a ratio-model as a potential useful tool for allocating FIB loading to different fecal sources (Wang et al. 2013). Also, an evaluation study was conducted to establish consensus that a standardized approach is needed to assess the extent of human fecal contamination at a site (Cao et al. 2013d). SCCWRP and its partners also developed a standardized and mathematically defined human fecal score (HFS) to integrate human marker results from multiple samples at a site. The HFS provides a basis for management decisions on prioritization of sites for remediation (Cao et al. 2017).

Priorities for Future Research

In the future, SCCWRP intends to (1) demonstrate field application of the above described quantitative source tracking tools for management goals, such as remediation and site prioritization, BMP treatment effectiveness, QMRA eligibility studies, and cost-benefit analysis; (2) develop similar indices such as a gull fecal index and cow fecal index, and relate these indices to public health risks; and (3) adapt source apportionment and a human fecal index using viral MST markers under potential new water quality criteria based on viral indicators, such as coliphage.

Case Studies

Accomplishments

For the past decade, SCCWRP has been conducting field MST studies in collaboration with management agencies and research universities (Layton et al. 2015), and has developed a source identification manual that has been adopted by California and referenced around the world (Griffith et al. 2013). SCCWRP conducted a wet-weather MST study in the San Diego River watershed to delineate sources of fecal contamination, in support of the Surfer Health Study (see next section: Risk Assessment). The MST study identified that human fecal contamination, as measured by the HF183 human fecal marker, was widespread throughout the San Diego River watershed. Follow-on studies identified both faulty sewer infrastructure and septic systems in the Los Coches sub-watershed.

Priorities for Future Research

Although general principles govern field MST studies, each study site is unique and varies in terms of hypothesis development, study design, and interpretation of results, depending on site conditions and study goals. SCCWRP expects case studies to remain a valuable resource for member agencies, particularly case studies at sites with conditions not previously investigated, such as wet weather, and physical systems such as water and wastewater collection/distribution networks and beach sand.

Risk Assessment

Epidemiology

Accomplishments

SCCWRP has conducted multiple standard-setting studies in collaboration with epidemiologist collaborators at Avalon, Mission Bay, Doheny, and Malibu (Colford et al. 2007, Colford et al. 2012, Arnold et al. 2013, Yau et al. 2014) that have measured the risk to beachgoers from exposure to microbially contaminated water. Also, as EPA moves forward with developing new water quality criteria for coliphages, SCCWRP's research is being utilized by EPA as evidence linking coliphage and health risk (Griffith et al. 2016). Via the Surfer Health Study, SCCWRP also has conducted paired epidemiological data with the QMRA framework (see next section: Using QMRA to Establish Site-Specific Objectives) and ground-truthed the risk modeling results to the empirical illness risk (Arnold et al. 2017, Steele et al. 2018).

Priorities for Future Research

Because of the importance of measuring illness risk (especially when conducting natural source exclusions, assessing treatment effectiveness, setting TMDLs or ground-truthing QMRA), SCCWRP intends to continue to perform epidemiology studies at marine beaches in California. These case studies will be particularly useful in light of changing health risks due to emerging microbes and climate change. Further, SCCWRP intends to extend these measures of illness risk to freshwater beaches and streams to support efforts to develop viable QMRA modeling.

> ***Future focus area*: QMRA ground-truthing**
> Epidemiological studies will be necessary to provide empirical risk for tuning QMRA studies to actual illness data in new locations in southern California, including new beaches, variable geography and hydrology, and in freshwater sites such as swimming holes. Especially because EPA is now basing its criteria on risk-based estimates and

empirical illness data, this information will be necessary to understand risk and ground-truth risk models in the region's diverse water bodies and geographies.

***Future focus area*: Epidemiologic relationship between exposure to beach water and colonization of humans by anti-microbial resistant bacteria**
Antimicrobial resistant bacteria and genes have been reported in sand and water at beaches. However, the threat of exposure, and the risks of colonization or infection for swimmers and surfers, are not yet known. Studies tracking the proportion of swimmers or surfers who are colonized by antimicrobial resistant bacteria while quantifying the concentration of antimicrobial resistant bacteria and genes will provide a better understanding of the potential public health risk.

