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# 2017 Five-Year Review of the 2012 Recreational Water Quality Criteria

**U.S. Environmental Protection Agency**
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# Acronyms

| | |
|---|---|
| AMR | antimicrobial resistence |
| AMRB | antimicrobial resistant bacteria |
| ARG | antimicrobial resistant genes |
| AWQC | ambient water quality criteria |
| BAV | Beach Action Value |
| BEACH | Beaches Environmental Assessment and Coastal Health |
| CAFO | concentrated animal feeding operation |
| CAWS | Chicago Area Waterways System |
| CCE | calibrator cell equivalent |
| CDC | Centers for Disease Control and Prevention |
| cfu | colony forming unit |
| CHEERS | Chicago Health, Environmental Exposure, and Recreation Study |
| CRE | carbapenem-resistant Enterobacteriaceae |
| Ct | cycle threshold |
| CWA | Clean Water Act |
| D-HFUF | dead-end hollow fiber ultrafiltration |
| DNA | deoxyribonucleic acid |
| EMM | Environmental Master Mix |
| EnDDaT | Environmental Data Discovery and Transformation |
| EPA | Environmental Protection Agency |
| ESBL | extended-spectrum β-lactamase |
| FIB | fecal indicator bacteria |
| FSI | fecal source identification |
| GBM | generalized boosted modeling |
| GI | gastrointestinal |
| GM | geometric mean |
| HAB | hazardous algal bloom |
| HCGI | highly credible gastrointestinal illness |
| HGT | horizontal gene transfer |
| hr | hour |
| IEM | integrated environmental modeling |
| IFTAR | Interagency Task Force on Antimicrobial Resistance |
| L | liter |
| mL | milliliter |
| MLR | multiple linear regression |
| MRSA | methicillin-resistant *Staphylococcus aureus* |
| MST | microbial source tracking |
| NEEAR | National Epidemiological and Environmental Assessment of Recreational Water |
| NGI | NEEAR-GI illness |
| NIST | National Institute of Standards and Technology |
| NPDES | National Pollutant Discharge Elimination System |
| ORD | Office of Research and Development (U.S. EPA) |
| PCR | polymerase chain reaction |

| | |
|---|---|
| pdu | PCR-detectable units |
| PLS | partial least squares |
| QMRA | quantitative microbial risk assessment |
| qPCR | quantitative polymerase chain reaction |
| RfD | reference dose |
| RNA | ribonucleic acid |
| RT-PCR | reverse transcriptase-polymerase chain reaction |
| RT-qPCR | reverse transcriptase-quantitative polymerase chain reaction |
| RWQC | Recreational Water Quality Criteria |
| SAL | single-agar layer |
| SCCWRP | Southern California Coastal Water Research Project |
| SSM | single sample maximum |
| STV | statistical threshold value |
| TMDL | Total Maximum Daily Load |
| TSA | temporal synchronization analysis |
| UCB | University of California, Berkeley |
| UIC | University of Illinois Chicago |
| UNC | University of North Carolina |
| U.S. | United States |
| USGS | United States Geological Survey |
| VB | Virtual Beach |
| WQS | water quality standards |
| WWTP | wastewater treatment plant |

# Executive Summary

The United States (U.S.) Environmental Protection Agency (EPA) has conducted a five-year review of its 2012 Recreational Water Quality Criteria (RWQC), as required by the Beaches Environmental Assessment and Coastal Health (BEACH) Act amendments to the Clean Water Act (CWA) section 304(a)(9)(B). In conducting this review, the EPA considered several factors, including the availability and evaluation of new science, the review of information related to the underlying science used to develop the 2012 RWQC, additional implementation support needs, and perceived barriers to state adoption.

An important goal of this review and report is to document the assessment of whether revisions to the 2012 RWQC are necessary. The EPA's review included compiling the relevant scientific information published since 2010, gathering updated information on recreational criteria implementation tools and summarizing the information received from implementers of recreational water quality monitoring and improvement programs across the country. The EPA also conducted outreach to the recreational water quality community in the course of this review.

The report contains extensive information in each of the topic areas, and the conclusions derived from the report are summarized below.

## Science Review

**Health Studies.** Findings on health studies are generally consistent with the findings of studies that formed the basis for the 2012 RWQC, and enhance the depth and strength of the evidence underlying the RWQC. A growing body of evidence suggests that children can be disproportionately susceptible to health effects resulting from exposure to pathogens in recreational waters. There are opportunities for further resolution of epidemiological relationships, especially in the area of children's health protection and wider application of *Enterococcus* spp. qPCR.

Priorities for Further Work: Re-analysis of epidemiological data to assess potential differences in risk to children. Re-analysis of *Enterococcus* spp. qPCR data for consideration in criteria development, especially to address effluent sources. Also, evaluate how QMRA can be used to address risk to children from swimming exposure, and other regulatory purposes.

**Coliphage as an indicator.** Because evidence strongly suggests most illnesses in recreational waters are due to enteric viruses, development and implementation of viral indicators, such as coliphage, may yield advances in public health protection.

Priorities for Further Work: Completion and publication of coliphage methods and development of coliphage-based RWQC for inclusion into the "tool box."

**Indicators and Performance of qPCR Methods.** The advances in qPCR methodology since 2010 have brought greater reliability and utility to beach monitoring programs where they have been implemented, yet opportunities remain for further refinement of qPCR methodologies.

*Enterococcus* spp. measured by qPCR is a better predictor of swimming-associated GI illness and more timely than current culturable bacterial indicators. These factors coupled with a greater distribution of qPCR-capable laboratories in the future could lead to enhanced public health protection if implemented under the current criteria.

Priorities for Further Work: Completion of method validation and publication for the *E. coli* qPCR method (*Draft* Method C), development of alternative site-specific criteria for *Draft* Method C, additional training and capacity-building in qPCR laboratories in states, tribes, and localities.

**Microbial Source Tracking.** Accurate and reliable MST technologies could markedly improve future water quality management in the U.S., possibly allowing for the development of alternative site-specific criteria based on pollution sources present, strategic remediation planning based on fecal pollution levels from human sources. Use of alternative water quality metrics, such as human-associated MST technologies, may also be helpful to inform public health risk levels under wet weather conditions.

Priorities for Further Work: Completion and publication of standardized methods for EPA human-associated MST methods (HF183/BacR287 and HumM2) and completion of a DNA reference material development with NIST. Development and validation of virus-based human fecal source identification procedures. Further investigation of MST application in recreational water quality management settings such as prioritizing polluted sites for remediation based on human waste levels, identification of non-point pollution sources, and the development of alternative water quality metrics based on wet and dry weather scenarios.

**Antimicrobial Resistance.** The complex issue of antimicrobial resistance is becoming of increasing interest, creating a demand for more data to both inform our understanding of the forces driving this resistance and the actions needed to preserve bacterial susceptibility to our first-line medications. There is an increasing body of literature available on the environmental occurrence of AMRB/ARG and potential exposure in recreational waters. To develop a more complete picture regarding the threat and risks associated with antibiotic resistance, research is needed to better understand the role the environment plays in transferring AMRB/ARG to primary contact recreators. For example, additional research is needed on the incidence, associated risks, and transfer mechanisms in recreational waters, as well on the removal of AMRB/ARG by wastewater treatment processes. The EPA is in the early stages of developing a broader surveillance strategy and looking for meaningful opportunities to improve human health relating to exposures to AMRB/ARGs.

Priorities for Further Work: Development and standardization of AMRB/ARG detection methodologies. Collect information on the occurrence of AMRB/ARG in environmental waters, wastewater influent/effluents, and other potential reservoirs. Develop wastewater treatment and disinfection processes for AMRB/ARG targets. Characterize potential associated public health risks and mitigation strategies.

## Implementation Review

Although not essential in terms of their association with current or potentially revised RWQC, implementation activities are crucial to applying the advancing science to protect public health in recreational waters.

**Sanitary Surveys.** Sanitary Surveys continue to serve as an important tool for informing site remediation, characterizing waters for QMRA and site-specific criteria development, and can be linked with integrated environmental modeling.

Priorities for Further Work: Conversion of current marine sanitary survey tablet-based application to a web-based application, additional outreach on available sanitary survey applications, collaboration with Great Lakes beach programs on fresh water sanitary survey application and opportunities for integration with environmental modeling.

**Predictive/Statistical Modeling.** Predictive models offer states, territories, and tribes an alternative for same-day notification and resulting public health protection with lower capital investment and unit costs than other rapid methods.

Priorities for Further Work: Additional support to develop predictive models in marine environments as well as models paired with newer indicators such as qPCR-based indicators.

**Deterministic Process Modeling for Recreational Beach Site Assessment and Enhancement/Remediation.** These models provide a means of understanding physical forces influencing the movement of contaminants for problem definition and remediation and can include QMRA health-based models to develop site-specific criteria or evaluate remediation.

Priorities for Further Work: Development of additional training and tools to make process models and integrated environmental modeling more accessible to states, tribes and other interested stakeholders.

**Quantitative Microbial Risk Assessment (QMRA).** QMRA can enhance the interpretation and application of new or existing epidemiological data by characterizing various exposure scenarios, interpreting potential etiological drivers for the observed epidemiological results, and accounting for differences in risks posed by various sources of fecal contamination. Progress since 2010 includes new QMRA software infrastructure developed to provide risk estimates within a standard microbial watershed assessment.

Priorities for Further Work: Development of additional training and tools to make QMRA models more accessible to states, tribes and other interested stakeholders. Completion and publication of remaining QMRA guidance.

**Criteria Implementation: Adoption Status and Perceived Barriers.** The 2012 RWQC include many new elements that strengthen overall health protection in recreational waters and promote more consistent implementation. Many states that have some, but not all, of the elements of the

2012 RWQC in their water quality standards have been reluctant to adopt the new criteria due to the initial administrative burden associated with rulemaking and other resource concerns.

Priorities for Further Work: Continued funding of BEACH Act grants. Consider additional implementation guidance and explore reconsideration of addressing differences based on frequency of use.

## Cyanotoxins in Recreational Water

Recreators exposed to cyanotoxins in ambient recreational waters are at risk. The EPA is working to develop human health recreational ambient water quality criteria or swimming advisories for microcystins and cylindrospermopsin. The EPA expects to revise and publish a final criteria document in 2018.

Priorities for Further Work: Completion and publication of recreational criteria for the cyanotoxins, microcystins, and cylindrospermopsin.

## Assessment of the Need to Revise the 2012 RWQC

Based on the review of the existing criteria and developments in the available science described in this report, and consistent with CWA section 304(a)(9)(B), the EPA has decided not to revise the 2012 Recreational Water Criteria during this review cycle. The Agency believes, however, that further research and analysis as identified in this Report will contribute to the EPA's future review of the 2012 RWQC. The EPA will work with the environmental public health community as the Agency moves forward with its research efforts. The use of qPCR and ongoing research in methods and indicators continue to strengthen and augment the tools available to support the current criteria.

# I. Introduction

The United States (U.S.) Environmental Protection Agency (EPA) has conducted a 5-year review of its 2012 Recreational Water Quality Criteria (RWQC), as required by the Beaches Environmental Assessment and Coastal Health (BEACH) Act amendments to the Clean Water Act (CWA) section 304(a)(9)(B). In conducting this review, the EPA considered several factors, including the availability of new science and evaluation of the underlying science used to develop the 2012 RWQC, additional implementation support needs, and perceived barriers to state adoption. The Agency used the information in this "state-of-the science" report to assess whether new or revised RWQC are necessary at this time.

The development of the 2012 RWQC and this review are both requirements of the BEACH Act of 2000, which has provided grants to states, territories, and tribes to implement water quality monitoring and notification programs for coastal recreation waters[1] (including the Great Lakes) since 2002. The 2012 RWQC included development of a beach advisory threshold for use in posting swimming advisories and the ambient water quality criteria (AWQC) for use in a variety of other CWA programs (e.g., deriving National Pollutant Discharge Elimination System [NPDES] permits). Advisory decisions based on water quality monitoring are intended to reduce the risk to recreators and other users of these waters from illness associated with exposure to human fecal contamination and provide the public with information to make decisions about their actions. AWQC that are developed under CWA section 304(a) are recommendations on the latest science, which states and authorized tribes can adopt as part of their water quality standards (WQS). In the case of the 2012 RWQC, the EPA's recommendations were designed to protect primary contact recreational waters, not just coastal recreation waters. It is important to note that Congress required states and authorized tribes with coastal recreation waters to adopt new or revised WQS addressing pathogens in such waters within 36 months of the EPA's publication of the 2012 RWQC (CWA section 303(i)(1)(B)). The criteria, once adopted by states and authorized tribes and approved by the EPA under CWA section 303(c), become part of the regulatory structure of the state/authorized tribe and are intended to protect primary contact uses for the applicable waters. The recreational criteria values that are part of a state's or authorized tribe's approved WQS have a direct bearing on the issuance of NPDES discharge permits, waterbody assessments, the decisions regarding attainment of WQS under CWA sections 303(d) and 305(b), and the development of targets for Total Maximum Daily Loads (TMDLs) for restoring impaired waters.

[1]The BEACH Act of 2000 defines coastal recreation waters as follows:
The term 'coastal recreation waters' means—
(i) the Great Lakes; and
(ii) marine coastal waters (including coastal estuaries) that are designated under section 303(c) by a State for use for swimming, bathing, surfing, or similar water contact activities.
The term 'coastal recreation waters' does not include—
(i) inland waters; or
(ii) waters upstream of the mouth of a river or stream having an unimpaired natural connection with the open sea.

The criteria values specified in the RWQC are for densities of culturable fecal indicator bacteria (FIB) in water. The FIB, enterococci and *Escherichia coli* (*E. coli*), are not pathogenic under usual circumstances, but their presence in water above specified levels can indicate the presence of viral, bacterial, or protozoan pathogens associated with an elevated risk of illness. Therefore, ensuring that the RWQC are consistent with the current state of the science and are protective of human health is key to protecting the health of users of all waters designated for primary contact recreation.

The EPA identified the following objectives for this review of the 2012 RWQC:

- Inventory and evaluate health study information published since 2010 on public health impacts associated with exposure to fecal contamination in recreational waters.
- Review the 2012 RWQC based on internal EPA input on the science, taking into consideration feedback from the greater beach water quality community and stakeholders.
- Identify additional indicators and methods, including those that have become more refined or feasible since the issuance of the 2012 criteria, and assess their applicability for predicting potential adverse human health effects from recreational exposure.
- Provide information on the state of the science with respect to source tracking methods, sanitary survey design, predictive modeling for both fresh and marine waters, and other implementation tools.
- Include the latest science and information pertaining to the development of other criteria, such as coliphage and cyanotoxin criteria, that have the potential to protect recreational uses.
- Assess factors affecting state/authorized tribe adoption of the RWQC including perceived barriers to adoption and how states have implemented the criteria to meet their specific circumstances.

An important goal of this review and report is to document the basis for the assessment of whether revisions to the 2012 RWQC are necessary. That assessment was based on the overall review findings described later in this report, including internal EPA evaluation of the latest science, input from the greater beach water quality community, state agency representatives, and feedback from other stakeholders.

## II. Background – 1986 Criteria

FIB densities have long served as the surrogate measure of fecal contamination and, by inference, the presence of pathogens commonly associated with fecal material. The 1986 Criteria relied on a series of epidemiological studies that the EPA conducted in the late 1970s and early 1980s to evaluate culturable indicators of fecal contamination and illness in swimmers. These studies included *E. coli*, enterococci, and fecal coliforms, which had been the basis of recreational criteria recommendations before 1986. These epidemiological studies showed that enterococci are good predictors of gastrointestinal (GI) illnesses in fresh and marine recreational waters, *E. coli* is a good predictor of GI illnesses in fresh waters, and fecal coliforms were poor predictors of GI illness (Cabelli et al., 1982; Cabelli, 1983; Dufour, 1984).

**Table 1. The 1986 Criteria Provided Geometric Mean (GM) and Single Sample Maximum (SSM) (75th %ile) Values**

| | GM (cfu[b]/100 mL) | SSM[a] (cfu/100 mL) | Illness Rate n/1,000 |
|---|---|---|---|
| *In Fresh Waters* | | | |
| Enterococci | 33 | 61 | 8 |
| *E. coli* | 126 | 235 | 8 |
| *In Marine Waters* | | | |
| Enterococci | 35 | 104 | 19 |

[a] The 1986 Criteria also provided SSM values for three other lower intensity levels of beach use.
[b] cfu = colony forming unit.

One of the stated goals of the BEACH Act of 2000 was to move beyond the perceived limitations of the EPA's previously recommended 1986 *Ambient Water Quality Recreational Criteria for Bacteria* (the 1986 Criteria) in place at the time. The lag between sample collection and the receipt of analytical results was considered a potential impediment to public health protection, and the BEACH Act of 2000 envisioned "improving detection in a timely manner in coastal recreation waters of the presence of pathogens that are harmful to human health" (106th Congress of the United States).

### A. State of the Science in 2000–2010

The EPA planned and subsequently conducted epidemiological investigations at U.S. beaches in 2003, 2004, 2005, 2007, and 2009, known collectively as the National Epidemiological and Environmental Assessment of Recreational Water (NEEAR) study. The NEEAR study enrolled 54,250 participants, encompassed nine locations, and collected and analyzed numerous samples from a combination of freshwater, marine, tropical, and temperate beaches (U.S. EPA, 2010c; Wade et al., 2008, 2010). Health studies were also conducted by other entities during the period, such as the Southern California Coastal Water Research Project (SCCWRP), but not all were published prior to the development of the RWQC (Colford et al., 2007; Till et al., 2008; Marion et al., 2010; Sinigalliano et al., 2010).

The EPA also held a 5-day scientific workshop in 2007 to obtain a broad range of external scientific input to support the development of the 2012 RWQC. The report from this workshop, *Report of the Experts Scientific Workshop on Critical Research Needs for the Development of New or Revised Recreational Water Quality Criteria* (U.S. EPA, 2007a), served as the scientific roadmap for new 2012 RWQC and implementation guidance. The EPA used the report from the Experts Scientific Workshop to develop the *Critical Path Science Plan for the Development of New or Revised Recreational Water Quality Criteria* (U.S. EPA, 2007b), which was externally peer reviewed. The EPA completed 32 projects to inform the development of the 2012 RWQC. The 2012 RWQC document (U.S. EPA, 2012a) lists these projects and provides a description of the science used to develop the elements of the 2012 RWQC including:

- Epidemiological studies and quantitative microbial risk assessments (QMRAs)
- Site characterization studies
- Indicators/Methods development and validation studies
- Refining and validating both EPA and other models for fresh and marine beaches
- Developing recommended levels of public health protection.

## B. Key Points of the 2012 RWQC

The 2012 RWQC use enterococci and *E. coli* as predictors of GI illnesses in recreational waters, and include eight major elements, described below.

### 1. Magnitude, Duration, and Frequency: Geometric Mean and Statistical Threshold Value

The 2012 RWQC consist of three primary components: magnitude, duration, and frequency.

***Magnitude:*** The magnitudes of the bacterial indicators are the measured densities of the FIB from the water quality density distribution used for the criteria, expressed both as a geometric mean (GM-50th percentile value) and as a statistical threshold value (STV-90th percentile value).

***Duration:*** The duration is the period over which excursions of the magnitude values are recorded and calculated. The EPA recommended a duration of 30 days in the criteria for both the GM and the STV.

***Frequency:*** The frequency is how often the GM or the STV are exceeded. The EPA recommended no exceedances for the GM over the period of the duration.

Because the STV reflects the 90th percentile of the distribution of values used to determine the RWQC, the RWQC allowed for a 10-percent exceedance of the STV (1 in 10 samples). The EPA selected the estimated 90th percentile of the water quality distribution to account for the expected

variability in water quality measurements, while limiting the percentage of samples allowed to exceed the STV as a threshold of water quality impairment.

The EPA was clear that "both the GM and the STV would be part of the WQS, and, therefore both targets would be used to determine whether a waterbody attains the WQS for primary contact recreation" (U.S. EPA, 2012a).

**Table 2. 2012 RWQC Recommended GM and STV Values for 36 and 32 Illnesses/1,000 Recreators (NEEAR-GI Illness [NGI]) for Marine and Fresh Waters**

| Criteria Elements | Estimated Illness Rate (NGI): 36 per 1,000 Primary Contact Magnitude | | | Estimated Illness Rate (NGI): 32 per 1,000 Primary Contact Magnitude | |
|---|---|---|---|---|---|
| **Indicator** | **GM (cfu/100 mL)[a]** | **STV (cfu/100 mL)[a]** | **OR** | **GM (cfu/100 mL)[a]** | **STV (cfu/100 mL)[a]** |
| Enterococci – marine and fresh water | 35 | 130 | | 30 | 110 |
| **OR** | | | | | |
| *E.coli* – fresh water | 126 | 410 | | 100 | 320 |

**Duration and Frequency:** The waterbody GM should not be greater than the selected GM magnitude in any 30-day interval. These should not be greater than a 10-percent excursion frequency of the selected STV magnitude in the same 30-day interval.

[a] The EPA recommends using EPA Method 1600 (U.S. EPA, 2002a) to measure culturable enterococci, or another equivalent method that measures culturable enterococci, and using EPA Method 1603 (U.S. EPA, 2002b) to measure culturable *E.coli* or any other equivalent method that measures culturable *E.coli*.

### 2. NEEAR Gastrointestinal Illness Rate

The EPA's use of the NGI definition for illness rate in the 2012 RWQC reflected a change in the GI definition of illness to capture a broader range of milder symptoms compared to the definition the EPA used as the basis for the 1986 Criteria (highly credible gastrointestinal illness or HCGI). Whereas HCGI required fever along with gastrointestinal symptoms to be considered a case, fever was not required for NGI. The equivalent rate of occurrence of NGI is approximately 4.5 × HCGI, so that the comparable base illness rate in the 2012 RWQC is 36 illnesses/1,000 swimmers vs. 8 illnesses/1,000 swimmers in the 1986 Criteria (Wymer et al., 2013). The 36 illnesses/1,000 NGI does not represent an increase in risk of illness over the 8 illnesses/1,000 HCGI, but has led to an incorrect perception of an increase in some instances (NRDC, 2014).

### 3. The 2012 RWQC Includes Two Sets of Recommended Criteria Values

Criteria values were provided for culture- and quantitative polymerase chain reaction (qPCR)-enumerated FIB at two illness rates, 32 and 36 illnesses per 1,000 swimmers (NGI illness rate). Based on the EPA's analysis of the available information, either set of thresholds protects the designated use of primary contact recreation and, therefore, protects the public from the risk of exposure to harmful levels of pathogens from fecal contamination. The two sets of numeric concentration thresholds included in the 2012 RWQC provide states and authorized tribes flexibility to make risk-management decisions based on local conditions.

### 4. No Marine/Fresh Water Illness Rate Differential

The recommendations for criteria illness rate are consistent for both marine and fresh waters, which was not the case for the 1986 Criteria.

### 5. A Single Level of Beach Use

The 1986 Criteria included four SSM values appropriate for different levels of beach use intensity corresponding to the 75th, 82nd, 90th, and 95th percentiles of the distribution of values from the water quality sampling distributions observed in the EPA's epidemiological studies. In the 2012 RWQC, the Agency removed those use intensity recommendations. Accordingly, the 2012 RWQC includes criteria values for two different illness rates, but a single level of beach use intensity. For further discussion of the elimination of the use intensity values in the 1986 Criteria for the 2012 RWQC, please refer to Section 3.6.1 in the 2012 RWQC document (U.S. EPA, 2012a).

### 6. Beach Action Values

In addition to recommending criteria values, the EPA also provided states and authorized tribes with Beach Action Values (BAVs) for use in notification programs. The BAV was defined as the 75th percentile of the water quality distribution of values of *E. coli* and *Enterococcus* spp. in the epidemiological studies. The EPA's intent was to provide the BAV for states and authorized tribes as a precautionary tool for beach management decisions. The EPA recommended the BAVs as beach notification values for adoption by the states in their public health programs, but not as part of the 2012 RWQC recommendations under CWA section 304(a).