Quantitative Microbial Risk Assessment to Establish Site-Specific Objectives

Although Quantitative Microbial Risk Assessment has only recently begun to be used to assess health risk at recreational beaches, EPA's 2012 recreational water criteria (US EPA 2012) has opened the door for beaches with high FIB and demonstrated low risk to perform a site-specific analysis and potentially adopt site-specific standards. QMRA requires quantification of the pathogens in the water, a measure of exposure to the pathogens, and a dose-response relationship in order to generate an estimate of the illness risk. SCCWRP and its member agencies are in a unique position to work together to determine the requirements for successful identification of potentially eligible beaches, including identification of likely contamination sources affecting the beaches, source tracking, and ground-truthing to empirical risk, where required. This work is analogous to the SCCWRP beach epidemiology studies that have been performed over the past 15 years (Colford et al. 2007, Colford et al. 2012, Arnold et al. 2013, Yau et al. 2014), which were conducted concurrently with water quality methods. Establishing site-specific objectives via QMRA will provide a method for producing the exposure and source quantification data needed for risk assessment, and a means for calibrating QMRA models to empirically determined rates of illness.

Accomplishments

SCCWRP helped develop the standard protocol for QMRA studies at California beaches, including identifying checkpoints that were incorporated into the study design to direct resources where they will be most effective. This process has been tested on three beaches in Southern California – Kidde Beach in Ventura, and Ocean Beach and Tourmaline Surfing Park in San Diego – providing baseline information used to determine whether each site was eligible to adopt modified water-quality objectives that would be higher than – but as protective as – current numerical water-quality thresholds. QMRA also was an integral component of the Surfer Health Study in San Diego, which involved combining water quality, epidemiology, and QMRA study elements to determine storm-related illness risk for the surfer population during the winter. The results of this study are expected to have a profound impact on future QMRA studies (Soller et al. 2017, Arnold et al. 2017, Steele et al. 2018).

Priorities for Future Research

The creation of site-specific objectives will hinge not only on the successful application of QMRA, but also on changes in storm water management and treatment. This necessarily requires the adoption of sensitive techniques for measuring pathogens, optimization of exposure and dose-response estimates, and the identification of recreational water sites that are expected to present a low illness risk and yet still have high FIB concentrations. To apply risk modeling widely, aspects of the beaches themselves must be characterized, and the hydrology, geography, and infrastructure must be investigated, along with the sources of microbial contamination at these beaches.

***Future focus area*: Setting site eligibility standards for QMRA**
With no clear guidelines for determining which waterbodies are eligible for site-specific objectives based on QMRA, there is a need for improvements in quantification of pathogens and FIB, which could increase the number of beaches that may qualify as candidates for site-specific objectives based on risk assessment. Any fresh or marine waterbody with low to no human source or pathogen input should be able to use QMRA to define the risk to swimmers and to be eligible for site-specific criteria. Technological improvements will assist in testing and mapping storm drain and sewer infrastructure at potential sites, while molecular assays that allow for improved detection will increase the confidence in the sites containing some contamination.

***Future focus area*: Assessing the suitability of current microbial standards for recreation shellfish harvest in California**
SCCWRP has begun evaluating and comparing paired water column and shellfish tissue levels of indicators and pathogens in Newport Bay (see Exposure Assessment section, New Targets subsection, Project: Alternative indicators for recreational shellfish harvesting waters). Results will be used to evaluate the appropriateness of pursuing development of alternative indicators and/or thresholds. If study findings support pursuing alternative indicators and/or thresholds, these alternative shellfish WQOs will need to be validated prior to general application to California coastal and estuarine waters. Thus, further research will be necessary to compare current vs. proposed alternative criteria, and ultimately to evaluate the health risk associated with these options.

***Future focus area*: Groundwater as a source of human contamination**
Groundwater has been implicated as source of human fecal contamination in previous beach water quality studies. Human fecal indicators and pathogens have variable fate and transport, making risk assessment of surface waters associated with groundwater-derived pathogens challenging. SCCWRP intends to develop and optimize methods to measure pathogens in groundwater and incorporate these measurements into a QMRA-based framework to evaluate risk to recreators in receiving waters.