### 7. qPCR Rapid Quantitation Methods

The EPA developed and validated a molecular testing method using qPCR as a rapid analytical technique for the detection and quantitation of enterococci in recreational water (EPA Method 1611). The EPA included qPCR-based values for the GM, STV, and BAV for both illness rates in the 2012 RWQC document. Due to potential matrix interference issues in water types other than those studied at the NEEAR effluent-affected beach sites, the EPA encouraged states and authorized tribes to conduct a site-specific assessment of the local appropriateness of qPCR before using this method for purposes of beach monitoring.

### 8. More Tools for Assessing and Managing Recreational Waters

The EPA provided additional information on tools for evaluating and managing recreational waters, such as predictive modeling and sanitary surveys, and stressed the need for a tiered approach to developing beach monitoring plans in the *2014 National Beach Guidance and Required Performance Criteria for Grants*. The Agency also provided Technical Support Materials for developing site-specific criteria and for adopting the use of alternative indicators or methods at recreational beaches.

## III. Scope and Methods of the Review

This section describes the measures the EPA has taken to assess advances in the state of the science supporting the 2012 RWQC since 2010 and the process of its implementation. The measures include an inventory of the relevant scientific information published since 2010, a description of recreational criteria implementation tools applied at recreational settings, information on sources of information and how information was accessed, and a summary of information received from implementers of recreational water quality monitoring and improvement programs across the country.

### A. Inventory of Scientific Information Published Since 2010

A thorough inventory of scientific information published since 2010 for topics central to recreational waters monitoring and assessment is the core of this review. Three general categories of relevant information were identified:

i. Performance and Implementation of qPCR Methods for FIB 2010 to present
ii. Health Studies, including epidemiological studies, refinement of analyses of data from previous studies, and the application of QMRA to water quality data and complex settings at recreational beaches
iii. Microbial source tracking (MST), including human and non-human fecal source markers and tracking.

### B. Recreational Criteria Implementation Tools

A further category of activities and tools related to water quality monitoring and contextual assessment of beach settings was identified as highly relevant to the implementation of the BEACH Act and activities related to the 2012 RWQC. This category of implementation tools includes:

i. Sanitary surveys and watershed assessments
ii. Statistical approaches for predictive estimates of water quality
iii. Deterministic modeling for recreational beach site assessment, enhancement, and remediation of adverse infrastructure impacts to sites.

### C. Sources of Information and How Information Was Accessed

The collection and analysis of information in each of these categories included accessing post-2010 information from three broad sources:

- EPA recreational water research and publications relating to that research
- External (non-EPA) academic research conducted by researchers at academic institutions and government organizations that have focused on recreational water activities and science related to the BEACH Act

- Implementers of recreational water quality monitoring and improvement programs across the country, including the EPA Regional Beach Program coordinators and state, municipal, and county officials in health and environmental agencies who often are direct or indirect recipients of BEACH Act grant funds or whose activities those funds leverage.

## D. How the Assessment Was Conducted

### 1. EPA Recreational Water Research

For this review, offices within the EPA inventoried recreational water research. This information is presented along with other information in Section IV below.

### 2. A Systematic Review of Available Peer Reviewed Literature

The EPA performed systematic searches of the peer-reviewed literature for articles pertaining to qPCR Methods for FIB; health studies, including epidemiological studies of recreational water contact activities; the application of QMRA to water quality data and complex settings at recreational beaches; and human and non-human fecal source markers and tracking (MST). Multiple sets of search terms applicable to the topic were applied to references in Web of Science and PubMed (http://www.ncbi.nlm.nih.gov/pubmed). Abstracts were screened for relevance to the scope of the search. The literature search was limited to English-language, peer-reviewed citations, published between 2010 and March 2017. Following the abstract screening, the full text of articles passing scope was reviewed for specific information related to each topic. Search terms and databases searched are provided in Appendix A.

For qPCR methods, the literature search returned 337 unique results, of which 54 were relevant based on the abstract screening. An additional 13 studies were identified through other sources (e.g., cited in another paper). For the qPCR methods review, 32 studies were summarized. For the health studies, the literature search returned 2,018 unique results, of which 98 were relevant based on the abstract screening (15 of these were then excluded based on the full text review). An additional 23 studies were identified through other sources (e.g., cited in another paper). For the health study review, 106 studies were summarized. Results of the systematic reviews are included in Appendix A and Appendix C.

### 3. Supplemental Review of Relevant Materials by the EPA

The EPA reviewed literature resulting from the systematic searches and from materials available from other sources such as technical documents from states and the United States Geological Survey (USGS). Summaries of this review are included in Section IV.

## E. Collection of Information from Practitioners, Academics, and Stakeholders Involved in Beach Monitoring

The EPA conducted informal interviews with recreational water public health practitioners; members of the academic community, particularly those with expertise in methods and epidemiology; and federal, state, and local government officials. Topics were discussed

according to the role those individuals played in applying the RWQC, for example, state implementer or local beach practitioner of beach program management. In addition, recent issues, events, and trends in recreational water science were discussed with academic scientists. The EPA also held discussions with other stakeholders.

The EPA interviewed practitioners from SCCWRP, the State of Michigan Department of Health, the City of Racine Wisconsin Department of Health, and USGS science centers in the Great Lakes region. EPA staff also spoke with researchers from the University of South Florida, University of North Carolina, University of California – Davis, University of Miami, University of Puerto Rico, and University of Hawaii.

The role of the EPA Beach Program Coordinators in the eight EPA regional offices with BEACH Act Programs (Regions 1–6, 9, and 10) is central to the ongoing operation, funding, and technical support of state, territorial, and tribal beach monitoring programs: The EPA Beach Program coordinators provide technical advice and oversee the BEACH Act grants for the qualifying entities within their region. Responsible state, tribal, and territorial agency contacts and managers in those regions not only coordinate and operate monitoring and advisory programs, but also move regulatory actions pertaining to the adoption of the criteria at the state or tribal level through the their respective regulatory and, in many cases, legislative processes. The EPA invited the Beach Program Coordinators and the respective states, tribes, and territories to discuss the 2012 RWQC, their implementation, and the quality of experiences they had implementing the RWQC.

The EPA conducted outreach to address the interests of various sectors of the recreational water stakeholder community. In addition to informal outreach to trade associations and non-governmental organizations that were key stakeholders in the development of the 2012 RWQC, The EPA held a public webinar in July 2017 on the review for any interested stakeholders. Participants included stakeholders from across the spectrum of environmental, industry, local government, and public health stakeholder groups. The webinar provided an overview of the review the EPA has undertaken and enabled stakeholders to provide input on the topics included in the review. The EPA communicated some of the initial findings of the review of the science and the timeline for completing the review.

# IV. Findings of the Review

## A. Inventory and Evaluation of Recreational Water Information

### 1. Introduction

Between 2002 and 2009, the EPA conducted a series of epidemiological studies at beach sites across the United States and Puerto Rico, collectively known as the National Epidemiologic and Environmental Assessment of Recreational (NEEAR) Water Studies. These studies were a collaboration between the EPA and the Centers for Disease Control and Prevention (CDC). The studies were designed to address amendments to the CWA known as the BEACH Act. The BEACH Act includes requirements for the EPA to study new, more rapid measures of fecal contamination in recreational waters and their associations with health effects among beach-goers, including non-gastrointestinal effects such as respiratory illness, skin rash, eye irritation, and ear infection.

The 2012 Recreational Water Quality Criteria were based on literature published before 2010. Research that EPA investigators have contributed to since 2010 has focused primarily on publications based on the NEEAR data and publications that used combined datasets from the NEEAR study and similar studies. These studies were conducted by the University of California, Berkeley (UCB); SCCWRP; and the University of Illinois Chicago's (UIC) Chicago Health, Environmental Exposure, and Recreation Study (CHEERS). Although the EPA did not lead most of these studies, the Agency made significant contributions, including providing data and assistance in interpretation, analysis, and publication of the studies. Additionally, as part of the EPA's 5-year review of the 2012 RWQC, the EPA completed the *Expert Consultation Report,* summarizing health studies published from 2010 to 2017, which included EPA and non-EPA epidemiological studies, exposure assessments, and quantitative microbial risk assessments (QMRAs) (Appendix C). This Chapter summarizes results from health studies by topic area: water ingestion and children, coliphage, additional alternative indicators, etiologic agents, tropical waters, non-point sources, wet weather, health burden, and non-enteric illnesses.

### 2. Water Ingestion and Children

A growing body of scientific evidence suggests that children can be disproportionately susceptible to health effects from pathogen exposures in ambient waters compared to adults. The risk differential could be due to one or more of the following: 1) children's immunological, digestive, and other bodily systems that are still developing; 2) children's greater exposure because they ingest more water and breathe more air in proportion to their body weight than adults; and 3) children's behavior, such as increased time spent in water and more vigorous activity, that might result in increased exposure in comparison to adults.

Historically, risk assessors have had limited data for evaluating children's potential exposures and health outcomes relative to adults as a result of exposure to fecal pathogens found in contaminated recreational waters. Few epidemiological studies and microbial risk assessments have explored child-specific risks from microbial contaminants found in water, although this is changing in recent years. The EPA identified four publications based on three studies published

since 2010 that evaluated and reported incidental ingestion while recreating that included children. These studies are summarized in subsequent paragraphs.

In 2006, the EPA conducted a pilot study to quantify the rate of water ingested among pool swimmers (including children six years and older). Water ingestion was quantified by measuring cyanuric acid in the pool water before swimming and in the urine after swimming (Dufour et al., 2006). This study was expanded to include a larger sample with a wider range of ages beyond that of the pilot study, and those results were published in 2017 (Dufour et al., 2017). The main findings built on the previous study and demonstrated that children between six and 10 years of age swallowed more water than adults, and male adults swallowed more water than female adults. In both Dufour studies, the actual amount of time spent swimming in water was a strong predictor of the volume of water ingested (Dufour et al., 2006, 2017).

To develop additional estimates of the volume of water ingested per swimming event considering both rate of ingestion and time spent in the water, the EPA applied data distributions of age, gender and time spent in the water from the over 60,000 observations at 12 freshwater and marine beaches in the combined NEEAR/UCB data set (excluding tropical beaches). Age- and gender-specific rates of ingestion from both Dufour studies were combined with these data in a simulation study to develop detailed, age-specific estimates of the volume of water ingested per swimming event (DeFlorio-Barker et al., 2017a). The authors reported that children (aged 6–12 years) swallow a median of 36 mL (90th percentile = 150 mL) of water, while adults aged 35 years and older swallow 9 mL (90th percentile = 64 mL) per swimming event, with male children swallowing more water compared to female children of the same age.

A study by Schets et al. (2011) provides incidental ingestion volumes for children aged 0 to 14 years in different types of waters based on surveys of parents' estimates of the amount their children incidentally ingested. Of the 8,000 adults who completed the questionnaire, 1,924 additionally provided estimates for their eldest child (<15 years of age). On average, depending on the water type, children and adult men ingested at a greater rate than women. For example, in swimming pools, children (38 mL/hour [hr]) ingested at a greater rate than adults (males 30 mL/hr; females 21 mL/hr). The exposure rates were not adjusted for body weight.

Like Dufour et al. (2017), Suppes et al. (2014) used cyanuric acid as an indicator of pool-water ingestion to evaluate the rate of water ingested by 16 children aged five to 17 years. They found children, on average, ingested pool water at a higher rate than adult participants. Total time in water, quantified by viewing videos, was used to adjust pool-water ingestion volumes to obtain rates. After adjustments for false-positive measurements were applied, the mean rate at which adults ingested water was 3.5 mL/hr (range 0–51 mL/hr). The mean rate at which children ingested water was 26 mL/hr (range 0.9–106 mL/hr).

In addition to greater exposure, the EPA NEEAR study provided some evidence that children were at a greater risk of swimming-associated illness following exposures to fecally-contaminated recreational water (Wade et al., 2008). Using the combined NEEAR/UCB data set representing over 80,000 observations from 13 beach sites, UCB researchers led an additional

analysis to provide summary estimates of gastroenteritis risks and illness burden associated with recreational water exposure and determine whether children have higher risks. Participants were classified as non-swimmers, swimmers below culturable enterococci criteria and swimmers above culturable enterococci criteria (U.S. EPA, 2012a). Authors concluded that children aged 0–4 and 5–10 years had the most water exposure, exhibited stronger associations between levels of water quality and illness, and accounted for the largest attributable illness burden (Arnold et al., 2016).

Several other studies also evaluated the risk to children in the beach environment. Cordero et al. (2012) characterized the variation in the risk of GI illness at an urbanized tropical beach during the dry and rainy seasons. Enterococci were below the water quality standard during the study, but were higher in the autumn rainy season. GI illness was reported more often during the rainy season compared to the dry season and a much higher risk of GI illness occurred among children <5 years of age compared to other age groups (Cordero et al., 2012).

Lamparelli et al. (2015) conducted a prospective-cohort epidemiological study at five beaches in Sao Paulo, Brazil affected by human sewage. At all five beaches, children ≤10 years of age had increased incidence of GI illness compared to recreators >10 years of age. Rates of GI illness among children ≤10 years of age ranged from approximately 10 to 20% at the five beaches. The pattern of elevated enterococci and elevated illness incidence across the five beaches, however, was inconsistent.

Sanborn and Takoro (2013) identified children younger than five years as being a high risk group for illness from recreational water exposure, especially if they have not been vaccinated for Rotavirus. de Man (2014) noted markedly higher risks of infection with agents of GI illness per flood event in urban floodwaters for children, relative to adults exposed to the same waters that were contaminated variously with *Giardia* spp. (35%, 0.1–142 cysts/liter [L]), *Cryptosporidium* (30%, 0.1–9.8 oocysts/L), Noroviruses (29%, $10^2$–$10^4$ PCR-detectable units [pdu]/L) and Enteroviruses (35%, $10^3$–$10^4$ pdu]/L). Although not comparable to recreational water exposure in the density ranges of thresholds of the RWQC, these findings underscore the contrast between adult and children's illness rates in a given setting.

In summary, increased water ingestion among children documented by Dufour et al. (2017) and DeFlorio-Barker et al. (2017a) support the epidemiological evidence from Arnold et al. (2016) and Wade et al. (2008) that children are more highly susceptible to swimming-associated GI illness, likely in part due to increased water ingestion rate per swimming event. Increased understanding of exposure of children gained since 2010 will be used in conjunction with epidemiological data and other health studies to further refine estimates of risk to children. These additional analyses are required to sufficiently quantify risks and to potentially revise the criteria.

## 3. Health Relationships and Coliphage

Since the 2012 Recreational Criteria were issued, the EPA has been evaluating development of recreational criteria for coliphage, a viral indicator (See Chapter IV). As part of these efforts, UCB led a comprehensive reanalysis of the NEEAR/UCB/SCCWRP data using data from beach sites where coliphage was measured. Questions addressed by this reanalysis were 1) Is coliphage associated with GI illnesses among swimmers? 2) How does coliphage compare to standard FIB (culturable enterococci) as a health indicator? and 3) Does coliphage presence affect the association between culturable enterococci and GI illness?

The studies included observations at six marine beach sites (two from NEEAR and four from UCB/SCCWRP in California) and over 40,000 beach goers and 1,818 water samples. Four of these beaches were classified for at least part of the study duration as human-impacted due to the known presence of fecal discharges. Two beaches were classified as not human-impacted because of no known sources of fecal discharge at those sites (Benjamin-Chung et al., 2017). The water samples were assayed for male-specific or somatic coliphage by EPA Method 1601 or 1602. Assays conducted to detect indicators varied by beach.[2] Somatic coliphage was detected more frequently than male-specific coliphage, and some beach sites had a low frequency of detection. Overall, no association between the presence of coliphage (or culturable enterococci) and GI illness was found among swimmers nor did the presence of coliphage affect the association between culturable enterococci and GI illness. Under "high-risk" conditions, defined as those for which human fecal contamination was likely impacting the beach, however, associations between both culturable enterococci and coliphage and GI illness among swimmers were observed.

This pooled analysis represents the largest evaluation to date of the association between coliphage in recreational water and GI illness. The findings provide evidence that the presence of coliphage is associated with GI illness among swimmers under conditions when human fecal contamination is present. Compared to associations with culturable enterococci, associations were similar for somatic coliphage and there was some evidence for a stronger association with male-specific coliphage. This work highlights the potential utility of coliphage as a predictor of GI illness when human fecal contamination is likely present. Potential limitations include a relatively high frequency of non-detects at all six marine beach sites, which could have been attributable in part to the use of 100-mL water samples rather than larger volume samples (Benjamin-Chung et al., 2017).

With regard to the SCCWRP studies (Griffith et al., 2016), male-specific coliphage (EPA Method 1603) exhibited a stronger association with GI illness compared to culturable enterococci (EPA Method 1600) at Avalon and Doheny Beaches (Griffith et al., 2016). At

[2]Somatic coliphage (EPA Method 1601) was analyzed at Avalon, Doheny, and Mission Bay; somatic coliphage (EPA Method 1602) was analyzed at Avalon and Doheny; male-specific coliphage (EPA Method 1601) was analyzed at all six beaches; male-specific coliphage (EPA Method 1602) was analyzed at Avalon and Doheny.

Malibu, a site where septage seeps at one end of the beach, F+ ribonucleic acid (RNA) coliphage genotype II was the only indicator significantly associated with GI illness (Colford et al., 2012).

## 4. Health Relationships and Additional Alternative Indicators

Although observations show that development and use of alternative fecal indicators is a rich and evolving field, no strong case has been made for changing the indicators currently recommended in the 2012 RWQC. As stated by numerous studies, however, alternative method-indicator combinations might be supported in certain situations (fecal sources, source dynamics, waterbody type) (Savichtcheva and Okabe, 2006; U.S. EPA, 2007a,b; Boehm et al., 2009; Schoen et al., 2011; Ashbolt, 2015; Griffith et al., 2016) and warrant further study especially in specific settings, such as tropical waters.

In the NEEAR studies, no indicators beyond culturable enterococci and *Enterococcus* spp. measured by qPCR were tested at every site. *Enterococcus* spp. measured by qPCR was strongly and consistently associated with GI illness among swimmers across the NEEAR studies in both marine and fresh waters. These associations led to the development of supplemental criteria in the 2012 RWQC. In addition to the associations with GI illness among swimmers and male-specific coliphage at two of the NEEAR marine sites, associations between GI illness and Bacteroidales measured by qPCR (Wade et al., 2010) and GI illness and *Clostridium* spp. measured by qPCR were also observed. Archived NEEAR water samples were recently tested for the presence of human-specific *Bacteroides* markers of fecal contamination. Although detections of one marker (BsteriF1) showed patterns of positive associations with swimming-associated GI illness, consistent associations between the presence of other human-specific *Bacteroides* markers and GI illness were not observed among swimmers due to frequent non-detects and generally low levels of detection (Napier et al., 2017). The authors state that quantitative measures for the human markers could be needed to assess the relationship between risk and human fecal pollution.

Studies conducted by UCB and SCCWRP at Doheny, Avalon, and Malibu beaches in California tested a broader range of indicators than did the NEEAR studies (Colford et al., 2012; Arnold et al., 2013; Yau et al., 2014; Griffith et al., 2016). Although all three beaches were affected by human fecal contamination, the contamination dynamics were complex and differed significantly from site to site. The indicator results are summarized by Griffith et al. (2016). At all three study sites, F+ coliphage was more strongly associated with GI illness than culturable enterococci (see Coliphage section). At Doheny Beach and Avalon Beach, associations between swimming-associated GI illness and *Enterococcus* spp. measured by qPCR were similar to those observed for culturable enterococci. For the other multiple indicators assessed, positive associations were observed only when these beaches were thought to be impacted by human fecal contamination (e.g., when the berm was open at Doheny Beach and under conditions of high submarine groundwater discharge at Avalon). Arnold et al. (2016) also observed the positive associations with health effects for both culture and qPCR-enumerated enterococci at beaches with known point sources of human fecal pollution, but not at beaches lacking those sources. Arnold et al.

(2016) also reported increased illness risks for children for *Enterococcus* spp. measured by both qPCR and culturable enterococci.

## 5. Etiologic Agents

Researchers have long suspected viruses as possible etiological agents in swimming-associated illness (Cabelli et al., 1982; WHO, 2003; Sinclair et al., 2009). Most types of enteric human viruses are generally unlikely to occur in animal feces (Feachem et al., 1983; Halaihel et al., 2010), although pigs and birds periodically carry zoonotic waterborne viruses (Meng, 2011; Raoult, 2011). Moreover, Wong et al. (2009) reported that water samples from both Silver Beach and Washington Park Beach (both NEEAR beaches) contained human adenoviruses. Other results indicate that enteric viruses can be highly infectious even at low doses (Teunis et al., 2008, Wade, et al., 201) and are relatively resistant to standard sewage treatment processes (Laverick et al., 2004; Lodder and de Roda Husman, 2005; Pusch et al., 2005; van den Berg et al., 2005; Haramoto et al., 2006). These studies collectively highlight the potential importance of human enteric viruses as etiological agents of concern in recreational waters contaminated by human fecal sources and, in particular, treated and disinfected effluent.

The understanding of the human health effects from pathogens in ambient waters has grown (e.g., detection methodologies, epidemiological study designs, risk assessment approaches, evaluation of risk management). For example, as part of the 2009 NEEAR study at Boquerón Beach, Puerto Rico, the EPA collected saliva samples from a subsample of study participants to test using a multiplex salivary immunoassay for evidence of infection among swimmers. This assay, developed by EPA scientists, can detect infection from several potentially waterborne pathogens including common variants of norovirus (Augustine et al., 2017; Griffin et al., 2011, 2015). Of 1,298 participants who provided three samples, 34 (2.6%) had antibody responses indicative of a potential infection with norovirus genogroup I or II. The infection rate was over four times higher among swimmers who immersed their heads in water compared to participants who did not immerse their heads in water. Very few of the infections were associated with self-reported symptoms, indicating these infections were likely asymptomatic or produced mild symptoms that were unnoticed or not reported. The findings provide some of the first direct evidence that enteric viruses (norovirus) are transmitted during swimming even without the presence of symptoms (Wade et al., 2016). QMRA analyses support these results, which indicate enteric viruses are likely the most important etiologic agent in waters affected by human fecal sources (Soller et al., 2010a). A pathogen monitoring program at this location during the epidemiological study detected enteric viruses in beach water and a QMRA conducted incorporating the pathogen data showed that enteric viruses could account for almost all of the illnesses reported (Soller et al 2016).

## 6. Tropical Waters

Researchers and regulators have long expressed concern regarding the applicability of FIB in tropical environments due to their potential to regrow and persist in the water, sand, and soil in these environments (Boehm et al., 2009). Recent studies in tropical locations found levels of *E. coli* and enterococci four to five logs higher compared to coliphages and enterophages,

suggesting a natural source of the FIB indicators in pristine tropical waters (Santiago-Rodriguez et al., 2016).

An EPA epidemiological study conducted in 2009 in Boquerón Beach, Puerto Rico did not provide conclusive evidence of the applicability of FIB in tropical environments due to interference of the qPCR signal and relatively low levels of fecal contamination (Wade et al., 2010). Similarly, a study in Luquillo, Puerto Rico found a higher risk of GI illness compared to non-swimmers and a higher risk of GI illness observed among children <5 years of age (Cordero et al., 2012), but no consistent associations with levels of fecal contamination. Sanchez-Nazario et al. (2014) also conducted an epidemiological study at three tropical beaches with point and non-point sources of fecal pollution. Although they found an increased risk of illness among swimmers compared to non-swimmers, including when water quality met the current microbial standard, indicators were not predictive of GI illness. Coliphages were found to be the best predictors of respiratory illness followed by *E. coli* (Sanchez-Nazario et al., 2014).