QMRA Optimization

Adapting QMRA for use at California beaches involves research needs that extend beyond the site-specific investigations. For example, the relationship between molecular quantification and published dose-response relationships is just beginning to be investigated, as is the applicability of these dose-response models to exposure by swimming, refinements to estimates of exposure in beachgoers, and fate and transport of pathogens in California recreational waters. For QMRA models to be relied on by beach managers and regulators, these issues must be addressed.

Accomplishments

SCCWRP already has begun to apply QMRA to complex environmental samples, with QMRA used for the first time at marine beaches in Ventura, San Diego and Los Angeles Harbor (Soller et al. 2017). Also, SCCWRP has pioneered new molecular methods such as digital PCR that have increased sensitivity and enabled direct measurement of pathogens in stormwater (Steele et al. 2018).

Ongoing Research

The Surfer Health Study in San Diego represents the first adaptation of QMRA to a marine beach with stormwater discharge during wet weather. Following direct quantification of pathogens including viruses, bacteria, and protistan parasites in storm water using molecular assays, SCCWRP has begun working to determine the relationship between the molecular-based concentrations of pathogens and the dose-response curve. Many of the dose-response curves were generated using culture-based techniques, which not only may be unreliable in complex environmental samples, but were estimated using specific pathogenic strains. SCCWRP is applying sensitive, molecular-based techniques, developed collaboratively with the world's experts in these cutting-edge technologies.

Priorities for Future Research

Optimization of QMRA methods will rely on development of new molecular assays and new targets (see the Exposure Assessment section), improved dose-response relationships from epidemiological and direct feeding studies, and a more detailed understanding of the fate and transport of pathogens in the water bodies studied. These will be enhanced by the site-specific QMRA studies described in the previous section and by case studies performed in different water bodies. There are simplifying assumptions made in these cases, but it is not clear if these assumptions are valid. SCCWRP plans to test and address these assumptions through collaborations with leading scientists and through case studies that apply improved techniques and technologies to optimize these risk analyses.

***Future focus area*: QMRA of *Vibrio* spp. in shellfish tissues**
SCCWRP and its partners are evaluating levels of *Vibrio* spp. and pathogenic subpopulations in shellfish tissues using cultivation and molecular methods. Moving forward, SCCWRP intends to use a QMRA framework to evaluate the health risks from vibrios associated with the consumption of raw oysters at the time of harvest. These models will utilize the empirical data collected, comparing results of *Vibrio* and pathogenic subpopulation levels measured by digital PCR and culture-based methods.

***Future focus area*: Quantifying viable bacteria**
SCCWRP intends to use assays that target the RNA from genes turned on by active bacteria to quantify viable bacteria. This will enable not only the inclusion of active pathogens, but also provide a means to detect those pathogenic bacteria that are viable but would be missed by conventional culture techniques. This should provide a more accurate estimate of the microbial risk in the water being studied.

***Future focus area*: Understanding human norovirus dose-response**
The dose-response estimates for human norovirus are based on feeding studies from norovirus GI, but norovirus GII is responsible for as many or more illnesses. Future research in collaboration with clinical virologists is needed to zero in on the infectivity of norovirus strains. This is a critical part of the QMRA design, as there are few data about norovirus infectivity.

***Future focus area*: Understanding fate and transport of pathogens**
Estimating exposure to waterborne pathogens is a critical piece of the QMRA framework, along with measuring pathogen concentration and dose-response illness models. Pathogens in the environment likely do not share the fate of FIB. Pathogens

and human markers may decay rapidly compared to FIB, which would result in an underestimate of risk, or they may persist, which would result in an overestimate of risk. SCCWRP plans to conduct research not only on the decay or persistence of pathogens in the environment, but also to attempt to determine their transport in stormwater and receiving water. Because these systems are both notoriously complex, SCCWRP plans to estimate their fate and transport through empirical measurement (e.g., dye studies or biological markers) and through coupled physical-biological modeling.