Lamparelli et al. (2015) reported the findings of a prospective-cohort epidemiological study at five beaches in Sao Paulo, Brazil affected by partially (primary treatment and chlorination), poorly, or non-treated human sewage. Highly significant exposure-response relationships between levels of *E. coli* and enterococci bacteria and self-reported GI illness were found among swimmers. The geometric mean for enterococci ranged from 16 to 64 cfu/100 mL and for *E. coli* from 42 to 234 cfu/100 mL, and three of the five beaches had geometric means below the EPA's current recommendations. The findings of the study provide some of the first published evidence that FIB are predictive of swimming-associated GI illness in tropical environments at sites impacted by sources of human fecal contamination. Other measures of fecal contamination (i.e., molecular measures, coliphage), however, were not available.

Additionally, conditions in more tropical regions, especially Hawaii, are such that the 2012 RWQC may be more protective when used in conjunction with QMRA. This is due to the propensity for enterococci to be associated with contaminated soil (Vijayavel et al., 2010) and to exhibit higher decay rates in the environment (Kirs et al., 2016), and the enhanced possibility of enterococci regrowth in a tropical setting. One QMRA study reported that GI illness risks from viral exposures were generally orders of magnitude greater than bacterial exposures in Hawaiian waters impacted by stream discharges (Viau et al., 2011). Researchers found a positive, significant association between GI illness rates predicted by QMRA and *Clostridium perfringens* densities; no other microbial indicators correlated to risk (Viau et al., 2011). Another QMRA study found a correlation between densities of indicator bacteria and rainfall in an urbanized tropical stream, but not between rainfall and a human fecal marker (Kirs et al., 2017). The stream studied is chronically affected by human sewage inputs, such as illegal cross-connections and leaking sewer systems, during dry and wet weather periods. Kirs, et al. note that water management decisions in Hawaii should not rely solely on enumeration of enterococci or *E. col*i (Kirs et al., 2017). In Hawaii, where Enterrococci are found at high densities in soils, multiple lines of evidence, including *Clostridium perfringens*, indicator bacteria, and F+-specific coliphage, were required to identify sewage as the cause of water quality impairment in an

urbanized tropical watershed. Again, this represents an ongoing challenge in tropical waters for traditional indicators.

## 7. Non-Point Sources

Beach sites with known human sources of fecal contamination are considered to have the highest risk of swimming-associated illness due to the large numbers of microorganisms and the high potential for pathogenic microorganisms in human sewage and human fecal contamination. In addition, associations with FIB are most reliable and consistent at beach sites dominated by human point source fecal contamination. Studies with non-point, non-human, diffuse, and sporadic sources often have failed to identify significant associations between FIB density and illness. An EPA study conducted in 2009 at a marine beach site (Surfside Beach, South Carolina), with no identified point sources of human fecal contamination, found no strong or consistent associations between levels of FIB and swimming-associated illness (Wade et al., 2010). Similarly, at a beach with no known point sources, a dose-response relationship was observed between skin infections and culturable enterococci, but was not observed between GI illness and any FIB (Sinigalliano et al., 2010). Additionally, a series of three large epidemiological studies by UCB and SCCWRP with EPA contribution confirmed that associations between FIB and illness are most robust and consistent when human fecal contamination impacts the beach.

At Malibu Beach, California, Arnold et al. (2013) found no association between any of the fecal indicator organisms measured and illness among swimmers. The beach is impacted by non-point source urban runoff, and during the study water quality was good, meeting or exceeding the EPA's and the State of California's criteria. At Avalon Beach, California, Yau et al. (2014) reported significant associations between culturable enterococci and GI illness among swimmers only when submarine groundwater (influenced by human fecal contamination from leaking septic and sewer systems) was likely impacting the beach. *Enterococcus* spp. measured by qPCR was also positively associated with GI illness among swimmers under conditions when submarine groundwater discharge was high. At Doheny Beach, California, associations between culturable enterococci and *Enterococcus* spp. measured by qPCR and GI illness were observed only when a "sand berm" was open, allowing potentially untreated human contamination from the San Juan Creek to impact the beach (Colford et al., 2012).

Collectively, these studies provide evidence that when human sources impact marine beach sites, enterococci (enumerated by both culture and qPCR) are associated with GI illness among swimmers. When impacts are not associated with known human sources, FIB densities are not as strongly associated with GI illness.

Regarding non-human sources, QMRA analyses found that exposure to animal fecal sources such as gull, chicken and pigs might pose a lesser risk compared to human fecal material (Soller et al., 2010a). Risk from bovine feces directly deposited into a recreational waterbody can result in risk similar to that posed by secondary treated and disinfected effluent. The EPA conducted a series of field experiments using land-applied cattle manure, pig slurry, and chicken litter to

evaluate runoff containing animal fecal material. Simulated rainfall mobilized FIB and pathogens from the study area. The EPA included the results from the mobilization experiments to modify the fecal loading of FIB and pathogen parameters in a QMRA analysis. Risk from all non-human sources including bovine feces can be less when the fecal material is land applied and reaches surface water via rainfall-induced runoff (Soller et al., 2010a; EPA 2010c; Soller et al., 2015). Risks from mixed sources are driven predominantly by the proportion of the contamination source with the greatest ability to cause human infection (potency), which is not necessarily the most abundant source(s) of FIB (Schoen and Ashbolt, 2010; Soller et al., 2014).

Risks from nonhuman fecal sources can be influenced, however, by the magnitude of contamination. One study in New Zealand comparing human-impacted waters with waters impacted by other animal wastes found both types of waters had similar potential for illness risks and both were higher than non-impacted "control" waters (McBride et al., 1998). This study included sites that were heavily impacted by animal waste (i.e., non-human) from rural watersheds. Considered together, this information suggests that both the nature and the magnitude of the fecal source impacting a waterbody influence the potential for human health risks.

## 8. Wet Weather

In recent years, several studies have highlighted the importance of significant rainfall in determining the degree of water contamination. For example, a recent epidemiological-coupled QMRA study in California surfers found that FIB measured in seawater (i.e., *Enterococcus* spp., fecal coliforms, and total coliforms) were strongly associated with illnesses, but only during wet weather. Urban coastal seawater exposure increased the incidence rates of many acute illnesses among surfers, with higher incidence rates after rainstorms (SCCWRP, 2016; Arnold et al., 2017). The QMRA component of the aforementioned study found that human enteric viruses are the pathogens of primary concern, based on site-specific pathogen monitoring data of storm water, site-specific dilution estimates, and literature-based data for ingestion pathogen dose-response and morbidity. Norovirus (genogroups I and II), enterovirus, and adenovirus were detected regularly in the stormwater discharges. No known permitted point-source discharges affect nearshore coastal waters in southern California; rather, wet weather facilitates discharges of raw human sewage to leak or overflow from malfunctioning infrastructure. To help improve water quality in both dry and wet weather conditions in southern California, alternative water quality metrics, like the human-associated fecal source marker HF183, are being used to inform decisions (SCCWRP, 2016).

Another study (Abia et al., 2016) noted that ingestion of 1 mL of river water from the Apies River in Gauteng, South Africa could lead to 0–4% and 1–74% probability of illness during the dry season and wet season, respectively. Authors noted that activities that disturb sediments lead to elevated risk of infection to users of the river. In the Chicago Area Waterways System (CAWS), wet-weather conditions also contributed to elevated pathogen loads (Rijal et al., 2011). A QMRA in Philadelphia, Pennsylvania waterways found dry-weather risk estimates to be significantly lower than those predicted for wet-weather conditions (Sunger et al., 2015).

## 9. Health Burden

Several studies provided evidence of the costs, burden, and severity resulting from swimming-associated illness. These studies relied on data collected as part of the NEEAR epidemiological study, the UCB/SCCWRP epidemiological studies, the UIC CHEERS or a combination of these data sets.

Collier et al. (2015) used the NEEAR data set to document the overall occurrence of illness and healthcare utilization among beachgoers and their swimming exposures and social and demographic characteristics. DeFlorio-Barker et al. (2017a) considered how alternative definitions of GI illness, including severity of the episode, affected associations among swimming exposures. In a second paper, DeFlorio-Barker et al. (2017b) developed a range of the health burden costs resulting from swimming-associated GI illness. In this analysis, the authors found that each case of swimming-associated GI illness resulted in costs (due to medications, time lost from work, etc.) ranging from $46 to $263 (U.S. dollars).

In addition to reanalyses of the NEEAR data, CDC and the EPA summarized information on recent outbreaks in recreational waters for 2010–2011. The National Outbreak Reporting System (NORS) is a passive reporting system through which state and local health officials voluntarily report outbreaks to the CDC. During this time, 21 outbreaks associated with untreated recreational water occurred, resulting in 479 cases and 22 hospitalizations. Seven outbreaks were caused by *E. coli* O157-H7 or O111; two outbreaks by norovirus; and one outbreak by adenovirus. Twenty outbreaks were in fresh water (e.g., lakes), and one outbreak was in marine water (Hlavsa et al., 2015). Due to the voluntary nature of this surveillance system, which relies on individual states and localities to report outbreaks, the outbreaks reported to CDC are likely an underestimate of the actual number of outbreaks. In addition, the number of cases reported due to outbreaks represent only a small fraction of the total cases that occur in the population because most cases, especially for relatively mild and self-limiting illnesses, are not reported.

## 10. Non-Enteric Illness

In the 2000 BEACH Act amendments to the CWA, the EPA was required to study illnesses other than GI illness, the illness most commonly associated with recreational water exposure. Other endpoints include respiratory symptoms, skin rashes, and ear and eye infections. The NEEAR study found no associations between these other non-GI symptoms and levels of fecal contamination at beach sites (Wade et al., 2008, 2010). An analysis of earaches and ear infections reported from the NEEAR studies confirmed that, although swimmers had higher rates of earache and ear infections, these were not associated with fecal contamination (Wade et al., 2013). A meta-analysis by Yau et al. (2009) combined the NEEAR and UCB/SCCWRP data to study skin-related symptoms and found that although swimmers reported higher rates of skin-related symptoms, there was no association with levels of fecal contamination.

These studies provide additional evidence that GI illness is the most frequent and most consistent illness associated with fecal contamination at beach sites. Although other illnesses such as eye, skin, respiratory, and ear infections can be caused by exposure to fecally contaminated

recreational waters, they occur less frequently and have inconsistent associations with indicators of fecal contamination.

## B. Summary of Coliphage Advancements for RWQC

### 1. Introduction

Over the past few years, the EPA has been working to develop RWQC for coliphage, a viral indicator, to ensure public health protection from water sources that have been influenced by viral fecal contamination (U.S. EPA, 2015f, 2017a). Increasing evidence through microbial risk assessments (Schoen and Ashbolt, 2010; Soller et al., 2010a,b, 2015) and epidemiological studies (Lee et al., 1997; Colford et al., 2005, 2007; Wiedenmann et al., 2006; Wade et al., 2010; Griffith et al., 2016; Cabelli et al., 1982) illustrate that viruses cause most illnesses associated with primary contact recreation in surface waters impacted by human sources. Further, U.S. outbreak surveillance data collected by CDC points to viruses as the leading pathogen group responsible for untreated ambient recreational water outbreaks (Jiang et al., 2007; Sinclair et al., 2009; Hlavsa et al., 2015).

Human enteric viruses enter recreational surface waters from both treated and untreated human sources. A driving issue is that current wastewater treatment and disinfection processes specifically target the removal and inactivation of bacteria, not viruses (U.S. EPA, 2015f, 2017a). Numerous studies have identified the presence of viruses in wastewater treatment effluent, often when traditional fecal indicator bacteria are nondetectable (U.S. EPA, 2015f).

Although the EPA recommends coliphage as an option for evaluating fecal contamination in groundwater, the Agency currently has no coliphage recommendations applied to surface waters for protecting primary contact recreation. Coliphages are a subset of bacteriophage viruses that infect *E. coli*. In particular, male-specific (or F+ specific) and somatic coliphages have been proposed as more reliable indicators of human viral pathogens associated with fecal contamination than traditional fecal indicator bacteria (Gerba, 1987; Palmateer et al., 1991; Havelaar et al., 1993; Cabelli et al., 1982). Coliphages exhibit numerous desirable indicator characteristics. For example, they are abundant in domestic wastewater, raw sewage sludge, and polluted waters; are physically similar to viruses causing illnesses associated with primary contact recreation; originate almost exclusively from the feces of humans and other warm-blooded animals and undergo only very limited multiplication in sewage under some conditions (i.e., high densities of coliphages and susceptible host *E. coli* at permissive temperatures); are nonpathogenic; amenable to overnight culture methods and can be counted cheaply, easily, and quickly; in some studies show correlations to GI illness among swimmers; and are similarly resistant to sewage treatment and environmental degradation as enteric viruses of concern (Funderburg and Sorber, 1985; Havelaar et al., 1990, 1993; Sobsey et al., 1995; Gantzer et al., 1998; Grabow, 2001; Mandilara et al., 2006; Nappier et al., 2006; Pouillot et al., 2015; U.S. EPA, 2001a,b, 2015f).

As part of the coliphage criteria development process, the EPA has 1) conducted a series of literature reviews; 2) refined somatic and male-specific (or F+) coliphage enumeration methods

(culture and molecular), including completing single-laboratory and multilaboratory validation studies on culture-based methods for wastewater treatment effluent and ambient waters; 3) conducted the 2016 Coliphage Experts Workshop; and 4) participated in an analysis of NEEAR/UCB epidemiological data, specifically evaluating coliphage-health associations. This section summarizes the results from the EPA's literature reviews, information about the EPA'scoliphage enumeration methods, and conclusions on the 2016 Coliphage Experts Workshop. The epidemiological studies where coliphage was measured as an indicator are discussed above.

## 2. Literature Reviews

In 2015, the EPA published *Review of Coliphages as Possible Indicators of Fecal Contamination for Ambient Water Quality* (U.S. EPA, 2015f), a peer-reviewed literature review of the scientific information that the EPA will evaluate to develop coliphage-based AWQC for the protection of swimmers. The review generally illustrates the currently available data support the conclusion that coliphages are good alternative indicators of fecal contamination to the EPA's currently recommended criteria for *E. coli* and enterococci. In addition, coliphages are better indicators of viruses in treated wastewater than bacteria.

Additionally, the EPA has conducted a series of systematic literature reviews of viruses in raw sewage and in ambient waters (Eftim et al., 2017a; U.S. EPA, 2017a). The work indicates that pathogenic viruses (norovirus) are found in raw sewage at $\log_{10}$ mean densities of 4.7 ($\log_{10}$ standard deviation of 1.5) genome copies/L (Eftim et al., 2017a). The systematic literature review of male-specific and somatic coliphage densities in raw sewage and ambient waters are in progress, but the work has been presented at the 2016 and 2017 University of North Carolina (UNC) Water Microbiology Conferences and 2015 Coliphage Experts Workshop. Collectively, the data will be used to assist in the criteria derivation for the coliphage-based RWQC (Eftim 2016; 2017b).

Finally, fate and transport of bacteriophage (and other indicators) were reviewed (U.S. EPA, 2015f; McMinn et al., 2017). As part of this effort, the EPA evaluated inactivation through the wastewater treatment processes based on the published literature (McMinn et al., 2017) and investigated decay in marine environments (Wanjugi et al., 2016). The results indicate that $\log_{10}$ reduction of coliphage was more similar to that of viral pathogens than FIB to human viruses, suggesting they might be better surrogates for removal of viral pathogens than FIB (U.S. EPA, 2015f; McMinn et al., 2017). Additionally, bacteriophage exhibit differential decay patterns in marine waters that appear influenced by several biotic and abiotic factors and by bacteriophage type (Wanjugi et al., 2016).

## 3. Methods

The EPA's culture-based assay uses dead-end hollow fiber ultrafiltration (D-HFUF) paired with the single-agar layer (SAL) procedure as described in EPA Method 1602 to concentrate and enumerate culturable somatic and F+ specific coliphage from large volumes (>1 L) of surface waters (McMinn et al., 2017a). Application of the method to a variety of surface waters resulted

in average percent recoveries of greater than 50%. Analyses to date indicate that D-HFUF-SAL is a robust and sensitive method that can be used for routine measurements of culturable somatic and F+ coliphage from surface waters (McMinn et al., 2017a). The D-HUF-SAL method (2 liters [L]) for the enumeration of F-specific and somatic coliphage has undergone multi-laboratory validation for use in ambient waters and in advanced treated wastewater effluent. Similarly, EPA Method 1602 (100 mL) has undergone multi-laboratory validation for use in secondary wastewater (no disinfection) effluent.

The EPA has additionally developed molecular assays to target four genogroups of F-specific (F-RNA [ribonucleic acid]) coliphages via reverse transcriptase-polymerase chain reaction (RT-PCR) and reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR), which can provide information regarding the source of fecal pollution (human vs. other animals) (Friedman et al., 2009, 2011, 2017). Specificity of the assays for their respective fecal sources was evaluated on F-RNA coliphages (n = 49) originating from various warm-blooded animals, sewage and combined sewage overflow, demonstrating its usefulness in discriminating coliphage from different sources (Friedman et al., 2009). In addition, successful evaluation on a panel of environmental F-RNA strains demonstrated their utility in the assessment of sanitary quality of recreational waters (Friedman et al., 2011). Further evaluation of RT-qPCR methods signified that the F-RNA genotyping procedure successfully indicated possible human fecal contamination, but the methodology has challenges and would require substantial refinements and improvements before considering its use for routine measurements of coliphage densities in surface waters (Paar et al., 2015).

## 4. 2016 Coliphage Experts Workshop

The EPA held the Coliphage Experts Workshop in March 2016 as part of the Agency's ongoing efforts to build the scientific basis for developing coliphage-based water quality criteria. The EPA brought together a group of 12 internationally recognized experts on the state of the science of coliphages and their usefulness as a viral indicator for the protection of public health in recreational waters. Experts represented a spectrum of perspectives from academia, federal agencies (EPA, CDC, Food and Drug Administration), and the wastewater industry. The EPA recently published a peer-reviewed meeting proceedings report on the workshop (U.S. EPA, 2017a). The goal of the workshop was not to reach consensus; instead, it was designed to be a critical thinking and information-gathering exercise. Agenda discussion topics included the need for a viral indicator, coliphage as a predictor of GI illnesses, how coliphage could be useful as an indicator of wastewater treatment performance, male-specific versus somatic coliphage, a systematic literature review of viral densities, and future research.

During these discussions, individual experts had common views that viruses are a source of illness in recreational water exposures and that those viruses enter surface waters via wastewater treatment plant (WWTP) effluent, especially during wet weather events and when WWTPs exceed design flows. Additionally, experts noted that coliphages are more similar to human pathogenic viruses than traditional FIB and they more closely mimic the persistence of human pathogenic viruses. Experts also suggested that future epidemiological studies specifically

include coliphages as measured indicators. As an indicator of WWTP performance, coliphages are consistently present in municipal sewage and provide a baseline for examining different WWTP processes under varied conditions. Experts indicated that the literature suggests coliphage and human viruses have more similar log-reductions during wastewater treatment, compared to traditional FIB. Opinions ranged, however, on whether somatic, male-specific coliphage, or both would be better for various applications. Evidence shows a relationship to GI illness in epidemiological studies for both coliphage types in some studies. A fact sheet and the proceedings for the 2016 Coliphage Experts Workshop are available online (U.S. EPA, 2017a).

## C. Summary of Scientific Advancements in FIB qPCR

### 1. Introduction

U.S. EPA's 2012 RWQC (2012 RWQC; U.S. EPA, 2012a) included qPCR EPA Method 1611 (U.S. EPA, 2012b) as a supplemental indicator method to detect and quantify *Enterococcus* spp. in ambient water on a site-specific basis. The qPCR methodology offers the advantage of providing rapid detection results (2–6 hours), allowing beach managers to make same-day decisions to protect beachgoers. In contrast, water quality results for traditional culturable indicator methods are not available until 24–48 hours after sampling. In addition to providing rapid results, The EPA's *Enterococcus* spp. qPCR (Method A, Draft of EPA Method 1611) was more strongly associated with GI illness enterococci measured by culture in the NEEAR study (Wade et al., 2008; U.S. EPA, 2010c). At the time of the 2012 RWQC publication, however, the EPA still had limited experience with the method's performance across a broad range of environmental conditions. The 2012 RWQC contain this cautionary language: "EPA has limited experience with its performance across a broad range of environmental conditions. States should be aware of the potential for qPCR interference (see Section 3.1.1) in various waterbodies, which may vary on a site-specific basis. Thus, the EPA encourages a site-specific analysis of the method's performance prior to use in a beach notification program or adoption of WQS based on the method" (U.S. EPA, 2013d).

Interference is any process that results in lower quantitative estimates than actual values. For qPCR-based enumeration methods, interference can occur when substances bind to the target deoxyribonucleic acid (DNA), which can prevent the primers from binding, inhibit polymerase function, or cause the DNA to precipitate prior to amplification. Examples of substances causing interference include humic acids, coral sands, calcium, and certain types of clay particles; however, many other unidentified substances likely also contribute to qPCR interference.

Since 2010, however, the EPA has made significant advancements in the performance of the EPA's FIB qPCR methods. These advancements are articulated through peer-reviewed manuscripts, EPA Method documents, technical support materials, and a systematic literature review of qPCR methods (see Appendix A). Two key developments include the publication of an improved qPCR-based method for enumeration of *Enterococcus* spp. (EPA Method 1609) and the development of a draft EPA qPCR-based method for enumeration of *E. coli* (*Draft* Method "C"), both of which were included in the EPA's 2015 Great Lakes Beaches study. Finally, calculation tools to facilitate data analysis by the user are also available. This section briefly

describes improvements in EPA qPCR methods (EPA Method 1609 and *Draft* Method C) and technical support information available to stakeholders. The EPA notes that no change is needed in the criteria to apply these revised methods.

## 2. *Enterococcus* spp. qPCR EPA Method 1609

To address the potential for high interference levels, the EPA developed EPA Method 1609 (U.S. EPA, 2013b), which uses a custom designed reagent for environmental sample testing called Environmental Master Mix (EMM) (TaqMan; Applied Biosystems, Foster City, CA), that results in lower levels of interference in undiluted samples (Haugland et al., 2012, 2016; Cao et al., 2012; Sivaganesan et al., 2014). Like EPA Method 1611, EPA Method 1609 requires the sample processing control (SPC) interference control assay using Sketa 22 and recommends the internal amplification control (IAC) assay.

Appendix A (Table A-4) summarizes the EPA's systematic literature review results of the application of EPA *Enterococcus* spp. qPCR methods in ambient waters. EPA Method 1609 has a qPCR interference range of 0–14% in undiluted samples in both temperate marine and fresh waters, based on the SPC and IAC controls. In contrast, EPA Method 1611 has a much higher interference rate in undiluted samples ranging from 0 to 53% in both temperate marine and fresh waters, using both SPC and IAC for controls. For both methods, a five-fold dilution of the water samples reduces the interference rate in fresh and marine waters, and routinely performing this dilution is recommended in Method 1611.

Overall, EPA Method 1609 is recommended over EPA Method 1611. EPA Method 1609 has an overall more robust performance, with no sample dilution required in most instances, and a lower overall interference rate, as compared to other EPA methods (*Draft* Method A, EPA Method 1611). Sample dilution and use of the EMM addressed inhibition at the nine marine and 23 of the 25 potentially problematic freshwater sites in 10 states comprehensively investigated by the EPA since 2010 (Haugland et al., 2014, 2016).

Based on these results, use of EPA Method 1609 is appropriate when the required and suggested controls are employed. Use of the EMM, the Sketa 22 SPC assay, and optional use of the IAC assay both reduces interference and identifies whether interference was observed in the qPCR sample. These controls are not available for culture methods.

Revisions to Method 1609 (and 1611) have been published by the EPA as Methods 1609.1 and 1611.1, respectively, that are available online (U.S. EPA, 2015e). These updates were introduced to further standardize absolute *Enterococcus* spp. CCE density estimates across laboratories and to relate them to 2012 RWQC values (Haugland et al., 2014). Greater standardization can be achieved through the suggested use of EPA-provided DNA reference materials and data calculation support materials (see below Available Technical Support Information).