Case studies

SCCWRP is at the forefront of applying QMRA to California marine beaches. These case studies are establishing and testing the proper framework for a successful application QMRA, and testing QMRA results under different conditions, recreational water types, and geographies. SCCWRP is testing the procedures developed during the first recreational water QMRAs on marine beaches in dry and wet weather; these beaches are both open and protected, and have both large and small watersheds.

Accomplishments

Although SCCWRP has conducted one case study in dry weather at a protected beach in a small harbor (Kidde Beach), the QMRA itself was postponed until infrastructure problems that were discovered during the initial phases of the study could be repaired.

Priorities for Future Research

SCCWRP expects case studies to continue as a necessary part of risk assessment at California beaches. These will test the protocols, sampling and quantification technologies, and exposure assumptions, and also will provide the potential for meta-analysis of the QMRA studies, which may be used to improve on the dose-response estimates, exposure estimates, and molecular quantification of pathogens. The applications to other geographies and recreational water types (e.g., freshwater lakes) will provide an important test of QMRA where it has not been used, including freshwater beach types, such as along rivers, streams, lakes, and swimming holes.

Treatment Effectiveness

Stormwater

With a renewed focus in California on stormwater retention and treatment for drinking water, SCCWRP has an opportunity to apply the strategies developed in its years doing microbial source tracking and exposure assessment to quantify the reduction in both fecal indicator bacteria (or viruses) and pathogens. The molecular tools that have been developed for exposure assessment, along with future research that will reveal viability or infectivity, will allow for case studies to quantify the ability of best management practices to improve microbial water quality while capturing stormwater for reuse or infiltration.

Priorities for Future Research

While more information is available on BMP treatment effectiveness on other common stormwater contaminants, such data are much more limited for microbial contaminants. A majority of available information focuses on FIB, with little data on pathogen removal. Given the biological nature of microbial contamination, interactions between removal of microbial contaminants and nutrients (often co-existing in stormwater) are important for treatment efficiency, but have received limited investigation.

SCCWRP will partner with member agencies to measure the reduction of FIB and pathogens in different stormwater treatment systems. This will be accomplished through case studies built around BMPs designed for multiple beneficial uses, such as water capture, nutrient reduction, and pathogen reduction. SCCWRP envisions studies involving treatment wetlands, retention basins, biofiltration, and small-scale package treatment plants. SCCWRP does not have the engineering expertise to design these BMPs, but through collaboration with member agencies, SCCWRP will supply the expertise in quantifying pathogens and fecal indicators. Knowledge gained through such research will facilitate optimizing BMP planning and design for achieving various management goals at various physical scales (i.e., both local areas and entire watersheds).

***Future focus area*: Risk associated with exposure to irrigation with captured stormwater**

There is potential for captured stormwater to be used for irrigation purposes. SCCWRP intends to evaluate background levels of human fecal markers and pathogens in stormwater to better understand the human health risk associated with reuse of captured stormwater and if and to what extent pre-treatment is necessary.

Treated Wastewater

Recent evidence shows that wastewater treatment, which is optimized to remove bacteria, may increase the concentration of viruses or other emerging microbial contaminants, such as antibiotic resistance genes. Thus, the molecular tools adapted by SCCWRP and its collaborators are being used to measure the abundance and viability of pathogens, as well as to track the spatial distribution and removal of bacteria and viruses from treated effluent.

Ongoing Research

***Project*: Tracking pathogen abundance in Southern California**

SCCWRP is working to gain an understanding of the abundance of viruses in the general population in Southern California, and how it would relate to virus abundance in stormwater. Given recent evidence of a potential increase in coliphage and other viruses in treated effluent, this project is being expanded to measure viral pathogens in both influent and effluent to see whether there is an increase in phage (including coliphage) and/or pathogenic viruses after treatment.

***Project*: Comparison of wastewater treatment level and removal of antimicrobial resistant bacteria and genes**

SCCWRP and its four wastewater treatment member agencies are examining whether viable antibiotic-resistant bacteria – and the genes that code for antibiotic resistance – are being discharged into the environment following the wastewater treatment process. This study will explore whether differences in wastewater treatment regimens and effluent discharge practices across Southern California affect the viability of antibiotic-resistant bacteria and the concentration of antibiotic resistant genes. This information will inform recreational water exposure and water reuse projects, including direct potable reuse.

Priorities for Future Research

***Future focus area*: Using PCR to study pathogen viability**

> SCCWRP will apply digital PCR techniques developed for pathogen viability and infectivity to wastewater in collaboration with member agencies. The goal will be to use combinations of digital PCR and integrated cell culture PCR to study the removal and inactivation of viruses. This may provide a means to quantitatively compare the effect of new treatment processes and to gauge any increases in pathogens during the treatment processes already in use. Pathogen decay models can also be applied to treated effluent to provide insight into the long-term fate of pathogens and FIB. This work will be vitally important when treated effluent becomes the primary input for direct potable reuse.

Potable Water

Potable water reuse, either indirect or direct, is expected to become a reality in southern California in the near future. To build confidence in the system, the effectiveness of treatment must be tested. Potable water poses different challenges than stormwater and treated wastewater, as there will be a search for exceedingly rare but real targets. SCCWRP is focused on ensuring a clean system can continue to operate pathogen-free, and on monitoring the system to identify problems before they can spread throughout the system.

Priorities for Future Research

The increasing popularity of direct potable reuse will require monitoring for pathogens to ensure safety and build confidence. SCCWRP will leverage molecular assays described in more detail in previous sections and the automated digital PCR system to monitor microbial water quality in distributions systems and in source waters. This could be accomplished automatically with an automated sampler designed to filter large volumes. Monitoring pathogens in the source water would provide a test for the treatment effectiveness of the water exiting tertiary treatment and the water entering drinking water treatment plants. In addition, using automated digital PCR to measure RNA from active genes of toxin-producing cyanobacteria in source water would serve as an early warning for potentially toxic drinking water.

Technology Transfer

An exemplary achievement by SCCWRP is its decades-long effort to bring molecular testing capacity to the recreational water monitoring community. As new tools become available for assessments of exposure, causation, risk and treatment effectiveness, SCCWRP will continue to spearhead the transition of these tools from the research arena into practical use by the management community. This technology transfer will generally involve three distinct stages: (1) End users acquire the technology via training, guidance documents and workshops; (2) end users enact quality control measures via intercalibration, method acceptance and certification; and (3) end users demonstrate routine usage in the field. Additionally, because cost is an important consideration in adopting technology, cost-benefit analyses can aid in selecting alternative management strategies that may involve choosing different types of technology.

Training, Guidance Documents, and Workshops

Accomplishments

SCCWRP has a long track record of successfully training member agencies to conduct microbial water-quality assessments. SCCWRP's involvement started with ushering in the use of IDEXX methods to beach water quality monitoring. SCCWRP also standardized methods to measure FIB in beach sand

(Boehm et al. 2009). More recently, SCCWRP has helped get molecular methods such as qPCR to be used for beach monitoring; additionally, qPCR is now the dominant technology to measure exposure and causal assessment. Over the years, SCCWRP has held workshops, conducted method evaluation studies, produced qPCR training packages and videos, and held three qPCR training workshops (2009, 2011, and 2013). The most recent included 14 laboratories spanning the coast from San Francisco to San Diego that were trained on qPCR methods for quantifying both general fecal (*Enterococcus* spp.) and human fecal (the HF183 human marker) contamination. SCCWRP also produced a guidance document, the California Microbial Source Identification Manual, that has been adopted in California and referenced around the world for conducting source identification studies. Additionally, in 2012, on behalf of the California Beach Water Quality Work Group and California Clean Beach Task Force, SCCWRP held a state-of-the-science workshop to disseminate current knowledge on fecal source identification and associated risk assessment tools.

Ongoing Research

***Project*: Digital PCR training**

Digital PCR is poised to become the dominant, next-generation quantification technology. With SCCWRP at the forefront of developing and validating digital PCR methods for quantifying FIB, MST markers and pathogens, SCCWRP is continuing to publish method demonstration videos and standardized digital PCR protocols, and assisting member agencies in achieving proficiency in running digital PCR assays (Cao et al. 2016a).