The EPA has developed a draft qPCR method for *E. coli* (*Draft* Method C; Chern et al., 2011), which incorporates the same interference modifications and controls as EPA Method 1609. A multi-laboratory validation study is currently underway, and results are expected by the end of

2018. Appendix A describes the systematic literature review results of 13 studies by the EPA and others that have evaluated qPCR methods for *E. coli*. Overall, results illustrate low rates of inhibition (<10%) at the locations sampled. The number of sites and samples reported, however, is significantly smaller than for *Enterococcus* spp. qPCR. The EPA's *Draft* Method C shows promise for use on a site-specific basis, but, no peer-reviewed demonstrations of its use in routine monitoring are presently available.

## 3. Available Technical Support Information

Since 2010, the EPA has also developed a series of support materials and information for stakeholders interested in using qPCR in their waterbodies.

### *qPCR Standards*

Evaluation of reference materials used in the qPCR-based methodology highlighted the importance of using standardized protocols and reference materials (Shanks et al., 2012; Cao et al., 2013a; Haugland et al., 2014). Efforts are ongoing with the National Institute of Standards and Technology (NIST) to establish an interagency agreement to develop DNA reference materials for the EPA's qPCR methods. In the meantime, Agency laboratories have prepared DNA reference materials for *Enterococcus* spp. (EPA Methods 1609.1 and 1611.1) and *E. coli* (*Draft* Method C) that can be used for the standardization of these methods and has made these materials available to the public. The EPA contact information for obtaining these materials is currently undergoing revision.

### *Training Sessions*

Successful application of the qPCR-based methods requires sufficient laboratory capability and proficiency. Proficiency is affected by the experience of laboratory personnel, and sufficient training of personnel is needed to ensure adequate method performance. The EPA has held multiple "train-the-trainer" sessions to assist states in learning qPCR techniques. The continued availability of standards will also help facilitate consistency of results within and between laboratories. Additionally, the EPA has an ongoing collaboration with Michigan Department of Environmental Quality to assess the implementation of the *E. coli* qPCR method in state public health and water testing laboratories. Additional stakeholder troubleshooting and guidance are expected to result from this effort.

### *qPCR Acceptability Criteria*

The EPA has provided guidance on how to evaluate the acceptability of EPA *Enterococcus* spp. qPCR EPA Methods 1611 or 1609 at a specific beach. The guidance assumes that the testing laboratory has been able to perform one of these methods within the acceptance criteria, and now wishes to ascertain whether qPCR would be acceptable for use at a particular site. Site acceptability is based on the demonstration that a sufficiently high percentage of multiple samples, collected from the site over time, show an absence of sample matrix interference, as determined by the qPCR methods controls. It is important to note that EPA Method 1609 reduces the frequency of interference compared to EPA Method 1611 and allows analyses of undiluted extracts for greater analytical sensitivity at many sites. A recent multi-laboratory study of

potentially problematic sites across the United States revealed that 20 of the 22 sites met the EPA site acceptability guidelines when using EPA Method 1609 (Haugland et al., 2016).

### *Calculation Spreadsheets*

The EPA has provided Excel spreadsheet workbooks that can be used for the standardized for the calculation of *Enterococcus* spp. calibrator cell equivalent (CCE) densities in test samples in Methods 1609.1 and 1611.1 (U.S. EPA, 2015e). The workbooks will automatically perform the calculations employing formulas that are derived from EPA Methods 1611.1 or 1609.1 and require only inputs of raw cycle threshold (Ct) measurements of the methods standards, control samples, and test samples. The workbooks also identify test samples that fail the acceptability criteria for the interference controls in the methods.

### *Detection and Quantification of EPA Enterococcus* spp. *qPCR Methods*

The EPA has provided results and conclusions from an EPA Office of Research and Development (ORD) study to determine the limit of detection and lower limit of quantification of EPA Method 1611. The analyses were performed on 5×-diluted DNA extracts of samples (as specified in this method) containing known quantities of enterococci cells. The lower limit of quantification was reported at different thresholds of acceptable variability: 10%, 20%, and 33% coefficient of variation. At 10% coefficient of variation, the estimated lower limit of quantification was 179 cells/sample and at 33%, 125 cells/sample. The 99% frequency limit of detection was between 75 and 150 cells/sample. The conclusion from this study was that the overall method should normally be sensitive enough to support the EPA RWQC values except possibly when less than recommended sample volumes are collected or when total DNA recoveries from the samples are extremely low. Extrapolation of these results to EPA Method 1609, which recommends analyses of undiluted extracts, suggests that this method should support the RWQC values in virtually all samples of recommended volume that pass the acceptability criteria for the controls in the method (U.S. EPA, 2013d). The 33% coefficient of variation lower limit of quantification value from this study has been incorporated into the EPA Excel spreadsheet workbook for Methods 1609.1 or 1611.1.

## D. Human/Non-Human Fecal Source Identification

### 1. Fecal Source Identification

***Background***

FIB currently recommended for management of fecal contamination in ambient waters are found in the feces of warm-blooded and some cold-blooded animals. FIB methods are commonly used for water quality management because procedures are typically straightforward and inexpensive, especially cultivation-based protocols. Ideally, FIB information provides valuable information on the total level of fecal pollution in the waterbody of interest. FIB approaches, however, have several limitations that can reduce their utility for water quality management. For example, naturalized FIB populations have been reported to exist in some non-fecal sources, such as beach sands, soils, and sediments, and are associated with aquatic algae and plants (Badgley et al., 2010, 2011; Gordon et al., 2012; Byappanahalli et al., 2007, 2012a,b; Eichmiller et al., 2013; Bradshaw et al., 2016). In addition, FIB results provide no information about different pollution sources present (Hagedorn et al., 2011; McLellan and Eren, 2014). Many impaired waters are polluted by multiple fecal sources originating from human waste treatment facilities, agricultural practices, and wildlife. FIB are not always well correlated with pathogens, potentially limiting protection of public health (Savichtcheva et al., 2006; Wu et al., 2011; Harwood et al., 2014). In addition, culturable indicator densities might not reflect potentially high-risk scenarios when disinfected effluents affect a waterbody (Wade et al., 2008; Wong et al., 2009; Soller et al., 2010b; Schoen et al., 2011).

Fecal source identification (FSI) techniques are used to characterize different fecal sources potentially present in polluted waters (McLellan and Sauer, 2009; Harwood et al., 2014). FSI methods rely on the detection of host-identifiers, which are typically chemical or microbial targets highly associated with a particular pollution source (Hagedorn et al., 2011). Research attempts to link FIB occurrence trends to host-identifier measurements often show poor correlations (Hagedorn and Weisberg, 2009). It is important to note, however, that waters can be polluted by multiple fecal sources. As a result, FIB can represent a cumulative measure of multiple fecal pollution sources, and some non-fecal sources of indicator, while a host-identifier is targeting a particular source. Furthermore, fecal contamination from human and animal sources contribute different pathogens to ambient waters resulting in variable relationships between FIB, pathogens, and illness outcomes (Soller et al., 2010b; McLellan and Eren, 2014). For example, human fecal contamination, such as untreated sewage and even disinfected effluent, is associated with the highest potential risk of GI illness (Soller et al., 2010b, Schoen et al., 2011).

The notion that some fecal pollution sources represent a higher public health risk is not new. The World Health Organization's recreational water guidelines highlight the pollution source risk differential and incorporate a water classification scheme that emphasizes fecal contamination from humans (WHO, 2003). This realization has led to a large body of research exploring the application of host-identifiers for water quality management. For example, Bradshaw et al. (2016) found that incorporating a combination of FIB, host-identifiers, and other water quality measurements improved water quality assessment in a mixed land use area in a watershed. Other

research groups have focused on potential health-predictive associations between host-identifiers and illness outcomes (Boehm et al., 2015; Brown et al., 2017). The ability to make a direct association between a host-identifier and the risk of an illness outcome could substantially improve public health protection in recreational water management scenarios.

### *Fecal Source Identification with Chemical Host-Identifiers*

Many organic and inorganic chemical compounds are reported to be highly associated with human fecal contamination, including chemicals that are closely associated with human feces (e.g., fecal steroids, caffeine, or synthetic chemicals found in products specific to household and community waste streams) (NRC, 2004; Hagedorn and Weisberg, 2009). The steroid used most frequently as a human host-identifier is coprostanol, produced by catabolism of cholesterol in the intestinal tract (Leeming and Nichols, 1996). A panel of multiple fecal sterols can provide even more information than coprostanol alone in the presence of human fecal contamination (U.S. EPA, 2007a). Caffeine is another common chemical host-identifier of human fecal contamination. The main sources of caffeine in U.S. waters is likely human fecal waste and coffee waste disposal activities. Optical brighteners, added to laundry detergents, are also reported to be useful for detecting sources of human fecal pollution in municipal effluent (Hagedorn et al., 2005; Hartel et al., 2007). Optical brighteners can be measured with a hand-held fluorometer, which can provide immediate and relatively inexpensive monitoring results in the field (Hagedorn and Weisberg, 2009). Linear alkylbenzenes, residues of surfactants commonly used in detergents, are another potential chemical host-identifier of human contamination in surface waters (Phillips et al., 1997; Gustafsson et al., 2001). Chemicals from other personal care products and some pharmaceuticals might also prove useful as human fecal waste host-identifiers and could be a valuable management tool for groups with the appropriate resources and expertise (U.S. EPA, 2007a).

### *Fecal Source Identification with Microbial Host-Identifiers*

Microbe-based FSI, often referred to as MST, targets enteric microbial species closely associated with the gut of a particular animal group. To date, a wide range of technologies is reported to identify these host-associated microorganisms, ranging from canine scent detection to next-generation sequencing (Yan and Sadowsky, 2007; U.S. EPA, 2011; Hagedorn et al., 2011; Santo Domingo et al., 2011; Boehm et al., 2013). The most widely used technologies use molecular methods such as the polymerase chain reaction (PCR) (Stewart et al., 2013). Molecular methods refer to protocols used in genetics, microbiology, biochemistry, or other related fields to study biologically important molecules such as DNA, RNA, and proteins. Before the widespread use of molecular detection and quantification techniques, studies examined the enumeration of specific groups of bacteria, such as the fecal anaerobes Bifidobacteria spp. and Methanobrevibacter smithii, as potential host-identifiers of human and other animal fecal contamination sources (Harwood et al., 2009; Ballesté and Blanch, 2011; McLellan and Eren, 2014).

Over the past decade, the field of molecular biology has advanced significantly. By combining the concept of host-associated bacteria with molecular methodologies, a central MST hypothesis has emerged suggesting that host-associated genetic markers can act as a metric of fecal contamination from a particular animal group. As a result, considerable amounts of time and

resources have been dedicated to the development, testing, and performance validation of molecular MST technologies (Shanks et al., 2008, 2009, 2010; Haugland et al., 2010; Boehm et al., 2013, 2015; Ebentier et al., 2013; Stewart et al., 2013). Researchers have also focused on examining potential relationships among molecular host-identifiers, FIB, pathogens, and public health outcomes (Harwood et al., 2014, Dubinsky et al., 2016; Kirs et al., 2017).

Accurate and reliable MST technologies could dramatically improve water quality management in the United States. Some applications include enhanced characterization of fecal contamination trends in waterbodies impacted by multiple pollution sources, increased understanding of potential public health risks in recreational water settings, and targeted remediation of fecal contamination. To date, MST has aided the identification of fecal pollution sources in impaired waters (Kirs et al., 2017) and urban-impacted recreational beaches (Molina et al., 2014), and helped identify pollution sources during wet-weather-related overflows of human sewage impacting U.S. coastal waters (SCCWRP, 2016). MST tools have been applied in the development of TMDL management plans as part of CWA requirements and in the evaluation of best management practice effectiveness (U.S. EPA, 2005). MST methods have also been combined with high-resolution digital mapping strategies to successfully identify non-point sources of human fecal pollution in a large watershed (Peed et al., 2011). Significant advances made in this area now allow the potential for application of MST to facilitate decision-making for water quality managers. Successful implementation should rely on a "tool box" approach, where MST methods are combined with other established water quality assessment methods to enhance management activities. In addition, the growing body of scientific evidence suggesting that public health risks due to exposure from fecal contamination in recreational waters can be quite different when pollution sources are human compared to non-human sources suggests MST methodologies could play a key role in future public health risk assessments (Soller et al., 2010a, 2014, 2015; U.S. EPA, 2014b). While research in this area progresses, with continuing advances and broader application of these technologies, MST clearly has great potential to improve water quality management and help protect public health.

## 2. EPA MST Research

Human sources of fecal contamination pose a greater potential risk to human health compared to many animal sources (Soller et al., 2010a). Therefore, understanding the sources of fecal contamination is important to protect beachgoers from exposure to poor microbial water quality. The 2012 RWQC has provisions that recommend FSI technologies for use as a sanitary characterization tool (EPA 820-F-12_058) and evidence to support alternative criteria eligibility (EPA 820-R-14-010). Since 2012, The EPA has made significant progress toward the implementation of human source identification technologies, particularly in regard to HF183/BacR287 and HumM2 qPCR methods.

The EPA's ORD maintains an active research program to advance science in human FSI technologies to support implementation of the EPA's RWQC. U.S. recreational waters may be affected by human fecal waste originating from numerous sources such as leaking sewer lines, faulty septic systems, combined sewer overflows (CSOs), sanitary sewer overflows (SSOs), or

illicit activities. Human fecal waste can harbor disease-causing pathogens that contribute to poor public health outcomes and economic burdens. Since currently recommended FIB methods do not discriminate between human and other potential pollution sources in recreational environments due to local wildlife and agricultural activities, human FSI methods can complement general fecal indicators to improve recreational water quality management. ORD has invested considerable resources to develop, validate, standardize, and implement human FSI technologies.

Since 2010, EPA researchers have published 16 peer-reviewed studies and developed a series of technology transfer tools focusing on human FSI method validation, standardization, and implementation.

The EPA collaborated with SCCWRP and 25 other expert laboratories to conduct an international blinded study to identify top performing human FSI technologies (Boehm et al., 2003). Findings demonstrated that most experts (>90%) favor PCR-based technologies (Stewart et al., 2013), that qPCR methodologies are highly reproducible only with standardized protocols (Ebentier et al., 2013), and that HF183 and HumM2 qPCR technologies are top-performing human FSI methods (Layton et al., 2013). As a result, the EPA and collaborators performed a technical review of the HF183 qPCR technology resulting in the optimized HF183/BacR287 method for recreational water applications (Green et al., 2014). HF183/BacR287 and HumM2 qPCR methods were then subjected to an EPA-led 16-laboratory national validation for fresh and marine recreational water use (Shanks et al., 2016). Currently, draft EPA Methods for human FSI are under internal review.

A series of studies was conducted to support implementation of qPCR human FSI applications. Notable contributions include experiments demonstrating the uniformity in sewage microbial communities across the United States (Shanks et al., 2013), how the unit of measure (e.g., enterococci cell count, total DNA mass) for qPCR can alter reported concentrations of human fecal pollution (Ervin et al., 2013), in situ human waste decomposition varies by pollution source and indicator type (Wanjugi et al., 2016), and QMRA modeling can be used to predict links between HF183 and public health risk in raw sewage (Boehm et al., 2015).

The demand for human FSI is rapidly increasing. In response, EPA scientists have developed a series of tools to help facilitate technology transfer of HF183/BacR287 and HumM2 qPCR human FSI technologies. Tools include standardized data acceptance metrics, draft EPA Method procedures, a self-administered method proficiency test, and an automated data analysis tool (Shanks et al., 2016).

Field demonstrations are necessary to provide real-world examples of human FSI qPCR method application. EPA scientists have conducted multiple field studies focusing on identification of diffuse human fecal pollution sources from urban runoff (Molina et al., 2014) and septic field discharge in recreational beach, stream, and river settings (Peed et al., 2011).

### *Key Implementation Gaps*

The EPA advances in national method validation, standardization, and implementation combined with key scientific studies and technology transfer tools provide necessary information to support the use of human FSI qPCR technologies in recreational settings. Two key implementation gaps remain, however, including: 1) formal publication of EPA Methods for HF183/BacR287 and HumM2; and 2) the development of a DNA reference material. To develop a national DNA reference material, the EPA recently entered into an Interagency Agreement with the National Institute of Standards and Technology (October 2017).

### *Emerging Science*

The EPA is actively conducting scientific experiments to support human FSI qPCR method implementation. Current efforts are focused on the development of a standardized procedure to prioritize recreational sites based on human fecal pollution levels (Cao et al., 2013b, Cao et al., 2018). In addition, two large-scale field studies are being conducted using FSI in conjunction with other water quality and climate parameters from nine Great Lake and 29 Tillamook watershed sites to identify occurrence patterns and impact on water quality management in recreational settings. Finally, the EPA and collaborators have developed new viral-based human FSI technologies to complement bacterial HF183/BacR287 and HumM2 methods (Stachler et al., 2017).

## 3. Selected External Research Contributions to MST Development

### *"Tool Box" MST Application Demonstrations*

The presence of microbial pollutants in surface waters can originate from several sources (e.g., wastewater effluent, sewage leaks, sewer overflows, illegal discharges, wildlife, and agricultural runoff). The presence of these pollutants can be influenced by several factors such as weather conditions, adjacent land use, local waste management infrastructure, and watershed characteristics. Currently no single method is used to define local water quality, discriminate between pollution sources, provide weather condition information, and include local land use practices. As a result, many researchers and management experts employ a "tool box" approach to build a comprehensive framework to interpret water quality conditions. For example, Litton et al. (2010) reported the use of a quantitative sanitary survey approach combined with a range of other analytical tools to identify potential sources of FIB contributing to local water impairment. Water quality metrics included FIB measurements of enterococci and *Escherichia coli* (IDEXX methods), select genetic markers (HF183 and *Enterococcus* spp.) determined by qPCR, and chemical identifiers of sewage and wastewater. Findings provided important insights on the benefits and limitations of specific methods, the value of a "tool box" approach to interpret water quality data, and the promising potential of human-associated MST methods. Another study performed in collaboration with scientists from the University of South Florida and the SCCWRP authority paired FIB and bacterial human-associated MST methods with virus-based water quality metrics to investigate non-point sources of fecal pollution at two California marine beaches (McQuaig et al., 2012). Findings illustrated limitations of FIB alone to characterize

sewage pollution sources and the presence of viral pathogens, underscoring the value of a "tool box" approach for water quality management.

***Technology Transfer***

An essential component of the successful implementation of MST method is technology transfer. Molecular MST methods can be technically demanding, requiring specialized equipment, detailed procedures, and specialized training. A useful MST technology must be transferable, with a high degree of reliability and reproducibility. Many MST methods have been developed and validated within individual laboratories. Inter-laboratory testing has been minimal, providing limited information on MST method reproducibility. Information on key factors that influence MST method reproducibility across laboratories is vital for successful implementation and public acceptance. To help address this gap, several organized studies have been conducted to evaluate molecular MST protocols. For example, scientists from five laboratories situated across the Gulf of Mexico region conducted a study to characterize the inter-laboratory performance of three human-associated MST end-point PCR methods (Gordon et al., 2012). Another group evaluated nine host-associated MST qPCR methods across five laboratories using standardized and non-standardized procedures (Layton et al., 2013). Findings demonstrated the successful technology transfer of MST molecular methods and the importance of standardized procedures.

***Advances in Virus-Based MST***

Most currently available human MST technologies target fecal bacteria. The presence of some viruses, however may also be used to distinguish human from non-human sources of fecal contamination. As a result, research efforts have been made to develop virus-based MST methodologies (McQuaig et al., 2009; Rosario et al., 2009) and compare performance to bacteria-based approaches (Staley et al., 2012). For example, Staley and colleagues performed side-by-side testing of sewage diluted in five water types (estuarine, marine, tannic, lake, and river) to evaluate the suitability of each method to estimate risk of GI illness. Findings demonstrated strengths and limitations of bacteria- and virus-based MST approaches and included a recommendation for a "tool box" approach incorporating both bacterial and viral methodologies in future studies.

***The Source Identification Protocol Project***

The Source Identification Protocol Project was an international effort to identify top performing MST methods and characterize the current state of the science. This effort was led by the SCCWRP authority and scientists from Stanford, the University of California – Los Angeles, University of California – Santa Barbara, and EPA-ORD. The effort was designed to engage the international scientific community to identify top performing MST technologies for human, ruminant, cattle, dog, and swine pollution sources by expert consensus; demonstrate the importance of procedure standardization; and explore emerging technologies based on microbial community methodologies. The study was centered on the construction of a 64-sample, blinded sample set challenging expert laboratories to correctly detect and quantify fecal pollution sources. Participating in the study were 27 expert laboratories from seven countries applying 41 MST methods. Findings were published in a special edition of the International Water Association journal of *Water Research* (Reis and Wuertz eds., 2013). The Source Identification

Protocol Project represents a critical step toward the public acceptance of MST technologies for water quality management by identifying top methods based on expert consensus.

***Emerging MST Technologies***

New technologies such as next-generation sequencing and digital PCR represent potentially important advances for MST. As a result, the research community has begun to explore the application of these new technologies for MST. For example, next-generation sequencing has been successfully used to track the movement of CSO events in the Great Lakes (Newton et al., 2013). Some MST methods have also been adapted to a digital PCR platform to explore potential advantages of this new technology for molecular testing of environmental samples (Cao et al., 2015). Emerging technologies will likely harness the power of high-throughput nucleic acid sequencing and other methodologies for the rapid and simultaneous measurements of multiple MST host-identifiers. These novel technologies will likely provide future water quality managers, public health officials, and researchers with powerful tools to improve water quality management.

***Importance of MST Genetic Marker Decay***

Understanding the decay of microorganisms associated with specific fecal pollution sources is necessary for implementation of MST molecular methods for water quality management. Unlike culture-based FIB methods, MST molecular methods target nucleic acids instead of live cells that need to be cultivated in a laboratory. This fundamental difference in method analyte between cultivated FIB measurements and genetic testing with MST technologies can result in different persistence behaviors in environmental settings. Thus, understanding how different environmental stressors influence FIB and MST genetic marker decay is imperative to interpret water quality results properly. As a result, researchers have investigated factors such as sunlight (Green et al., 2011), water type (Jeanneau et al., 2012), temperature (Kreader et al., 1998; Okabe and Shimazu, 2007; Dick et al., 2010), and influence of indigenous microbiota (Kreader et al., 1998; Dick et al., 2010) on FIB and MST genetic marker decay. Findings suggest that cultivated FIB decay trends are significantly different from MST genetic markers. Most studies agree that persistence of MST genetic markers is typically longer in colder water and in marine waters compared to fresh water.

***First State Manual on Implementation of MST Methods***

The state of California has spent approximately $100 million since 2001 to improve beach water quality at impaired recreational sites. Despite these efforts, several locations still frequently exceed local WQS based on FIB measurements alone, mostly due to poor information on contamination sources leading to inadequate cleanup strategies. Advances in science and the need for fecal source pollution information led the California Clean Beach Initiative program to fund research efforts with the aim to develop a state manual for implementing MST methods (Griffith et al., 2013). This pioneering manual outlines a tiered, “tool box” approach to implement MST methodologies based on a hypothesis-driven, science-based strategy that provides a foundation for implementing MST technologies on a national level.

## E. Emerging Issues: Evidence of Exposure to Antimicrobial Resistant Bacteria in Recreational Waters

### 1. Introduction

Within the past several years, an increasing body of research indicates the environment has become not only a recipient of drug-resistant bacteria, but also a reservoir for and a source of resistance genes (Martinez, 2009; Wright, 2010; U.S. EPA 2013e; Berendonk et al., 2015). Drug-resistant bacteria and associated genes have become an emerging concern regarding the protection of human health during recreational activities in surface waters. Contaminated wastewater effluents, a variety of non-point sources, and even naturally occurring bacteria, are potential origins of drug-resistant or antimicrobial resistant bacteria (AMRB) and antimicrobial resistant genes (ARG) in recreational waters. Environmental surveillance is a key tool in furthering the understanding of AMRB/ARG and in participating in the One Health approach to this growing global issue of concern that incorporates human health, animals, and the environment, as recommended in the National Antimicrobial Resistance Monitoring System[3] program. Additionally, future research on recreational waters should include: 1) human health risk assessment strategies for various exposure scenarios; 2) removal of AMRB/ARG from wastewater treatment processes and disinfection; 3) environmental selection for resistance; and 4) mitigation strategies for preventing the loading of AMRB/ARG into recreational waters.