***Project*: Coliphage Draft EPA Method 1642**

EPA recently released a draft method for culture-based enumeration of coliphage in recreational waters. SCCWRP hosted two trainings on the new method for member agencies. SCCWRP will continue to assist member agencies in achieving proficiency in the new method through additional training as needed, in development of user-friendly protocols, and by providing excel workbooks for interpreting raw environmental data and obtaining QA/QC parameters.

Priorities for Future Research

As new tools emerge, SCCWRP will continue to develop training material and guidance documents and hold workshops. SCCWRP will work closely with method and instrument developers, member agencies, and USEPA to develop effective procedures for training the community on the latest technology for improved exposure, causal, risk and treatment effectiveness assessment. Particularly as science enters the age of next-generation sequencing and the accompanying complex bioinformatics that are needed for data analysis, more automated data analysis pipelines will need to be developed, and new strategies/formats may need to be devised for transitioning this technology from research to practical use.

Intercalibration, Method Acceptance, and Certification

Accomplishments

SCCWRP has been a world leader in conducting interlaboratory calibration studies, collecting firsthand method performance and quality assurance data that have led to method acceptance by regulatory agencies and ultimately to adoption by end users. SCCWRP was instrumental in conducting the intercalibration study that enabled regulatory acceptance of the cheaper and faster IDEXX methods (an advancement over membrane filtration) for recreational water quality monitoring in US EPA Region 9, and eventually across nation. Following a 2013 qPCR training workshop, SCCWRP led an intercalibration study that evaluated proficiency of eight Southern California laboratories that perform qPCR methods, enabling regional assessment of general and human-associated fecal contamination by qPCR in the Southern California Bight. SCCWRP also conducted intercalibration studies among research and commercial laboratories to gather performance data for benchtop digital PCR platforms and automated qPCR modules, all in preparation for technology transfer to end users.

Priorities for Future Research

***Future focus area*: Seeking regulatory approval for equivalent monitoring methods**
For end users to take advantage of faster, cheaper and better analytical methods in monitoring microbial water quality, regulatory acceptance of the equivalency of alternative methods to EPA and state-approved methods is critical. Following the technical supporting material published by EPA for approving site-specific objectives based on alternative indicators and methods, SCCWRP will lead a consortium of regulators, municipalities and laboratories to work toward approval of qPCR for beach water quality monitoring, and concurrent approval of digital PCR as an equivalent method.

***Future focus area*: Getting ELAP certification for molecular methods**
SCCWRP will collaborate with state agencies to develop ELAP method certification for molecular methods such as qPCR and digital PCR. Such an accreditation program is essential for wide adoption and for continuous quality assurance of method performance by end users.

Case Studies

Accomplishments

In 2010, SCCWRP led the first study in the nation to demonstrate successful use of qPCR for same-day (i.e., by noon) notification of beach water quality (Griffith and Weisberg 2011). This study also revealed beach water sample collection as a critical time delay for achieving rapid notification, even with faster qPCR detection technology, and thus paved the way for development of mobile and real-time monitoring technologies. Meanwhile, SCCWRP coordinated with the Southern California Bight 2013 Regional Monitoring Program to assess the feasibility of using *Enterococcus* qPCR methods to monitor general fecal contamination; the study was conducted at 36 sites by nine 9 agencies. SCCWRP also worked with Bight ’13 to use the human marker qPCR assay to assess the extent of human fecal contamination; this work was done at 29 urban drainage sites by 10 agencies.

Priorities for Future Research

SCCWRP will continue to work with member agencies to identify projects that will push the science forward and apply the latest technologies and techniques at locations that will provide useful test cases for beach managers and regulators.

Cost-Benefit Analyses

Priorities for Future Research

SCCWRP will work with an environmental economist to use the data generated by the previous studies and those outlined in the exposure, causal, and risk assessment and treatment effectiveness sections of this document to construct the biological data for wet-weather TMDL cost-benefit analyses. The models created and proposed in the risk and causal assessment sections, combined with the data generated from measuring infectivity and viability and treatment effectiveness, will be offered to explore the benefits associated with different treatment or mitigation options. This will provide the information necessary for managers and regulators to make policy decisions backed by scientific data that have the biggest impact possible.

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