### 2. Antimicrobial Resistance Mechanisms

Although drug-resistance genes are naturally occurring, anthropogenic releases of antibiotics and ARG through clinical and agricultural use represent a much larger concern for human and ecological health. Contaminants such as heavy metals and pharmaceuticals are also reported to exert selective pressure that can result in co-selection for antibiotic resistance in the environment (Wellington et al., 2013; Baker-Austin et al., 2006). The dispersion of resistance throughout the environment occurs through loading of wastewater effluent and discharges, application of animal waste to land, horizontal gene transfer (HGT) among bacteria, and gene selection via environmental conditions. HGT is the primary mechanism of concern in the environmental dispersion of drug-resistant bacteria. When resistant bacteria are released into the environment, they can share their resistance genes with native bacteria and pathogens via HGT. HGT occurs in one of three ways: (1) uptake of genetic material from the environment—transformation; 2) direct transfer of genetic material from one cell to another—conjugation; or 3) movement of genetic material from one cell to another via a bacteriophage vector (Burmeister, 2015). Very small concentrations of antibiotics in polluted environments could enable the selection for resistant and multi-resistant genes using HGT mechanisms, which could result in the maintenance or increase of concentration of ARGs (Gullberg et al., 2014; Baquero and Coque, 2014).

[3]The National Antimicrobial Resistance Monitoring System is a Food and Drug Administration program to promote and protect public health. This ongoing collaboration with CDC and U.S. Department of Agriculture aims to work within a One Health approach to address AMR challenges.

## 3. Point Sources and Non-point Sources

The EPA categorizes pollutant sources as either point sources or non-point sources for regulatory purposes. Point sources can include discharges from WWTPs, industrial facilities, and concentrated animal feeding operations (CAFOs) (U.S. EPA, undated). Municipal sewage, which is treated in WWTPs, can contain waste from households, storm drains, and hospitals. A wide variety of products that contain antibacterial compounds, such as triclosan, are now sold and used in households beyond the more common antibacterial hand soaps and cleaning products, ranging from toys to kitchen tools (Aiello and Larson, 2003). Studies indicate that antibacterial agents in soap and other household products select for environmental microflora that allow ARGs to thrive (Levy, 2001).

Hospital wastewater carries diverse communities of pathogens and pharmaceuticals, entering surface waters, either directly or indirectly through municipal sewage systems or after hospital pretreatment. Originating in hospitals, pathogenic bacteria with resistance against all or almost all of our existing antibiotic treatments are of increasing concern. For example, carbapenem-resistant Enterobacteriaceae (CRE) has been declared the highest priority by CDC, a critical pathogen of concern. A healthcare-associated infection that infects hospitalized patients, CRE is caused by *Klebsiella* and *E. coli* bacteria (CDC, 2013) and has been found in significantly higher concentrations in hospital wastewater as compared to municipal wastewater (Lamba et al., 2017). CRE and other drug-resistant microbes can spread into surface water and from there into recreational waters, through insufficient treatment of hospital wastes. In some cases, before hospital wastes are discharged into sewage systems, local authorities may regulate pollutant levels via the National Pretreatment Program to prevent overloading publicly owned water infrastructure with heavy loads of contaminants.

Regarding WWTP controls, the extent to which drug-resistant bacteria might survive wastewater treatment or pretreatment processes is not well established. The potential is multifaceted—mobile elements, bacteriophage, naked DNA of killed cells, or bacterial cells that have survived chlorination could be released into waterways (Rizzo et al., 2013; Gantzer et al., 1998). Tertiary-treated effluent has been found to contain high levels of antibiotic resistance determinants, at 20 times the concentrations of background levels (Lapara et al., 2011). One study in northern China found that wastewater effluent contained a higher concentration of ARG than influent at the same plant (Mao et al., 2015). The effect was associated with heavy metals and antibiotic residues in wastewater, indicating that these conditions might select for a proliferation of resistance, not removal of it (Mao et al., 2015). Seasonal disinfection practices (e.g., chlorinating only in summer) at certain WWTPs could influence levels of bacteria (Mitch et al., 2010) and AMRB entering surface waters via effluent discharge. In addition, sewer overflows are of concern due to the concentration of untreated pharmaceuticals and AMRB/ARG flowing unrestricted into surface waterbodies during rain events (Scheurer et al., 2015).

CAFOs may receive high volumes of antibiotics used for animal growth promotion, regular disease prevention, and treatment (U.S. EPA, 2013e). Here, high usage of antibiotics selects for resistant bacteria both within the animals and environment (Hribar, 2010). Additionally, of all

antimicrobials used in food-animal production, an estimated 25–75% of the drugs is excreted unchanged into waste (Kummerer, 2004). In 2005, the U.S. Department of Agriculture reported that CAFOs produced roughly 44 times more solid waste than WWTPs (Graham and Nachman, 2010). The EPA estimates "nearly all" of produced CAFO waste is used for land application (U.S. EPA, 2004), which creates a potential risk that bacteria and antibiotic residues may run off to surface waters depending on compliance with established permitting requirements. Of the surface water samples taken in a farm environment study, 81% of *E. coli* isolates exhibited resistance to cephalothin (Sayah et al., 2005). Near a concentrated swine operation, Sapkota et al. (2007) found down-gradient surface water contained 33-fold higher median levels of enterococci and *E. coli* compared to up-gradient. These down-gradient samples also had a higher percentage of erythromycin and tetracycline resistance (Sapkota et al., 2007).

Non-point sources constitute diffuse sources of antibiotics, resistance genes, or resistant bacteria that generally enter a waterbody via runoff, drainage, seeping, or precipitation (U.S. EPA, 2017b). Animal feeding operations (facilities or lots that do not meet the regulatory definition of a CAFO are not considered a point source) may further contribute to the contamination and spread of AMRB/ARG in the environment (U.S. EPA, 2013e). Land application of waste and manure from animal feeding operations adds antibiotics and resistant genes to soils and water (Hamscher et al., 2002; De Liguoro et al., 2003). High-volume usage of these antibiotics, such as tetracycline, results in leakage to groundwater and surface water supplies, disturbing bacterial communities and promoting resistance (De Liguoro et al., 2003). Although non-point sources carry a smaller concentration of resistant strains compared to point sources, their role remains an important consideration (Parveen et al., 1997).

Birds, particularly seagulls, play a role in the movement of and exposure to AMRB/ARG. Seagulls have been shown to carry extended-spectrum β-lactamase (ESBL) producing *E. coli* (Simöes et al., 2010), and multidrug resistance has been found in other wild birds (Sjölund et al., 2008; Cole et al., 2005). Compared to other bird species, gulls (*Larus* spp.) are significantly more likely to carry disease-causing pathogens, due to their tendency to forage on anthropogenic waste (Fenlon, 1981; Alm et al., 2018; Belant et al., 1998). Bacterial transport by gulls is associated with sewage outfalls, indicating that effluents containing AMRB are more likely to cause dissemination of that bacteria via gulls (Fenlon, 1981). A study in France found that 9.4% of the gulls observed were carrying ESBL-producing bacteria (Bonnedahl et al., 2009). Additionally, Alm et al. (2018) found evidence that gulls act as transport vectors, picking up human pathogens from anthropogenic waste sites at landfills and wastewater outputs, and spreading these pathogens to recreational waters and beaches.

## 4. Evidence of Recreational Exposure

Exposures to AMRB/ARG have been documented at beaches and in recreational waters globally. Prospective cohort epidemiological studies on three California beaches correlated the detection of a variety of indicators, AMRB, and pathogens with incidence of gastrointestinal (GI) illness (Griffith et al., 2016). Methicillin-resistant *Staphylococcus aureus* (MRSA) was highly associated with GI illness, showing a stronger correlation than the EPA's current culture method

(EPA Method 1600) at the beach where it was measured, which was impacted by human sewage from faulty infrastructure (Griffith et al., 2016). This work highlights potential risks associated with AMRB in recreational waters impacted by human sewage and indicates that recreators could in some cases be exposed to MRSA (Griffith et al., 2016).

More recently, AMRB surveillance was combined with human exposure estimates to quantify the probability of exposure to *E. coli* harboring $_{blaCTX-M}$ genes in coastal waters. These genes represent nearly 80% of all ESBL-producing *Enterobacteriaceae*, which confer resistance to multiple antibiotics, such as fluoroquinoloenes, aminoglycosides, and tetracyclines. Authors conducted a cross-sectional epidemiology study comparing regular surfers and non-surfers to evaluate the association between water exposure and gut colonization by *E. coli* harboring gene $_{blaCTX-M}$. Results indicated that 6.3% of surfers were colonized by the gene, compared to 1.5% of non-surfers (risk ratio = 4.09; CI 1.02-16.4) (Leonard et al., 2018).

Schijven et al. (2015) measured concentrations of ESBL-*E. coli* (ESBL-EC) in recreational waters and in source waters, including ditches surrounding poultry farms and municipal wastewater. Using this information, they modeled the potential of EBSL-EC to reach recreational waters and thus the and the probability of human exposure through swimming. They found exposure to ESBL-EC by swimming is likely, when recreational waters are located downstream of wastewater treatment plants or livestock farms and noted that research is warranted for the evaluation of public health effects, such as colonization, infection, or horizontal gene transfer, upon exposure.

Studies have also shown *E. coli* and enterococci persisting in sand are capable of not only surviving in sandy environments but also replicating (Hartz et al., 2008: Haack et al., 2003: Whitman and Nevers, 2003: Alm et al., 2006). In recent years, evidence has grown that AMRB/ARG also survive and replicate in sand. Studies in Puerto Rico and the United Kingdom found recreational waters and sand could be reservoirs for resistance genes and estimated human exposures to resistant bacteria while swimming (Santiago-Rodriguez et al., 2013; Leonard et al., 2015). In 2014, a Michigan-based study captured HGT of resistance genes among *E. coli* within sand microcosms of recreational freshwater beaches of Lake Huron (Alm et al., 2014).

## 5. The EPA's Work on AMR for Recreational Waters

In 2001, the EPA and 10 other federal agencies formed the Interagency Task Force on Antimicrobial Resistance (IFTAR, 2001; Colson, 2010). The EPA has conducted surveillance research related to AMRB/ARG in wastewater and ambient waters. Initially, the EPA studied the occurrence of *E. coli* resistant to a variety of clinically relevant antibiotics in primary wastewater effluents (Boczek et al., 2006, 2007). Subsequent research has focused on the occurrence of *E. coli* isolates in primary wastewater effluents that meet the CDC definition of CRE and the presence of different carbapenemase genes associated with CRE globally (estimated completion date: Summer 2018).

As part of the National Rivers and Streams Assessment (NRSA 2013–2014), the EPA is enumerating ARG in rivers and streams across the United States using droplet digital PCR. The

EPA has begun investigating gene targets, including but not limited to, beta-lactam, carbapenem, tetracycline, and colistin, and other genetic markers of AMRB.

## F. Assessment of Recreational Criteria Implementation and Tools

This section discusses the advances in implementation tools, the status of 2012 RWQC adoption, and perceived barriers to adoption. In Section 6.0 of the 2012 RWQC document, the EPA discussed two important tools for evaluating and managing recreational waters, sanitary surveys, and predictive models. With the publication of the criteria document and the *National Beach Guidance and Required Performance Criteria for Grants, 2014 Edition* (U.S. EPA, 2014a), the EPA has encouraged states and beach managers to use both tools to protect public health.

### 1. Sanitary Surveys

As a widely used tool for investigating the sources of fecal contamination impacting a waterbody, sanitary surveys are important to understanding watersheds and beaches. Sanitary surveys involve collecting environmental, meteorological, physical, and water quality data at the beach and in the surrounding watershed. Sanitary surveys help state and local beach program managers and public health officials identify and characterize sources of beach water pollution. By identifying, assessing, and mitigating pollution sources, states can reduce or eliminate beach advisories and closures.

Beach managers can use sanitary survey results to prioritize state or local resources to help improve recreational beach water quality. Routine or daily sanitary survey data (e.g., bacteria levels, source flow, turbidity, rainfall) also can be used to develop models for predicting beach water quality using readily available data.

Since the publication of the 2012 RWQC, the EPA has published two new sanitary survey tools: a paper and an electronic version of the Marine Beach Sanitary Survey. In 2013, the Agency published the Marine Beach Sanitary Survey (U.S. EPA, 2013a) for marine waters. This survey is based on the Great Lakes Beach Sanitary survey (U.S. EPA, 2008) for fresh waters with minor modifications to include factors important in marine environments (e.g., tidal phase and flow, rip currents). Like the Great Lakes Beach Sanitary Survey, the Marine Beach Sanitary Survey consists of two forms—the Routine On-site Sanitary Survey (U.S. EPA, 2013b) and the Annual Sanitary Survey (U.S. EPA, 2013c) to help states conduct both short- and long-term beach assessments. The Routine On-site Sanitary Survey is conducted at the same time water quality samples are taken and includes a form for documenting the methods used to collect data during the survey. The Annual Sanitary Survey records information about factors in the surrounding watershed that might affect water quality at the beach, including, for example, information on septic tank locations and conditions, land use information, and other observations relating to long-term of water quality impacts within the watershed. Like the Great Lakes Sanitary Survey, the Marine Beach Sanitary Survey can be used to address a variety of beach management uses including:

- Characterizing risk and prioritizing beaches

- Beach and watershed planning
- Remediation
- Predictive modeling

In September 2016, the EPA released a mobile app of the routine marine beach sanitary survey form for use on Android and Apple tablets. Sanitary surveys routinely provide valuable and useful environmental information that can be paired with water quality data and to develop predictive models. The EPA's goal with the development of this app is to provide a tool that would make both the collection and transfer of environmental and water quality data easy for model development purposes.

Sanitary surveys are now a widely used element of state beach programs on both coasts and in the Great Lakes. With the EPA's Great Lakes Restoration Initiative and BEACH Act grant funds, Great Lakes states have been able to reduce significantly the percentage of sources previously identified as "Unknown" that impact their beaches using sanitary surveys. Once identified and characterized, sources can then be targeted and prioritized for remediation, leading to fewer exceedances and advisories and to overall improved local water quality.

## 2. Statistical Modeling for Predictive Estimates of Water Quality

In the 2012 RWQC, the EPA encouraged the use of predictive models to supplement water quality monitoring using culture methods and to enable timely beach notification decisions. Predictive modeling uses past water quality data and current observed hydrometeorological data to estimate water quality at a given time. Predictive models enable assessment of the risk to human health from exposure to both human and non-human sources impacting beaches and their associated watersheds.

### *Virtual Beach*

In developing the 2012 RWQC criteria, the EPA conducted research and published a two-volume survey report to advance the use of predictive models. Volume I (U.S. EPA, 2010a) describes the types of predictive tools (e.g., statistical models, rainfall thresholds, and notification protocols) that can be used to make beach notification decisions and how they are being used as part of beach management programs across the United States. Volume II (U.S. EPA, 2010b) provides the results of EPA research on the development of statistical models at research sites. Volume II also introduces Virtual Beach (VB), a software package and statistically based decision tool that allows users to build site-specific statistical models to predict FIB levels at recreational beaches. VB reads input data from a text or Excel file, assists users in preparing data for statistical analysis, and provides three analytical techniques for model development.

Since the publication of criteria, the EPA has released several versions of VB and has made several enhancements to this tool. The current version of VB, $VB_{3.0}$, includes statistical methods that provide users more flexibility in modeling datasets. In addition to multiple linear regression (MLR), which was the only original option, users can now use partial least squares (PLS) regression and generalized boosted modeling (GBM) algorithm to fit their data and make

predictions. PLS regression reduces overfitting in the presence of correlated predictors, an issue that can arise with MLR. GBM is a machine-learning technique that uses decision/regression trees instead of linear equations. It enables accurate predictions for new observations without overfitting and handles nonlinear relationships between response and independent variables without having to transform the independent variables. One drawback of GBM is that the model cannot be inspected graphically or expressed mathematically. Both PLS and GBM modeling use cross-validation to evaluate real-world prediction accuracy (Cyterski et al., 2013). GBM has been shown across a wide range of datasets to outperform an array of other statistical methods in recent years (Corsi et al., 2016).

Other improvements to VB include automated interaction with the USGS Environmental Data Discovery and Transformation (EnDDaT) system for site-based data acquisition; a genetic algorithm for intelligent search of parameter space for MLR modeling; probability of health criteria exceedance calculations for model predictions; and cross validation of MLR models to assess predictive capabilities (Cyterski et al., 2013).

### *Recursive Partitioning Based Models*

Recursive partitioning is an alternative to parametric regression methods. In recursive partitioning, a decision tree is used to model the response variable by splitting the observations into subgroups that share similar values of the response variable and similar values of associated covariates nodes. Modeled outcomes are obtained by answering an ordered sequence of questions, with the question asked at each step dependent on answers to previous questions. Recursive partitioning can be used to predict pathogen occurrence (categorical response) or concentration (continuous response) in a variety of applications using covariates that are easier to measure (e.g., FIB, water quality parameters) than direct pathogen analysis (Bradshaw et al., 2016; Wilkes et al., 2011).

### *Other Research and Guidance*

Other methods to achieve better predictions of FIB densities in recreational waters include temporal synchronization analysis (TSA). A paper by Cyterski et al. (2012) investigated improvements in empirical modeling performance using independent variables that had been temporally synchronized with the FIB response variable. TSA investigates whether the dependent (response variable) and independent (covariates) data series are temporally phase-shifted and examines if function (e.g., mean, sum, standard deviation) of the independent variables over some temporal window can produce a better correlation to the dependent variable. Using data collected from South Shore Beach in Milwaukee, Wisconsin, results show that TSA was useful for reducing mean square error of fitted data and improved predictive model performance (as measured by the mean square error of prediction) (Cyterski et al., 2012).

In 2016, the EPA published new guidance to encourage state and local beach managers to investigate the utility of predictive models for their beach monitoring and notification programs and to assist with developing these tools. The guidance, *Six Key Steps to Developing and Using Predictive Tools at Your Beach* (U.S. EPA, 2016b), provides a simple, straightforward approach

on how to develop a predictive model for beach water quality. The guidance walks the user through each step in the process, from deciding whether a model is needed or appropriate for the user's beach (Step 1) to developing (Step 4), validating (Step 5), and evaluating the model over time (Step 6). It also provides useful hints and tips.

The benefits of predictive models for beach monitoring and notification programs have been further demonstrated by the other studies (Shively et al., 2016; Francy et al., 2013) conducted since the publication of the 2012 RWQC. Predictive models offer states, territories, and tribes the potential for same-day notification and public health protection with considerably lower capital investment and unit costs than other rapid methods.

## 3. Deterministic Process Modeling for Recreational Beach Site Assessment and Enhancement/Remediation

Whereas predictive statistical models typically rely on the regression of predictive (observational) variables against water quality data determined in a related timeframe, benefit to understanding the occurrence and timing of pollution events also can be derived from the use of deterministic process models in recreational water settings. The models used to simulate and predict contaminant transport, attenuation, and concentration are hydrodynamic process models that apply fully understood and documented process equations populated with appropriate observed variables. Model outputs are useful in demonstrating variations and movement of contaminants in response to currents, wind, and other weather variables.

Nevers and Boehm (2010) provide an overview of using deterministic models to predict FIB densities in surface waters. Nevers and Boehm underscore the value of fate and transport models for increasing and refining the understanding of mechanisms that lead to observed variations in water quality but that are not well defined. Other instances where deterministic models have been applied to water quality at recreational venues are described in U.S. EPA (2010a).

## 4. Integrated Environmental Modeling and QMRA

Epidemiological and QMRA studies have shown that elevated levels of FIB in surface waters can be associated with an increased risk of illness. The epidemiological relationships are not consistent among different water and fecal source types, however, and QMRA analyses have shown that risks can differ depending on the source of fecal material that predominates in a waterbody (Fewtrell and Kay, 2015; Soller et al., 2010a,b, 2014, 2015). The pathogens responsible for the illnesses vary in their type (e.g., viruses, bacteria, protozoa) and occurrence in the source of fecal material affecting the waterbody (e.g., wastewater effluent, sewage overflows, feces from agricultural animals, wildlife). Additionally, the fate and transport characteristics of the various pathogens can differ and be affected by the way feces enters a waterbody and is transported within the waterbody and by the pathogen-host interactions at the receptor location. Integrated environmental modeling (IEM) is a framework that allows the characterization of these complex patterns by linking models, databases, and visualization tools in various ways to provide comprehensive and flexible solutions to environmental and risk management questions. IEM provides a science-based structure that develops and organizes multidisciplinary knowledge

and applies it to explore, explain, and forecast environmental system responses to natural and human-induced stressors (Whelan et al., 2014).

EPA scientists incorporated the health modeling associated with a QMRA into an IEM framework that includes automated data access retrieval and processing; integrated model databases; approaches for performing sensitivity, variability, and uncertainty analyses; and risk quantification, on a watershed scale (Whelan et al., 2014). A QMRA software infrastructure has been developed to automate the manual steps associated with a standard microbial watershed assessment (e.g., TMDL, sanitary surveys), as much as possible, to expedite the process (make faster), minimize resource requirements (save money), increase ease of use, and bring more science-based processes into the analysis. It supports watershed-scale microbial source-to-receptor modeling by focusing on animal- and human-impacted watersheds, and links to a user interface and workflow that automates data collection, collation of microbial sources, watershed delineation, and flow and microbial calibration at downstream receptors (Kim et al., 2013; Kim et al., 2016; Whelan et al., 2018). A process that normally has taken months or even years to complete now can be completed within days, depending on source data availability. The software contains source information to support a sanitary survey by linking pollution sources, physical features, land-use, etc., as they vary with time (Whelan et al., 2017a). By integrating watershed fate and transport models with the health models describing health risks and exposure, policy-related issues can be iteratively explored in ways that the traditional empirical approaches do not allow. Furthermore, the IEM framework with QMRA provides a platform that facilitates transparency and reproducibility, supporting the evaluation and management of watersheds. Finally, the software can be used to help develop site-specific water quality criteria that differ from the EPA's recommended criteria.

Some of the components necessary to integrate the QMRA into the IEM framework include:

***Microbial Source Module***

The Microbial Source Module determines microbial loading rates associated with 1) land-applied manure on undeveloped areas from domestic animals; 2) direct shedding (excretion) on undeveloped lands by domestic animals and wildlife; 3) urban or engineered areas; and 4) discharge to streams from leaking septic systems and from domestic animals in animal feeding operations (AFOs) including NPDES permitted facilities (Whelan et al., 2015a; Whelan et al., 2018).

***Microbial Release Model***

Mathematical models were developed to describe the physics to predict the release of microbes from fresh and aged manure—cattle solid pats, poultry dry litter, and liquid waste from swine—during rainfall events, which helps improve microbial loading estimates in mixed-use watersheds (Kim et al., 2013; Whelan et al., 2017b).

***Microbial Properties Database Editor***

A Microbial Properties Database Editor is an interface to a database that bridges the gap between monitoring and modeling, as it was developed to capture microbial-relevant data used by

QMRA-related process-based source, fate, transport, and risk models. Users can modify physico-microbial properties related to indicators and pathogens, such as mass of a microbe, excretion density of microbes in animal feces, prevalence, etc. Microbial properties are related to changes in or with the microbe such as inactivation rate, dose-response coefficients, attachment/detachment rates, etc. (Whelan et al., 2015b).

***Microbial Inactivation Model***

The solar photo-inactivation model provides FIB, virus, and pathogen inactivation rates required by predictive models and QMRA in recreational waters, accounts for ultraviolet wavelength effects, is based on aquatic and atmospheric parameters, and accounts for variability of photo-inactivation over space and time in Great Lakes and other recreational waters. For example, depth dependence and time dependence of inactivation rates of indicator enterococci were estimated following rainfall events in the Manitowoc River and Manitowoc, Wisconsin beaches using the model and data collected at river and beach sites. This model was also used to predict photo-inactivation rates at three beaches and tributaries located in southern Lake Michigan and Lake Erie.

EPA scientists have been applying the IEM framework to characterize fecal sources and water quality in specific watersheds (e.g., Manitowoc, Wisconsin, Tillamook, Oregon), including evaluating seasonal dynamics of sources and relative risks from various source in the watershed. These efforts have supported the development of the framework and calibration of the model with real, site-specific data.

## 5. Adoption Status and Perceived Barriers

As mentioned earlier in this report, Congress directed states and tribes with coastal recreation waters to adopt new or revised WQS within 36 months of the EPA's publication of the 2012 RWQC. (CWA Section 303(i)(1)(B)). WQS are the foundation for a wide range of programs under the CWA. They serve multiple purposes including establishing the water quality goals for a specific waterbody, or waterbody segment, and providing the regulatory basis for deriving water quality-based effluent limits beyond the technology-based levels of treatment required by CWA sections 301(b) and 306. WQS also serve as a target for CWA restoration activities such as TMDLs. The WQS regulation at 40 Code of Federal Regulations part 131 describes the requirements and procedures for states and authorized tribes to develop, adopt, review, revise, and submit WQS and the requirements and procedures for the EPA to review, approve, disapprove, or promulgate WQS as authorized by section 303(c) of the CWA.

In addition to recommending the FIB and criteria values for magnitude, duration, and frequency in the 2012 RWQC document, the EPA also provided states with BAVs for use in notification programs. The state, tribal, or local government entity responsible for ensuring public health typically uses BAVs to make decisions about whether swimming or engaging in other primary contact reaction in their waters is safe.

***Status of Adoption of the 2012 RWQC into WQS***

The EPA reviewed the current recreational WQS in effect for CWA purposes in all 50 states, five U.S. Territories, and the District of Columbia and for the three authorized tribes that receive BEACH Act grant funds. For this discussion, the term "jurisdiction" refers to these entities.

- Seventeen jurisdictions have adopted and the EPA has approved revised RWQC for all primary contact waters; three additional jurisdictions have adopted and the EPA has approved revised RWQC for just their coastal recreation (i.e., BEACH Act) waters).
- Of the 38 BEACH Act jurisdictions, 14 are using the recommended 2012 RWQC BAV (e.g., 70 enterococci, 235 *E. coli*) for their coastal recreation waters and the remaining 24 are using an alternative Beach Notification Threshold (BNT), often the SSM value derived from the 1986 Criteria (e.g., 104 cfu/100 mL enterococci, 235 cfu/100 mL *E. coli*).
- Four jurisdictions have only fecal coliform in their WQS as their FIB; nine additional jurisdictions use fecal coliform as the only FIB for some but not all of their waters designated for primary contact recreation (e.g., they use enterococci as the FIB in their marine waters and fecal coliform in their fresh waters).

For the elements of the 2012 RWQC (magnitude, duration, and frequency and FIB), the replacement of fecal coliform with either *E. coli* or enterococci as the FIB is the most commonly identified need. Most jurisdictions, however, have revised the FIB for their BEACH Act waters and are considering revising FIB for all waters in the near future. The STV is the second element most commonly found to need revision, and that is related to multiple use categories discussed in the next section. Use of a BAV/Alternate Beach Notification Threshold for swimming advisories has been almost universally implemented in BEACH Act jurisdictions.

***Barriers to Adoption of the 2012 RWQC***

The EPA interviewed 34 of the 38 BEACH Act jurisdictions and several additional inland states. The goal of these interviews was to discuss the status of the adoption of 2012 RWQC into their standards and to identify any barriers to adoption.

- Adoption is a lengthy process

Jurisdictions noted that revisions to WQS are an administrative burden, and jurisdictions have limited resources. The CWA requires revisions to be scientifically defensible and protective of public health. Once a jurisdiction has evaluated the revisions based on these factors and any state regulatory administrative requirements, they then seek public and EPA input on the draft revisions. In addition, because the RWQC are intended to protect public health, state/tribal processes often require two agencies to be involved in the revisions: the public health department and the state/tribal environmental agency.

- Pre-2012 RWQC are protective

Jurisdictions believe that RWQC based on the 1986 Criteria do not significantly differ from the 2012 RWQC. They have expressed concern that they offer little or no benefit to public health as

compared to the administrative burden needed to adopt because their current criteria are similar to the 2012 RWQC. The EPA continues to believe that all the elements of the 2012 RWQC meaningfully strengthen them as compared to previous criteria recommendations.

- Multiple magnitude values based on use intensity

Another barrier to adopting the jurisdictions mentioned is related to eliminating the use intensity paradigm that was part of the 1986 Criteria and the EPA's 2004 promulgation for coastal recreation waters. As discussed earlier in this document, in the 2012 RWQC, the EPA removed the use intensity categories that had differing magnitude values depending on how likely the waterbody was to be frequently visited. The categories included designated bathing beach, moderate use, light use, and infrequent use. Some jurisdictions have designated all their waters as primary contact recreation. States and tribes (and their stakeholders) are concerned that primary contact recreation might not be attainable in their less frequently used waters and these waters will have to be listed as impaired under CWA section 303(d). Revising a waterbody's designated use, however, is also a potentially administratively burdensome process. Therefore, these jurisdictions find the elimination of the use intensity categories as a barrier to statewide adoption of the 2012 RWQC.

- Need for additional criteria guidance

A few jurisdictions mentioned that the EPA's implementation documents released with the 2012 RWQC were helpful, but they are struggling with development of site-specific criteria and alternative Beach Notification Thresholds. They also noted that the release of the EPA's expected guidance documents on QMRA would help them evaluate the specific pathogenic risks at a particular primary contact recreation waterbody and support site-specific RWQC development.

Based on these discussions, the EPA concludes that additional support to jurisdictions through the continuation of BEACH Act grants that support program implementation and additional EPA guidance would encourage additional adoption of the 2012 RWQC.

## G. Recreational Criteria for the Cyanotoxins: Microcystins and Cylindrospermopsin

In December 2016, the EPA published draft recommended values for microcystins and cylindrospermopsin under CWA 304(a) for states to consider as the basis for swimming advisories for notification purposes in recreational waters to protect the public or for adopting new or revised WQS. The *Human Health Recreational AWQC or Swimming Advisories for Microcystins and Cylindrospermopsin* focuses on the health risks associated with recreational exposures in waters containing these cyanotoxins produced by cyanobacteria.

Cyanobacteria, also commonly referred to as blue-green algae, are photosynthetic bacteria that are ubiquitous in nature, including occurrence in surface waters. Microcystins, a class of cyanotoxins including over 100 congeners, and cylindrospermopsins can be produced by multiple genera of cyanobacteria commonly found in fresh waters of the United States (U.S. EPA, 2016a).

Cyanotoxin-producing cyanobacteria fit the definition of pathogenic (*i.e.*, disease-causing) organisms (Stewart et al. 2006). Cyanotoxins have the potential to cause direct damage to multiple targets within the body (*e.g.*, liver toxicity, kidney toxicity, adverse neurologic and reproductive effects, etc.) and can result in severe adverse outcomes for people who are exposed. For microcystins, the primary adverse health effect of concern is liver toxicity and for cylindrospermopsins, kidney toxicity; other potential health endpoints have been noted for both toxins (U.S. EPA, 2015c,d). Direct contact with cyanobacterial cells, either dermally, ingestion or by inhalation, can elicit an allergenic response in those exposed resulting in itchy rashes, eye irritation, gastrointestinal distress and respiratory symptoms (Bernstein et al. 2011; Levesque et al. 2014; Geh et al. 2015).

Environmental conditions that promote excessive growth of cyanobacteria in surface waters can lead to situations in which cyanotoxins are produced or cyanobacterial cell density is high, or both, known as harmful algal blooms (HABs). Environmental factors that play an important role in the development of cyanobacterial blooms and their production of cyanotoxins include the levels of nitrogen and phosphorus, the ratio of nitrogen to phosphorus, temperature, organic matter availability, light attenuation, and pH. Cyanotoxins can be produced before an HAB reaches visibly high cell densities and, once produced, these cyanotoxins can persist even after a bloom is no longer visible. Given that cyanobacterial blooms typically are seasonal events and can be short term, recreational exposures are likely to be episodic.

The EPA evaluated the health effects of microcystins and derived a reference dose (RfD) in its 2015 *Health Effects Support Document for the Cyanobacterial Toxin Microcystins* (U.S. EPA, 2015c). Exposure to elevated levels of microcystins could lead to liver damage. The critical study for the derivation of the microcystins RfD was conducted by Heinze et al. (1999) based on rat exposure to microcystin-LR in drinking water. The critical effect dose from this study was slight-to-moderate liver necrosis, and levels of liver enzymes associated with tissue damage. The EPA established the RfD based on microcystin-LR and used it as a surrogate for other microcystin congeners. The RfD was used to derive the EPA's previously published Drinking Water Health Advisories (U.S. EPA, 2015a,b) and the recommended values in this document. The critical dose and effects used to establish the RfD from Heinze (1999) are supported by a Guzman and Solter (1999) study, also conducted in rats.

The EPA evaluated the health effects of cylindrospermopsin and derived an RfD in its 2015 *Health Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin* (U.S. EPA, 2015d). The kidneys and liver appear to be the primary target organs for cylindrospermopsin toxicity. The critical study for the derivation of the cylindrospermopsin RfD was conducted by Humpage and Falconer (2002, 2003) based on drinking water exposure to mice. The critical effect was kidney damage, including increased kidney weight and decreased mouse urinary protein. Mouse urinary proteins are synthesized in the liver (U.S. EPA, 2015c).

Exposure to cyanobacterial cells has also been linked to multiple inflammatory health effects. At this time, available data are insufficient to develop nationally recommended recreational values for cyanobacterial cell density related to inflammatory health endpoints. The reported

epidemiological relationships in the literature are not consistent for specific health outcomes (e.g., dermal symptoms, eye/ear irritation, fever, GI illness, and respiratory symptoms) or for those health outcomes associated with specific cyanobacterial cell densities. The uncertainties related to the epidemiological study differences, such as study size, species, and strains of cyanobacteria present, and the cyanobacterial cell densities associated with significant health effects, do not support the development of a single cell value applicable to all recreational waters. For more information on HABs, see https://www.epa.gov/nutrient-policy-data/cyanobacterial-harmful-algal-blooms-water. See https://www.epa.gov/wqc/microbial-pathogenrecreational-water-quality-criteria for information on the draft Recreational AWQC for microcystins and cylindrospermopsin and the final AWQC when they become available. The EPA also recently made available information for recreational water managers to use for monitoring and responding to cyanobacteria and cyanotoxins in recreational waters, see https://www.epa.gov/nutrient-policy-data/monitoring-and-responding-cyanobacteria-and-cyanotoxins-recreational-waters.

# V. Summary and Priorities for Further Work

In this review of the 2012 RWQC, the EPA has assessed the extent of scientific progress in the field of human microbial health risks associated with exposure to fecal pollution from swimming and other use of recreational waters. There has been considerable progress in many areas. The EPA and other organizations have invested heavily in the science that was the basis for the 2012 RWQC. The progress since publication in 2012 has led to increases in the utility and level of function of technologies and approaches that provided data for developing the 2012 RWQC. A clear example of this progress is the development of qPCR EPA Method 1609 for the enumeration of *Enterococcus* spp. This refined method greatly reduces the impact of method interference encountered with EPA Method 1611. Further, the development of an qPCR method for *E. coli*, the indicator more commonly used and preferred by states in the Great Lakes, will leverage the use of qPCR in those important waters. Developments such as these will lead to more timely estimates of water quality and reduced risk to swimmers. This section provides highlights of the scientific and implementation reviews and describes the conclusions and priorities for further work. The additional work described below will help inform future reviews of the RWQC.

## A. Health Studies (see section IV.A for more information)

Additional health studies pertaining to the basis for the 2012 RWQC provided confirmation of these findings:

- Both epidemiological and QMRA-based studies provide scientifically defensible estimates of human health effects from exposure to waters contaminated by feces.
- In waters affected by human fecal contamination, GI illness is the most sensitive health endpoint reported in epidemiological studies.
- Children can be more highly exposed and have greater susceptibility to swimming-associated GI illness.
- Waters affected by some non-human sources could pose less risk compared to human fecal contamination.
- *Enterococcus* spp. qPCR and coliphage are associated with GI illness at sites impacted by human sources.
- Norovirus infection and transmission are associated with swimming.

Findings on health studies are generally consistent with the findings of studies that formed the basis for the 2012 RWQC, and enhance the depth and strength of the evidence underlying the RWQC. A growing body of evidence suggests that children can be disproportionately susceptible to health effects resulting from exposure to pathogens in recreational waters. There are opportunities for further resolution of epidemiological relationships, especially in the area of children's health protection and wider application of *Enterococcus* spp. qPCR.

Priorities for Further Work: Re-analysis of epidemiological data to assess differences in risk to children. Re-analysis of *Enterococcus* spp. qPCR data for consideration in criteria development, especially to address effluent sources. Also, evaluate how QMRA can be used to address risk to children from swimming exposure, and other regulatory purposes.

### B. Developments for Coliphage, Including Analytical Methods (see section IV.B for more information)

An area in which the EPA has invested considerable resources is the exploration of a viral indicator-based RWQC. The EPA has conducted a literature review for the prospects of coliphage RWQC and published *Review of Coliphages as Possible Indicators of Fecal Contamination for Ambient Water Quality* (U.S. EPA, 2015). Other important milestones completed include:

- Systematic literature reviews of viral densities in raw sewage and ambient waters
- Development of quantitation methods for coliphage
- Methods for culturable coliphage enumeration
    - Draft Method 1642 – ultrafiltration + single agar layer.
    - Draft Method 1643 – single agar layer.
- Presenting findings (e.g., at 2016/2017 UNC Water Microbiology Conferences)
- Application of methods to 2015 Great Lakes Study, and consulting outside experts in the field
    - 2016 Coliphage Experts Workshop.
    - Fact Sheet and Peer-reviewed Meeting Proceedings.

Because evidence suggests most illnesses in recreational waters are due to enteric viruses, the development and implementation of coliphage as a viral indicator will likely yield improvements in public health protection.

Priorities for Further Work: Completion and publication of coliphage methods and development of coliphage-based RWQC for inclusion into the "tool box."

### C. Analytical Methods (see section IV. C. for more information)

When introduced with the 2012 RWQC, EPA Method 1611 represented a major advance in microbial detection and quantitation methodology. Advances that produced EPA Method 1609 eliminated much of the concern about method interference and greatly increased the acceptance of qPCR methods by the recreational water community. The advances in methods include specific improvements:

***EPA Method 1609 for Enterococcus spp.***

- Provides the same results as EPA Method 1611 but with less sample interference in most situations and is recommended over EPA Method 1611. Recommendation to use undiluted samples compared to EPA Method 1611 for enhanced sensitivity.
- Updated EPA Method 1609.1 (and EPA Method 1611.1) facilitate standardization of results by referring to standardized DNA reference materials available from the EPA and a standardized Excel workbook for performing calculations.

***EPA method for E. coli (draft Method C)***

- Incorporates the same interference control modifications as EPA Method 1609.
- Recent nationwide field studies have suggested similar low frequencies of interference as with Method 1609.

The advances in qPCR methodology since 2010 have brought greater reliability and utility to beach monitoring programs where they have been implemented, yet opportunities remain for further refinement of qPCR methodologies. *Enterococcus* spp. measured by qPCR, is more predictive of swimming-associated GI illness and more timely than current culturable bacterial indicators. These factors coupled with a greater distribution of qPCR-capable laboratories in the future are likely to lead to enhanced public health protection.

Priorities for Further Work: Completion of method validation and publication for the *E. coli* qPCR method (*Draft* Method C), development of alternative site-specific criteria for *Draft* Method C, additional training and capacity-building in qPCR laboratories in states, tribes, and localities.

### D. Microbial Source Tracking (see section IV. D for more information)

Some of the most significant advances in RWQC research have occurred in the field of MST. A limitation of the current FIB paradigm for assessing the risk of illness in recreational settings is that indicator methods do not distinguish between pollution sources, which may indicate different human health risk. FIB from non-human sources could be present in recreational waters representing a potentially lower illness risk compared to the same pollution level originating from a human source alone. In such situations, the 2012 RWQC might be over-protective. Similarly, disinfected WWTP effluent could pose a higher risk of illness than reflected by the RWQC due to the survival of disease-causing viral pathogens. Under these circumstances, the 2012 RWQC thresholds may be under-protective. Measuring enterococci with qPCR methods at the threshold values included in the 2012 RWQC document can currently be used to address this concern. The EPA continues to develop criteria for coliphage, a viral indicator, which could also be used to address this concern. Information on fecal pollution sources becomes an essential element for management and protection of public health at beaches and other recreational waters.

Numerous advances in MST such as the development of host-associated genetic technologies (e.g., HF183/BACR287, and HumM2) with a high degree of specificity and sensitivity are improving recreational water management. A growing list of MST studies are demonstrating the

potential role of rainfall in fecal water pollution. In addition, multiple studies show that genetic MST methods can be highly reproducible when standardized procedures are used. It is clear that fecal source identification technologies are a valuable addition to the recreational water quality assessment "tool box."

Accurate and reliable MST technologies could markedly improve water quality management in the United States by allowing the development of alternative site-specific criteria based on the differences in risk across sources and identifying opportunities for source remediation. Use of alternative water quality metrics, such as human markers, might be helpful to inform risk levels in wet weather conditions.

Priorities for Further Work: Completion and publication of standardized methods for EPA human-associated MST methods (HF183/BacR287 and HumM2) and completion of a DNA reference material development with NIST. Development and validation of virus-based human fecal source identification procedures. Further investigation of MST application in recreational water quality management settings such as prioritizing polluted sites for remediation based on human waste levels, identification of non-point pollution sources, and the development of alternative water quality metrics based on wet and dry weather scenarios.

### E. Antimicrobial Resistance (see section IV. E for more information)

The complex issue of antimicrobial resistance is becoming of increasing interest, creating a demand for more data to both inform our understanding of the forces driving this resistance and the actions needed to preserve bacterial susceptibility to our first-line medications. There is an increasing body of literature available on the environmental occurrence of AMRB/ARG and potential exposure in recreational waters. To develop a more complete picture regarding the threat and risks associated with antibiotic resistance, research is needed to better understand the role the environment plays in transferring AMRB/ARG to primary contact recreators. For example, additional research is needed on the incidence, associated risks, and transfer mechanisms in recreational waters, as well on the removal of AMRB/ARG by wastewater treatment processes. The EPA is in the early stages of developing a broader surveillance strategy and looking for meaningful opportunities to improve human health relating to exposures to AMRB/ARGs.

Priorities for Further Work: Development and standardization of AMRB/ARG detection methodologies. Collect information on the occurrence of AMRB/ARG in environmental waters, wastewater influent/effluents, and other potential reservoirs. Develop wastewater treatment and disinfection processes for AMRB/ARG targets. Characterize potential associated public health risks and mitigation strategies.

## F. Implementation Tools and RWQC Adoption (see section IV.F for more information)

### 1. Implementation Tools

***Sanitary Surveys***

As a widely used tool for investigating the sources of fecal contamination impacting a water body, sanitary surveys are important to understanding watersheds and beaches.

Following a wave of Sanitary Survey development and promotion in the Great Lakes 2005-2010, recent developments in sanitary surveys include the publication of the:

- Marine Beach Sanitary Survey.
- Marine Beach Sanitary Survey Mobile Application (App)

Sanitary Surveys continue to serve as an important tool for informing site remediation, characterizing waters for QMRA and site-specific criteria development, and can be linked with integrated environmental modeling.

Priorities for Further Work: Conversion of current marine sanitary survey tablet-based application to a web-based application, additional outreach on available sanitary survey applications, collaboration with Great Lakes beach programs on fresh water sanitary survey application and opportunities for integration with environmental modeling.

***Predictive/Statistical Modeling***

Predictive/Statistical models provide a means for expanding the scope of coverage of water quality measurement in area and time. There have been substantial recent developments in this area, including:

- Virtual Beach model building tool has been enhanced with data acquisition (EnDDaT), and PLS (Partial Least Squares) and GBM (Generalized Boosted Modeling) predictive calculation capabilities.
- The EPA released new guidance, *Six Key Steps to Developing and Using Predictive Tools at Your Beach* (March 2016).

Predictive models offer states, territories, and tribes an alternative for same-day notification and resulting public health protection with lower capital investment and unit costs than other rapid methods.

Priorities for Further Work: Additional support to develop predictive models in marine environments as well as models paired with newer indicators such as qPCR-based indicators.

***Deterministic Process Modeling for Recreational Beach Site Assessment and Enhancement/Remediation***

Deterministic Process Models are useful to simulate and characterize contaminant transport and attenuation. Integrated Environmental Monitoring provides a science-based structure useful in developing and organizing information to explore and forecast environmental system responses

to varying conditions. The EPA is developing several new modules related to microbial sources, release, and inactivation. Progress since 2010 includes development of new QMRA software infrastructure developed to provide risk estimates within a standard microbial watershed assessment.

These models provide a means of understanding physical forces influencing the movement of contaminants for problem definition and remediation and can include QMRA health-based models to develop site-specific criteria or evaluate remediation.

Priorities for Further Work: Development of additional training and tools to make process models and integrated environmental modeling more accessible to states, tribes and other interested stakeholders.

***Quantitative Microbial Risk Assessment (QMRA)***

QMRA is a tool for assessing and managing risks to humans from exposure to pathogens in recreational waters. This tool is an alternative to assessing microbial risk in recreational waters based on epidemiology studies, which are costly and time-consuming. QMRA can also enhance the interpretation and application of new or existing epidemiological data by characterizing various exposure scenarios, interpreting potential etiological drivers for the observed epidemiological results, and accounting for differences in risks posed by various sources of fecal contamination. Integrated environmental modeling provides a means of understanding physical forces influencing the movement of microbial contaminants for problem definition and remediation and can include QMRA health-based models to develop site-specific criteria or evaluate remediation.

Priorities for Further Work: Development of additional training and tools to make QMRA models more accessible to states, tribes and other interested stakeholders. Completion and publication of remaining QMRA guidance.

## 2. Review of RWQC Adoption Status and Perceived Barriers

States and authorized tribes have taken a range of approaches in adopting the RWQC. States have used the flexibility of the RWQC to adopt a variety of protective strategies appropriate to local conditions. Great Lakes states had only minor adjustments to beach implementation. The adoption of the 2012 RWQC has been relatively slow (17 of 38 BEACH Act jurisdictions), despite the fact that use of an approved beach action threshold was widely accepted. As a result, no states or tribes had to forgo receiving BEACH Act grant funds for lack of an approved beach notification threshold. RWQC adoption in some states is lagging due to state processes such as involvement of two agencies (e.g., department of health and department of environment) or requirements for legislative approval. Some states expressed that changes between 1986 and 2012 RWQC are not substantive enough to take on the administrative burden of criteria adoption in WQS. In addition, many states still desire the paradigm that allows different criteria for different use intensities (high use beaches vs. infrequently used recreational waters). Jurisdictions also emphasized that they need BEACH Act grants to operate and maintain monitoring programs. The message from the managers of state recreational waters programs is that for some states, adoption is only justifiable with a more pronounced change in criteria

magnitude values. The EPA notes that although the criteria magnitudes were not drastically different in the 2012 RWQC as compared to the 1986 criteria, the 2012 RWQC included additional elements that strengthen overall health protection in recreational waters and promote more consistent implementation (https://www.epa.gov/sites/production/files/2015-10/documents/rec-factsheet-2012.pdf).

Priorities for Further Work: Continued funding of BEACH Act grants. Consider additional implementation guidance and explore reconsideration of addressing differences based on frequency of use.

### G. Recreational Criteria for Cyanotoxins (see section IV.G for more information)

Another critical water quality issue that the environmental community is facing is the growing number of water bodies in the U.S. and elsewhere affected by cyanotoxins and other products of harmful algal blooms (HABs). Recreators can be exposed to cyanotoxins in ambient recreational waters leading to increased health risks. Distinct from the 2012 RWQC for fecal indicators, The EPA is working to develop Human Health Recreational AWQC or Swimming Advisories for microcystins and cylindrospermopsin, having published a Draft in December 2016 and taken public comment (period closed 3/20/17). The EPA expects to revise and publish a final document in 2018. Other researchers have found that predictive models may be useful for estimating the probability of exceeding cyanobacterial levels related to HABs.

Additionally, the EPA has made materials available for Recreational Water Managers on public messaging and notification, monitoring plans, and means of networking with key partners. Water Quality Criteria Materials available with final criteria will include frequently asked questions for assessment, listing/TMDLs/NPDES permits, and information on adoption and implementation flexibilities for the criteria.

Priorities for Further Work: Completion and publication of recreational criteria for the cyanotoxins, microcystins, and cylindrospermopsin for inclusion into the “tool box”.

## VI. Assessment of the Need to Revise the 2012 RWQC

The scientific studies and progress described in this document detail robust continuing advances in the capability of the environmental public health community to protect primary contact designated uses at beaches and in other recreational waters. This scientific progress is the continuation of a major effort by the U.S. EPA and other entities to apply modern tools, such as molecular quantification (qPCR) methods for FIB, extensive epidemiological studies, and other integrative approaches that formed the scientific basis for the 2012 RWQC.

A foundational element of the 2012 RWQC is the use of the indicator paradigm. This approach employs FIB to detect the presence of fecal material and, therefore, the risk of illness from exposure to fecal pathogens. The studies described in this report underscore the protectiveness of the 2012 RWQC and its threshold values, especially when coupled with the use of qPCR as a means of quantification as is fully described in Section IV.A. Detection of FIB using qPCR methods represents a major advance in supporting this paradigm. Advances continue to unfold with refinements to existing methods and qPCR methods for additional organisms (e.g., *E. coli*). Efforts to develop viral indicators such as coliphage (described in Section IV.B) represent a further work-in-progress to develop indicators for other pathogens of concern.

Although this report includes descriptions of many areas of evolving scientific knowledge, it is clear that there needs to be further work to allow use of this new and emerging information in recreational water quality criteria development. For example, we describe data demonstrating children ingest greater volumes of water, given their body weight, relative to adults, and may be potentially at greater risk than the general population. Going forward, the EPA will further evaluate epidemiology data in combination with other health studies and exposure information regarding risks to children to determine if changes are needed in the future.

In another example, there is notable progress in the area of microbial source tracking (MST) and fecal source identification which is employing qPCR for identification of the presence of species-specific gene segments for pollution source identification (see Section IV.D). The use of reliable human source FIB markers brings with it the possibility to resolve the ambiguity and limitations of the indicator paradigm. However, although this technology continues to advance, further work still needs to be completed (e.g., completion of analytical methods and standardization of DNA reference material) before the EPA could use it in support of CWA 304(a) recommendations for recreational water quality criteria.

Additionally, new work is in progress, but not yet completed, to develop recreational criteria/swimming advisory values for cyanotoxins, which are contaminants of emerging concern in recreational waters. While not envisioned in the BEACH Act of 2000, these contaminants directly affect users of recreational waters, and their inclusion represents an integrative approach to health protection.

Specific areas remain where additional progress is needed to support potential future revisions of the 2012 RWQC, as are described in this review report:

- Re-analysis of epidemiological data and use of QMRA to assess differences in risk to children.
- Re-analysis of *Enterococcus* spp. qPCR data for consideration in criteria development, especially to address effluent sources.
- Completion and publication of coliphage methods and development of coliphage-based RWQC for inclusion into the recreational waters "tool box."
- Completion of method validation and publication for the *E. coli* qPCR method (*Draft* Method C).
- Completion and publication of standardized methods for EPA human-associated MST methods (HF183/BacR287 and HumM2) and completion of a DNA reference material development with NIST. Development and validation of virus-based human fecal source identification procedures.
- Conversion of current marine sanitary survey tablet-based application to a web-based application.
- Development of predictive models in marine environments as well as models paired with newer indicators such as qPCR-based indicators.
- Development of additional training and tools to make process models and integrated environmental modeling more accessible to stakeholders.
- Development of additional training and tools to make QMRA models more accessible.
- Completion and publication of recreational criteria for cyanotoxins (microcystin and cylindrospermopsin).

Based on the EPA's review of the existing criteria and developments in the available science, the EPA has decided not to revise the 2012 Recreational Water Criteria during this review cycle. The Agency believes, however, that further research and analysis as identified in this Report will contribute to the EPA's future review of the 2012 RWQC. The EPA will work with the environmental public health community as the Agency moves forward with its recreational water research efforts. The use of qPCR and ongoing research in methods and indicators continue to strengthen and augment the tools available to support the current criteria.

## VII. References


Abia, A.L.K., Ubomba-Jaswa, E., Genthe, B., Momba, M.N.B. 2016. Quantitative microbial risk assessment (QMRA) shows increased public health risk associated with exposure to river water under conditions of riverbed sediment resuspension. Science of the Total Environment, 566: 1143-1151.

Aiello, A., Larson, E. 2003. Antibacterial cleaning and hygiene products as an emerging risk factor for antibiotic resistance in the community. The Lancet: Infectious Diseases, 3: 501-506.

Alm, E.W., Burke, J., Hagan, E. 2006. Persistence and potential growth of the fecal indicator bacteria, *Escherichia coli,* in shoreline sand at Lake Huron. Journal of Great Lakes Research, 32: 401-405.

Alm, E.W., Zimbler, D., Callahan, E., Plomaritis, E. 2014. Patterns and persistence of antibiotic resistance in faecal indicator bacteria from freshwater recreational beaches, Journal of Applied Microbiology, 117: 273-285.

Alm, E.W., Daniels-Witt, Q.R., Learman, D.R., Ryu, H., Jordan, D.W., Gehring, T.M., Santo Domingo, J. 2018. Potential for gulls to transport bacteria from human waste sites to beaches. Science of the Total Environment, 615: 123-130.

Arnold, B.F., Schiff, K.C., Griffith, J.F., Gruber, J.S., Yau, V., Wright, C.C., Wade, T.J., Burns, S., Hayes, J.M., McGee, C. 2013. Swimmer illness associated with marine water exposure and water quality indicators: Impact of widely used assumptions. Epidemiology, 24(6): 845-853.

Arnold, B.F., Wade, T.J., Benjamin-Chung, J., Schiff, K.C., Griffith, J.F., Dufour, A.P., Weisberg, S.B., Colford Jr, J.M. 2016. Acute gastroenteritis and recreational water: highest burden among young US children. American Journal of Public Health, 106(9): 1690-1697.

Arnold, B.F., Schiff, K.C., Ercumen, A., Benjamin-Chung, J., Steele, J.A., Griffith, J.F., Steinberg, S.J., Smith, P., McGee, C.D., Wilson, R., Nelsen, C., Weisberg, S.B., Colford, J.M. Jr. 2017. Acute illness among surfers after exposure to seawater in dry- and wet weather conditions. American Journal of Epidemiology, 186(7): 866-875.

Ashbolt, N.J. 2015. Microbial contamination of drinking water and human health from community water systems. Current Environmental Health Reports: Water and Health, 2: 95-106.

Augustine SAJ, Simmons KJ, Eason TN, Curioso CL, Griffin SM, Wade TJ, Dufour A, Fout GS, Grimm AC, Oshima KH, Sams EA, See MJ, and LJ Wymer. Immunoprevalence to Six Waterborne Pathogens in Beachgoers at Boquerón Beach, Puerto Rico: Application of a

Microsphere-Based Salivary Antibody Multiplex Immunoassay. *Frontiers in Public Health*. 2017;5:84. doi:10.3389/fpubh.2017.00084.

Badgley, B.D., Nyak, B.S., Harwood, V.J. 2010. The importance of sediment and submerged aquatic vegetation as potential habitats for persistent strains of enterococci in a subtropical watershed. Water Research, 44(20): 5857-5866.

Badgley, B.D., Ferguson, J., Vanden Heuvel, A., Kleinheinz, G.T., McDermott, C.M., Sandrin, T.R., Kinzelman, J., Junion, E.A., Byappanahalli, M.N., Whitman, R.L., Sadowsky, M.J. 2011. Multi-scale temporal and spatial variation in genotypic composition of Cladophora-borne *Escherichia coli* populations in Lake Michigan. Water Research, 45(2): 721-731.

Baker-Austin, C., Wright, M.S., Stepanauskas, R., McArthur, J.V. 2006. Co-selection of antibiotic and metal resistance. Trends in Microbiology, 14(4): 176-182.

Ballesté, E., Blanch, A.R. 2011. Bifidobacterial diversity and the development of new microbial source tracking indicators. Applied and Environmental Microbiology, 77(10): 3518-3525.

Baquero F, *Coque* TM. *2014*. Widening the spaces of selection: evolution along sublethal antimicrobial gradients. mBio 5(6):e02270-14.Belant, J.L., Ickes, S.K., Seamans, T.W. 1998. Importance of landfills to urban-nesting herring and ring-billed gulls. Landscape and Urban Planning, 43: 11-19.

Benjamin-Chung, J., Arnold, B.F., Wade, T.J., Schiff, K., Griffith, J.F., Dufour, A.P., Weisberg, S.B., Colford, J.M. Jr. 2017. Coliphages and gastrointestinal illness in recreational waters pooled analysis of six coastal beach cohorts. Epidemiology, 28(5): 644-652.

Berendonk, T.U., Manaia, C.M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Burgmann, H., Sorum, H., Norstrom, M., Pons, M.N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F., Martinez, J.L. 2015. Tackling antibiotic resistance: The Environmental Framework. Nature Reviews Microbiology, 13(5): 310-317.

Bernstein, J. A., D. Ghosh, L. S. Levin, S. Zheng, W. Carmichael, Z Lummus, I. L. Bernstein. 2011. Cyanobacteria: An Unrecognized Ubiquitous Sensitizing Allergen? *Allergy and Asthma Proceedings* 32(2):106-110.

Brown, K.I., Graham, K.E., Boehm, A.B. 2017. Risk-based threshold of gull-associated fecal marker concentrations for recreational water. Environmental Science & Technology Letters, 4: 44-48.

Boczek, L.A., Johnson, C.H.,Rice, E.W. 2006. Trimethoprim-sulfamethoxazole resistance in sewage isolates of *Eschericia coli* Journal of Environmental Monitoring & Restoration 2: 19-25 2006

Boczek, L.A., Rice, E.W., Johnston, B., Johnson, J.R. 2007. Occurrence of antibiotic-resistance uropathogenic *Escherichia coli* Clonal Group A in wastewater effluents. Applied and Environmental Microbiology, 73: 4180-4184.

Boehm, A.B. 2003. Tiered approach for identification of a human fecal pollution source at a recreational beach: Case study at Avalon Bay, Catalina Island, California. Environmental Science and Technology, 37(4): 673-680.

Boehm, A.B., Ashbolt, N., Colford, J., Dunbar, L., Fleming, L., Gold, M., Hansel, J., Hunter, P.R., Ichida, A., McGee, C., Soller, J.A., Weisberg, S.B. 2009. A sea change ahead for recreational water quality criteria. Journal of Water and Health, 7(1): 9-20.

Boehm, A.B., Van De Werfhorst, L.C., Griffith, J.F., Holden, P.A., Jay, J.A., Shanks, O.C., Wang, D., Weisberg, S.B. 2013. Performance of forty-one microbial source tracking methods: A twenty-seven lab evaluation study. Water Research, 47: 6812 -6828.

Boehm, A.B., Soller, J.A., Shanks, O.C. 2015. Human-associated fecal quantitative polymerase chain reaction measurements and simulated risk of gastrointestinal illness in recreational waters contaminated with raw sewage. Environmental Science and Technology Letters, 2(10): 270-275.

Bonnedahl, J., Drobni, M., Gauthier-Clerc, M., Hernandez, J., Granholm, S., Kayser, Y., Melhus, A., Kahlmeter, G., Waldenstrom, J., Johansson, A., Olsen, B. 2009. Dissemination of *Escherichia coli* with CTX-M Type ESBL between humans and yellow-legged gulls in the South of France. PLoS One, 4(6): e5958.

Bradshaw, J. K., Snyder, B. J., Oladeinde, A., Spidle, D., Berrang, M. E., Meinersmann, R. J., Oakely, B., Sidle, R. C., Sullivan, K., Molina, M. 2016. Characterizing relationships among fecal indicator bacteria, microbial source tracking markers, and associated waterborne pathogen occurrence in stream water and sediments in a mixed land use watershed. Water Research, 101: 498-509.

Brown, K.I., Graham, K.E., Boehm, A.B. 2017. Risk-based threshold of gull-associated fecal marker concentrations for recreational water. Environmental Science & Technology Letters, 4: 44-48.

Burmeister, A.R. 2015. Horizontal gene transfer. Evolution, Medicine, and Public Health, 2015(1): 193-194.

Byappanahalli, M.N., Whitman, R.L., Shively, D.A., Ferguson, J., Ishii, S., Sadowsky, M.J. 2007. Population structure of Cladophora-borne *Escherichia coli* in nearshore water of Lake Michigan. Water Research, 41(16): 3649-3654.

Byappanahalli, M.N., Roll, B.M., Fujioka, R.S. 2012a. Evidence for occurrence, persistence, and growth potential of *Escherichia coli* and enterococci in Hawaii's soil environments. Microbes and Environments, 27(2): 164-170.

Byappanahalli, M.N., Yan T., Hamilton, M.J., Ishii, S., Fujioka, R.S., Whitman, R.L., Sadowsky, M.J. 2012b. The population structure of *Escherichia coli* isolated from subtropical and temperate soils. Science of the Total Environment, 417-418: 273-279.

Cabelli, V.J., Dufour, A.P., McCabe, L.J., Levin, M.A. 1982. Swimming associated gastroenteritis and water quality. American Journal of Epidemiology, 115: 606-616.

Cabelli, V.J. 1983. Health effects criteria for marine recreational waters, Technical Report. EPA 600/1-80-031. U.S. Environmental Protection Agency, Health Effects Research Laboratory: Research Triangle Park, NC.

Cao, Y., Griffith, J.F., Dorevitch, S., Weisberg, S.B. 2012. Effectiveness of qPCR permutations, internal controls and dilution as means for minimizing the impact of inhibition while measuring *Enterococcus* in environmental waters. Journal of Applied Microbiology, 113(1): 66-75.

Cao, Y., Griffith, J.F., Weisberg, S.B., Sivaganesan, M., Haugland, R.A., Kinzelman, J., Blackwood, A.D., Noble, R.T. 2013a. Effect of platform, reference material, and quantification model on enumeration of general fecal indicators by quantitative PCR methods. Water Research, 47(1): 233-241

Cao, Y., Hagedorn, C., Shanks, O.C., Wang, D., Ervin, J., Griffith, J.F., Layton, B., McGee, C., Riedel, T., Weisberg, S.B. 2013b. Towards establishing a human fecal contamination index in microbial source tracking. International Journal of Environmental Science and Engineering Research, 4: 46-58.

Cao, Y., Raith, M.R., Griffith, J.F. 2015. Droplet digital PCR for simultaneous quantification of general and human-associated fecal indicators for water quality assessment. Water Research, 70: 337-349.

Cao, Y., Sivaganesan, M., Kelty, C.A., Wang, D., Boehm, A.B., Griffith, J.F., Weisberg, S.B., Shanks, O.C. 2018. A human fecal contamination score for ranking recreational sites using the HF183/BacR287 quantitative real-time PCR method. Water Research, 128: 148-156.

CDC (Centers for Disease Control and Prevention). 2013. Antibiotic resistance threats in the United States, 2013. Retrieved October 20, 2017 from https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf

Chern, E.C., Siefring, S., Paar, J., Doolittle, M., Haugland, R.A. 2011. Comparison of quantitative PCR assays for *Escherichia coli* targeting ribosomal RNA and single copy genes. Letters in Applied Microbiology, 52(3): 298-306.

Cole, D., Drum, D.J.V., Stallknecht, D.E., White, D.G., Lee, M.D., Ayers, S., Sobsey, M., Maurer, J.J. 2005. Free-living Canada geese and antimicrobial resistance. Emerging Infectious Diseases, 11(6): 935-938.

Colford, J.M., Jr., Wade, T.J., Schift, K.C., Wright, C., Griffith, J.F., Sandhu, S.K., Weisberg, S.B. 2005. Recreational water contact and illness in Mission Bay, California. Southern California Coastal Water Research Project, Technical Report 449.

Colford, J.M., Jr., Wade, T.J., Schiff, K.C., Wright, C.C., Griffith, J.F., Sandhu, S.K., Burns, S., Sobsey, M., Lovelace, G., Weisberg, S.B. 2007. Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. Epidemiology, 18(1): 27-35.

Colford, J., Schiff, K.C., Griffith, J.F., Yau, V., Arnold, B.F., Wright, C.C., Gruber, J.S., Wade, T.J., Burns, S., Hayes, J., McGee, C., Gold, M., Cao, Y., Noble, R.T., Haugland, R., Weisberg, S.B. 2012. Using rapid indicators for *Enterococcus* to assess the risk of illness after exposure to urban runoff contaminated marine water. Water Research, 46(7): 2176-2186.

Colson, A. 2010. The Interagency Task Force on Antimicrobial Resistance: 10 years of Coordinated Federal Action. Extending the Cure: Policy Responses to the Growing Threat of Antibiotic Resistance. Policy Brief 9.

Cordero, L., Norat, J., Mattei, H., Nazario, C. 2012. Seasonal variations in the risk of gastrointestinal illness on a tropical recreational beach. Journal of Water and Health, 10(4): 579-593.

Corsi, S.R., Borchardt, M.A., Carvin, R.B., Burch, T.R., Spencer, S.K., Lutz, M.A., McDermott, C.M., Busse, K.M., Kleinheinz, G.T., Feng, X., Zhu, J. 2016. Human and bovine viruses and bacteria at three Great Lakes Beaches: Environmental variable associations and health risk. Environmental Science and Technology, 50(2): 987-995.

Cyterski, M., Zhang, S., White, E., Molina, M., Wolfe, K., Parmar, R., Zepp, R. 2012. Temporal synchronization analysis for improving regression modeling of fecal indicator bacteria levels. Water, Air, and Soil Pollution, 223(8): 4841-4851.

Cyterski, M., Brooks, W., Galvin, M., Wolfe, K., Carvin, R., Roddick, T., Fienen, M., Corsi, S. 2013. Virtual Beach 3: User's Guide. EPA/600/R-13/311. U.S. Environmental Protection Agency, Athens, Georgia.

DeFlorio-Barker, S., Arnold, B.F., Sams, E.A., Dufour, A.P., Colford Jr, J.M., Weisberg, S.B., Schiff, K.C., Wade, T.J. 2017a. Child environmental exposures to water and sand at the beach: Findings from studies of over 68,000 subjects at 12 beaches. Journal of Exposure Science and Environmental Epidemiology, 00: 1-8.

DeFlorio-Barker, S., Wade, T.J., Jones, R.M., Friedman, L.S., Wing, C., Dorevitch, S. 2017. Estimated costs of sporadic gastrointestinal illness associated with surface water recreation: A combined analysis of data from NEEAR and CHEERS studies. Environmental Health Perspectives, 125: 215-222.

De Liguoro, M., Cibin, V., Capolongo, F., Halling-Sorensen, B., Montesissa, C. 2003. Use of Oxytetracycline and Tylosin in intensive calf farming: Evaluation of transfer to manure and soil. Chemosphere, 52: 203-212.

de Man, H., van den Berg, H.H., Leenen, E.J., Schijven, J.F., Schets, F.M., van der Vliet, J.C., van Knapen, F., de Roda Husman, A.M. 2014. Quantitative assessment of infection risk from exposure to waterborne pathogens in urban floodwater. Water Research, 48: 90-99.

Dick, L.K., Stelzer, E.A., Bertke, E.E., Fong, D.L., Stoeckel, D.M. 2010. Relative decay of Bacteroidales microbial source tracking markers and cultivated *Escherichia coli* in freshwater microcosms. Applied and Environmental Microbiology, 76(10): 3255-3262.

Dubinsky, E.A., Butkus, S.R., Andersen, G.L. 2016. Microbial source tracking in impaired watershed using PhyloChip and machine-learning classification. Water Research, 105: 56-64.

Dufour, A.P. 1984. Health Effects Criteria for Fresh Recreational Waters. EPA 600/1-84-004.

Ebentier, D.L., Hanley, K.T., Cao, Y., Badgley, B.D., Boehm, A.B., Ervin, J.S., Goodwin, K.D., Gourmelon, M., Griffith, J.F., Holden, P.A., Kelty, C.A., Lozach, S., McGee, C., Peed, L.A., Raith, M., Ryu, H., Sadowsky, M.J., Scott, E.A., Santo Domingo, J., Schriewer, A., Sinigalliano, C.D., Shanks, O.C., Van De Werfhorst, L.C., Wang, D., Wuertz, S., Jay, J.A. 2013. Evaluation of the repeatability and reproducibility of a suite of qPCR-based microbial source tracking methods. Water Research, 47: 6839-6848.

Eftim, S.E. 2016. Systematic literature reviews and development of distributions of viral densities in raw wastewater. Presented at 2016 UNC Water Microbiology Conference. Chapel Hill, North Carolina, USA.

Eftim, S.E., Hong, T., Soller, J., Boehm, A., Warren, I., Ichida, A., Nappier, S.P. 2017a. Occurrence of norovirus in raw sewage – A systematic literature review and meta-analysis. Water Research, 111: 366-374.

Eftim, S.E. 2017b. Systematic literature reviews and development of distributions of viral densities in ambient water. Presented at 2017 UNC Water Microbiology Conference, Chapel Hill, North Carolina, USA.

Eichmiller, J.J., Hicks, R.E., Sadowsky, M.J. 2013. Distribution of genetic markers of fecal pollution on a freshwater sandy shoreline in proximity to wastewater effluent. Environmental Science and Technology, 47(7): 3395-3402.

Ervin, J.S., Russell, T.L., Layton, B.A., Yamahara, K.M., Wang, D., Sassoubre, L.M., Cao, Y., Kelty, C.A., Sivaganesan, M., Boehm, A.B., Holden, P.A., Weisberg, S.B., Shanks, O.C. 2013. Characterization of fecal concentrations in human and other animal sources by physical, culture-based, and quantitative real-time PCR methods. Water Research, 47(18): 6873-6882.

Feachem, R.G., Bradley, D.J., Garelick, H., Mara, D.D. 1983. Sanitation and disease: Health aspects of excreta and wastewater management. World Bank Studies in Water Supply and Sanitation 3.

Fenlon, D.R. 1981. Seagulls (*Larus* spp.) as vectors of salmonellae: An investigation into the range of serotypes and numbers of salmonellae in gull faeces. Journal of Hygiene (Cambridge), 86: 195-202.

Fewtrell, L., Kay, D. 2015. Recreational water and infection: A review of recent findings. Current Environmental Health Reports, 2(1): 85-94.

Francy, D.S., Brady, A.M.G, Carvin, R.B., Corsi, S.R., Fuller, L.M., Harrison, J.H., Hayhurst, B.A., Lant, J., Nevers, M.B., Terrio, P.J., Zimmerman, T.M. 2013. Developing and implementing predictive models for estimating recreational water quality at Great Lakes beaches. Scientific Investigations Report, 2013-5166. U.S. Geological Survey.

Friedman, S.D., Cooper, E.M., Casanova, L., Sobsey, M.D., Genthner, F.J. 2009. A reverse transcription-PCR assay to distinguish the four genogroups of male-specific (F+) RNA coliphages. Journal of Virological Methods, 159: 47-52.

Friedman, S.D., Cooper, E.M., Calci, K.R., Genthner, F.J. 2011. Design and assessment of a real time reverse transcription-PCR method to genotype single-stranded RNA male-specific coliphages (family Leviviridae). Journal of Virological Methods, 173(2): 196-202.

Friedman, S.D. 2017. Standard methods for the examination of waters and wastewaters. Coliphage Detection Methods. In Press.

Funderburg, S.W., Sorber, C.A. 1985. Coliphage as indicators of enteric viruses in activated sludge. Water Research, 19(5): 547-555.

Gantzer, C., Maul, A., Audic, J.M., Schwartzbrod, L. 1998. Detection of infectious enteroviruses, enterovirus genomes, somatic coliphages, and *Bacteroides fragilis* phages in treated wastewater. Applied and Environmental Microbiology, 64: 4307-4312.

Geh, E. N., D. Ghosh, M. McKell, A. A. de la Cruz, G. Stelma, and J. A. Bernstein. 2015, Identification of *Microcystis aeruginosa* Peptides Responsible for Allergic Sensitization and Characterization of Functional Interactions between Cyanobacterial Toxins and Immunogenic Peptides. Environ. Hlth Perspect. 123(11):1159-1166.

Gerba, C.P. 1987. Phage as indicators of fecal pollution, in Phage Ecology. Eds. Goyal, S.M., Gerba, C.P., Bitton, G. Wiley Interscience: New York, NY.

Gordon, K., Brownell, M., Wang, S., Lepo, J.E., Mott, J., Nathaniel, R., Kilgen, M., Hellein, K.N., Kennedy, E., Harwood, V.J. 2012. Relationship of human-associated microbial source tracking markers with enterococci in Gulf of Mexico Waters. Water Research, 47: 996-1004.

Grabow, W.O. 2001. Bacteriophages: Update on application as models for viruses in water. Water SA, 27(2): 251-268.

Graham, J.P., Nachman, K.E. 2010. Managing waste from confined animal feeding operations in the United States: The need for sanitary reform. Journal of Water and Health, 8(4): 646-670.

Green, H., Shanks, O.C., Sivaganesan, M., Haugland, R., Field, K. 2011. Differential decay of human faecal *Bacteroides* in marine and freshwater. Environmental Microbiology, 13: 3235-3249.

Green, H.C., Haugland, R., Varma, M., Millen, H.T., Borchardt, M.A., FIeld, K.G., Kelty, C.A., Sivaganesan, M., Shanks, O.C. 2014. Improved HF183 quantitative real-time PCR assay for characterization of human fecal pollution in ambient surface water samples. Applied and Environmental Microbiology, 80(10): 3086-3094.

Griffin SM, Chen M, Fout GS, Wade TJ, Egorov AI. Development of a multiplex microsphere immunoassay for the quantitation of salivary antibody responses to selected waterborne pathogens. J Immunol Methods 2011;364:83-93.

Griffin SM, Converse RR, Leon JS, et al. Application of salivary antibody immunoassays for the detection of incident infections with Norwalk virus in a group of volunteers. J Immunol Methods 2015;424:53-63.

Griffith, J.F., Layton, B.A., Boehm, A.B., Holden, P.A., Jay, J.A., Hagedorn, C., McGee, C.D., Weisberg, S.B. 2013. The California microbial source identification manual: A tiered approach to identifying fecal pollution sources at beaches. Southern California Coastal Water Research Project, Technical Report 804.

Griffith, J.F., Weisberg, S.B., Arnold, B.F., Cao, Y., Schiff, K.C., Colford Jr, J.M. 2016. Epidemiologic evaluation of multiple alternate microbial water quality monitoring indicators at three California beaches. Water Research, 94: 371-381.

Gullberg, E., Albrecht, L.M., Karlsson, C., Sandregren, L., Andersson, D.I. 2014. Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. mBio, 5: e01918-14.

Gustafsson, O., Long, C.M., MacFarlane, J., Gschwend, P.M. 2001. Fate of linear alkylbenzenes released to the coastal environment near Boston Harbor. Environmental Science and Technology, 35: 2040-2048.

Guzman, R.E., Solter, P.F. 1999. Hepatic oxidative stress following prolonged sublethal microcystin LR exposure. Toxicologic Pathology, 27(5): 582-588.

Haack, S.K., Fogarty, L.R., Wright, C. 2003. *Escherichia coli* and enterococci at beaches in the Grand Traverse Bay, Lake Michigan: Sources, characteristics, and environmental pathways. Environmental Science and Technology, 37: 3275-3282.

Hagedorn, C., Saluta, M., Hassall, A., Dickerson, J. 2005. Fluorometric detection of optical brighteners as an indicator of human sources of water pollution. Part II. Development as a source tracking methodology in open waters. Environmental Detection News, 2: 1-13.

Hagedorn, C., Blanch, A.R., Harwood, V.J. 2011. Microbial Source Tracking: Methods, Applications, and Case Studies. Published by Springer Press, New York.

Hagedorn, C., Weisberg, S.B. 2009. Chemical-based fecal source tracking methods: Current status and guidelines for evaluation. Reviews in Environmental Science and Bio/Technology, 8: 275-287.

Halaihel N., Masia R.M., Fernandez-Jimenez M., Ribes J.M., Montava R., De Blas I., Girones O., Alonso J.L., Buesa J. 2010. Enteric calicivirus and rotavirus infections in domestic pigs. Epidemiology and Infection, 138: 542-548.

Hamscher, G., Sczesny, S., Hoper, H., Nau, H. 2002. Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Analytical Chemistry, 74(7): 1509-1518.

Haramoto, E., Katayama, H., Phanuwan, C., Ohgaki, S. 2006. Quantitative detection of sapoviruses in wastewater and river water in Japan. Letters in Applied Microbiology, 46(3): 408-413.

Hartel, P.G., McDonald, J.L., Gentit, L.C., Hemmings, S.N., Rodgers, K., Smith, K.A., Belcher, C.N., Rivera-Torres, Y., Otero, E., Schiro, E.C. 2007. Improving fluorometry as a source tracking method to detect human fecal contamination. Estuaries and Coasts, 30(3): 551-561.

Hartz, A., Cuvelier, M., Nowosielski, K., Bonilla, T.D., Green, M., Esiobu, N., McCorquodale, D.S., Rogerson, A. 2008. Survival potential of *Escherichia coli* and enterococci in subtropical beach sand: Implications for water quality managers. Journal of Environmental Quality, 37: 898-905.

Harwood, V. J., Brownell, M., Wang, S., Lepo, J., Ellender, R.D., Ajidahun, A., Hellein, K. N., Kennedy, E., Ye, X., Flood, C. 2009. Validation and field testing of library-independent microbial source tracking methods in the Gulf of Mexico. Water Research, 43(19): 4812-4819.

Harwood, V.J., Staley, C., Badgley, B.D., Borges, K., Korajkic, A. 2014. Microbial source tracking markers for detection of fecal contamination in environmental waters: Relationships between pathogens and human health outcomes. FEMS Microbial Reviews, 38: 1-40.

Haugland, R.A., Varma, M., Sivaganesan, M., Kelty, C., Peed, L., Shanks, O.C. 2010. Evaluation of genetic markers from the 16S rRNA gene V2 region for use in quantitative detection of selected Bacteroidales species and human fecal waste by qPCR. Systematic and Applied Microbiology, 33(6): 348-357.

Haugland, R.A., Siefring, S., Lavender, J., Varma, M. 2012. Influences of sample interference and interference controls on quantification of enterococci fecal indicator bacteria in surface water by the qPCR method. Water Research, 46(18): 5989–6001.

Haugland, R.A., Siefring, S.D., Varma, M., Dufour, A.P., Brenner, K.P., Wade, T.J., Sams, E., Cochran, S., Braun, S. Sivaganensan, M. 2014. Standardization of enterococci density estimates by EPA qPCR methods and comparison of beach action value exceedances in river waters with culture methods. Journal of Microbiological Methods, 105: 59-66.

Haugland, R.A., Siefring, S., Varma, M., Oshima, K. H., Sivaganesan, M., Cao, Y., Raith, M., Griffith, J., Weisberg, S.B., Noble, R.T., Blackwood, A.D., Kinzelman, J., Ananeva, T., Bushon, R.N., Harwood, V.J., Gordon, K.V., Sinigalliano, C. 2016. Multi-laboratory survey of qPCR enterococci analysis method performance in U.S. coastal and inland surface waters. Journal of Microbiological Methods, 123: 114-125.

Havelaar, A.H., Pot-Hogeboom, W.M., Furuse, K., Pot, R., Hormann, M.P. 1990. F-specific RNA bacteriophages and sensitive host strains in faeces and wastewater of human and animal origin. Journal of Applied Bacteriology, 69(1): 30-37.

Havelaar, A.H., van Olphen, M., Drost, Y.C. 1993. F-specific RNA bacteriophages are adequate model organisms for enteric viruses in freshwater. Applied and Environmental Microbiology, 59(9): 2956-2962.

Heinze, R. 1999. Toxicity of the cyanobacterial toxin microcystin-LR to rats after 28 days intake with the drinking water. Environmental Toxicology, 14(1): 57-60.

Hlavsa M.C., Roberts, V.A., Kahler, A.M., Hilborn, E.D., Mecher, T.R., Beach, M.J., Wade, T.J., Yoder, J.S. 2015. Outbreaks of illness associated with recreational water—United States, 2011–2012. Morbidity and Mortality Weekly Report, 64(24): 668-672.

Hribar, C. 2010. Understanding concentrated feeding operations and their impact on communities. National Association of Local Boards of Health (NALBOH). Retrieved October 20, 2017 from https://www.cdc.gov/nceh/ehs/docs/understanding_cafos_nalboh.pdf.

Humpage, A.R., Falconer, I.R. 2002. Oral toxicity of Cylindrospermopsin: No observed adverse effect level determination in male Swiss albino mice. Research Report No. 13, 93.

Humpage, A.R., Falconer, I.R. 2003. Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: Determination of no observed adverse effect level for deriving a drinking water guideline value. Environmental Toxicology, 18(2): 94-103.

IFTAR (Interagency Task Force on Antimicrobial Resistance). 2001. A Public Health Action Plan to Combat Antimicrobial Resistance Part 1: Domestic Issues. Retrieved October 23, 2017 from: https://www.cdc.gov/drugresistance/actionplan/aractionplan.pdf.

Jiang, S.C., Chu, W., He, J.W. 2007. Seasonal detection of human viruses and coliphage in Newport Bay, California. Applied and Environmental Microbiology, 73(20): 6468-6474.

Jeanneau, L., Solecki, O., Wéry, N., Jardé, E., Gourmelon, M., Communal, P.Y., Jadas-Hécart, A., Caprais, M.P., Gruau, G., Pourcher, A.M. 2012. Relative decay of fecal indicator bacteria and human-associated markers: a microcosm study simulating wastewater input into seawater and freshwater. Environmental Science and Technology, 46(4): 2375-2382.

Kim, J., Lee, H., Lee, J.E., Chung, M.S., Ko, G.P. 2013. Identification of human and animal fecal contamination after rainfall in the Han River, Korea. Microbes and Environments, 28(2): 187-94.

Kim, K., Whelan, G., Molina, M., Purucker, S.T., Pachepsky, Y., Guber, A., Cyterski, M.J.,Ravenscroft, and D. H. Franklin. 2016. Rainfall-induced release of microbes from manure: model development, parameter estimation, and uncertainty evaluation on small plots. *J. Water Health DOI: 10.2166/wh.2016.239*

Kirs, M., Caffaro-Filho, R.A., Wong, M., Harwood, V.J., Moravcik, P., Fujioka, R.S. 2016. Human-associated *Bacteroides* spp. and human polyomaviruses as microbial source tracking markers in Hawaii. Applied and Environmental Microbiology, 82(22): 6757-6767.

Kirs, M., Kisand, V., Wong, M., Caffaro-Filho, R.A., Moravcik, P., Harwood, V.J., Yoneyama, B., Fujioka, R.S. 2017. Multiple lines of evidence to identify sewage as the cause of water quality impairment in an urbanized tropical watershed. Water Research, 116: 23-33.

Kreader, C.A. 1998. Persistence of PCR-detectable *Bacteroides distasonis* from human feces in river water. Applied and Environmental Microbiology, 64(10): 4103-4105.

Kummerer, K. 2004. Resistance in the environment. Journal of Antimicrobial Chemotherapy, 54: 311-320.

Lamba, M., Graham, D.W., Ahammad, S.Z. 2017. Hospital wastewater releases of carbapenem-resistance pathogens and genes in urban India. Environmental Science and Technology, 51(23): 13906-13912.

Lamparelli, C.C., Pogreba-Brown, K., Verhougstraete, M., Zanoli Sato, M.I., de Castro Bruni, A., Wade, Eisenberg, J.N.S. 2015. Are fecal indicator bacteria appropriate measures of recreational water risks in the tropics: A cohort study of beach goers in Brazil? Water Research, 87: 59-68.

Lapara, T.M., Burch, T.R., McNamara, P.J., Tan, D.T., Yan, M., Eichmiller, J.J. 2011. Tertiary-treated municipal wastewater is a significant point source of antibiotic resistance genes into Duluth-Superior Harbor. Environmental Science and Technology, 45: 9543-9549.

Layton, B.A., Cao, Y., Ebentier, D.L., Hanley, K., Ballesté, E., Brandão, J., Byappanahalli, M., Converse, R., Farnleitner, A.H., Gentry-Shields, J., Gidley, M.L., Gourmelon, M., Lee, C.S., Lee, J., Lozach, S., Madi, T., Meijer, W.G., Noble, R., Peed, L., Reischer, G.H., Rodrigues, R., Rose, J.B., Schriewer, A., Sinigalliano, C., Srinivasan, S., Stewart, J., Van De Werfhorst, L.C., Wang, D., Whitman, R., Wuertz, S., Jay, J., Holden, P.A., Boehm, A.B., Shanks, O., Griffith, J.F. 2013. Performance of human fecal anaerobe-associated PCR-based assays in a multi-laboratory method evaluation study. Water Research, 47(18): 6897-6908.

Laverick, M.A., Wyn-Jones, A.P., Carter, M.J. 2004. Quantitative RT-PCR for the enumeration of noroviruses (Norwalk-like viruses) in water and sewage. Letters in Applied Microbiology, 39(2): 127-136.

Lee, J.V., Dawson, S.R., Ward, S., Surman, S.B., Neal, K.R. 1997. Bacteriophages are a better indicator of illness rates than bacteria amongst users of a white water rafting course fed by a lowland river. Water Science and Technology, 35(11-12): 165-170.

Leeming, R., Nichols, P.D. 1996. Concentration of coprostanol that corresponds to existing bacterial indicator guideline limits. Water Research, 30: 2997-3006.

Leonard, A.F.C., Zhang, L., Balfour, A.J., Garside, R., Gaze, W.H. 2015. Human recreational exposure to antibiotic resistant bacteria in coastal bathing waters. Environment International, 82: 92-100.

Leonard, A.F.C., Zhang, L., Balfour, A.J., Garside, R., Hawkey, P.M, Murray, A.K., Ukoumunne, O.C., Gaze, W.H. 2018. Exposure to and colonization by antibiotic-resistance *E. coli* in UK coastal water users: Environmental surveillance, exposure assessment and epidemiological study (Beach Bum Survey). Environment International.

Lévesque B, Gervais MC, Chevalier P, Gauvin D, Anassour-Laouan-Sidi E, Gingras S, Fortin N, Brisson G, Greer C, and Bird D. 2014. Prospective study of acute health effects in relation to exposure to cyanobacteria. Sci Total Environ, 466-467, 397-403

Levy, S.B. 2001. Antibacterial household products: Cause for concern. Emerging Infectious Diseases Conference Presentation, 7(3): 512-515.

Litton, R.M., Ahn, J.H., Bramerscu, P.A., Holden, D.L., Sedlack, D.L., Grant, S.B. 2010. Evaluation of chemical, molecular, and traditional markers of fecal contamination in an effluent dominated urban stream. Environmental Science and Technology, 44: 7369-7375.

Lodder, W.J., de Roda Husman, A.M. 2005. Presence of noroviruses and other enteric viruses in sewage and surface waters in the Netherlands. Applied Environmental Microbiology, 71(3): 1453-1461.

Mao, D., Yu, S., Rysz, M., Luo, Y., Yang, F., Li, F., Hou, J., Mu, Q., Alvarez, P.J.J. 2015. Prevalence and proliferation of antibiotic resistance genes in two municipal wastewater treatment plants. Water Research, 85: 458-466.

Mandilara, G.D., Smeti, E.M., Mavridou, A.T., Lambiri, M.P., Vatopoulos, A.C., Rigas, F.P. 2006. Correlation between bacterial indicators and bacteriophages in sewage and sludge. Federation of European Microbiological Societies Microbiology Letters, 263(1): 119-126.

Martinez, J.L. 2009. The role of natural environments in the evolution of resistance traits in pathogenic bacteria. Proceedings of the Royal Society B, 276(1667): 2521-2530.

Marion, J.W., Lee, J., Lemeshow, S., Buckley, T.J. 2010. Association of Gastrointestinal Illness and Recreational Water Exposure at an Inland U.S. Beach. Water Research, 44(16): 4796-4804.

McBride, G.B., Salmond, C.E., Bandaranayake, D.R., Turner, S.J., Lewis, G.D., Till, D.G. 1998. Health effects of marine bathing in New Zealand. International Journal of Environmental Health Research, 8(3): 173-189.

McLellan, S.L., Eren, A.M. 2014. Discovering new indicators of fecal pollution. Trends in Microbiology, 22: 697-706.

McLellan, S. L., Sauer, E. P. 2009. Greater Milwaukee watersheds pathogen source identification. Great Lakes WATER Institute, University of Wisconsin-Milwaukee, Contract MO3016P02.

McMinn, B.R., B.R., Ashbolt, N.J., Korajkic. A. Bacteriophages as indicators of fecal pollution and enteric virus removal. Letters in Applied Microbiology, 65(1): 11-26.

McMinn, B.R., Ashbolt, N.J., Korajkic, A. 2017a. Bacteriophages as indicators of faecal pollution and enteric virus removal. Letters in Applied Microbiology, 65(1): 11-26.

McMinn, B.R., Huff, E.M., Rhodes, E.R., Korajkic, A. 2017b. Concentration and quantification of somatic and F+ coliphages from recreational waters. Journal of Virological Methods, 249: 58-65.

McQuaig, S. M., Scott, T. M., Lukasik, J. O., Paul, J. H., Harwood, V. J. 2009. Quantification of human polyomaviruses JC virus and BK virus by TaqMan quantitative PCR and comparison to other water quality indicators in water and fecal samples. Applied and Environmental Microbiology, 75(11): 3379-3388.

McQuaig, S., Griffith, J., Harwood, V. 2012. Association of fecal indicator bacteria with human viruses and microbial source tracking markers at coastal beaches impacted by nonpoint source pollution. Applied and Environmental Microbiology, 78(18): 6423-6432.

Meng, X.J. 2011. From barnyard to food table: The omnipresence of hepatitis E virus and risk for zoonotic infection and food safety. Virus Research, 161(1): 23-30.

Mitch, A.A., Gasner, K.C., Mitch, W.A. 2010. Fecal coliform accumulation within a river subject to seasonally-disinfected wastewater discharges. Water Research, 44: 4776-4782.

Molina, M., Hunter, S., Cyterski, M., Peed, L.A., Kelty, C.A., Sivaganesan, M., Mooney, T., Prieto, L., Shanks, O.C. 2014. Factors affecting the presence of human-associated and fecal indicator real-time quantitative PCR genetic markers in urban-impacted recreational beaches. Water Research, 64: 196-208.

Napier, M. D., Haugland, R., Poole, C., Dufour, A. P., Stewart, J. R., Weber, D. J., Varma, M., Lavender, J. S., Wade, T. J. 2017. Exposure to human-associated fecal indicators and self-reported illness among swimmers at recreational beaches: a cohort study 2017. Environmental Health.

Nappier, S.P., Aitken, M.D., Sobsey, M.D. 2006. Male-specific coliphages as indicators of thermal inactivation of pathogens in biosolids. Applied and Environmental Microbiology, 72(4): 2471-2475.

Nevers, M.B., Boehm, A.B. 2010. Modeling fate and transport of fecal bacteria in surface water. In The Fecal Bacteria. Eds. M.J. Sadowsky and R.L. Whitman, pp. 165-188. ASM Press, Washington, DC.

Newton, R.J., Bootsma, M.J., Morrison, H.G., Sogin, M.L., McLellan, S.L. 2013. A microbial signature approach to identify fecal pollution in the waters off an urbanized coast of Lake Michigan. Microbial Ecology, 65: 1011-1023.

NRC (National Research Council). 2004. Indicators for Waterborne Pathogens. National Academy of Sciences Press: Washington, DC.Natural Resources Defense Council. 2014, Testing the Waters 2014: A Guide to Water Quality at Vacation Beaches**.** New York, NY. https://www.nrdc.org/resources/testing-waters-2014-guide-water-quality-vacation-beaches

Natural Resources Defense Council. 2014 Testing the Waters New York, NY
https://www.nrdc.org/sites/default/files/ttw2014.pdf

Okabe, S., Shimazu, Y. 2007. Persistence of host-specific *Bacteroides-Prevotella* 16S rRNA genetic markers in environmental waters: Effects of temperature and salinity. Applied Microbiology and Biotechnology, 76(4): 935-944.

Paar, J. III, Doolittle, M.M., Varma, M., Siefring, S., Oshima, K., Haugland, R.A. 2015. Development and evaluation of culture-independent method for source determination of fecal wastes in surface and storm waters using reverse transcriptase-PCR detection of FRNA coliphage genogroup gene sequences. Journal of Microbiological Methods, 112: 28-35.

Palmateer, G.A., Dutka, B.J., Janzen, E.M., Meissner, S.M., Sakeellaries, M.G. 1991. Coliphage and bacteriophage as indicators of recreational water quality. Water Research, 25(3): 355-357.

Parveen, S., Murphree, R.L., Edmiston, L., Kaspar, C.W., Portier, K.M., Tamplin, M.L. 1997. Association of multiple-antibiotic-resistance profiles with point and nonpoint sources of *Escherichia coli* in Apalachicola Bay. Applied and Environmental Microbiology, 63(7): 2607-2612.

Peed, L.A., Nietch, C.T., Kelty, C.A., Meckes, M., Mooney, T., Sivaganesan, M., Shanks, O.C. 2011. Combining land use information and small stream sampling with PCR-based methods for better characterization of diffuse sources of human fecal pollution. Environmental Science and Technology, 45: 5652-5659.

Personal communication. 2017. UNC Water Microbiology Conference. Chapel Hill, North Carolina. May 15-19, 2017.

Phillips, C.R., Venkatesan, M.I., Bowen, R. 1997. Interpretations of contaminant sources to San Pedro Shelf sediments using molecular markers and principal component analysis. In E Ganhouse, R.P., ed. Molecular markers in environmental geochemistry. Washington, DC: American Chemistry Society, 242–260.

Pouillot, R., Van Doren, J.M., Woods, J., Plante, D., Smith, M., Goblick, G., Roberts, C., Locas, A., Hajen, W., Stobo, J., White, J., Holtzman, J., Buenaventura, E., Burkhardt 3rd, W., Catford, A., Edwards, R., DePaola, A., Calci, K.R., 2015. Metaanalysis of the reduction of norovirus and male-specific coliphage concentrations in wastewater treatment plants. Applied and Environmental Microbiology, 81(14): 4669-4681.

Pusch, D., Oh, D.Y., Wolf, S., Dumke, R., Schroter-Bobsin, U., Hohne, M., Roske, I., Schreier, E. 2005. Detection of enteric viruses and bacterial indicators in German environmental waters. Archives of Virology, 150(5): 929-947.

Raoult, D. 2011. Molecular, epidemiological, and clinical complexities of predicting patterns of infectious diseases. Frontiers in Microbiology, 2(25): 1-2.

Reis, M. and Wuertz, S., Eds. 2013. Microbial Source Tracking. Water Research, 47(18): 6811-6956.

Rijal, G., Tolson, J.K., Petropoulou, C., Granato, T.C., Glymph, A., Gerba, C., Deflaun, M.F., O'Connor, C., Kollias, L., Lanyon, R. 2011. Microbial risk assessment for recreational use of the Chicago Area Waterway System. Journal of Water and Health, 9(1): 169-186.

Rizzo, L., Manaia, C., Schwartz, T., Dagot, C., Ploy, M.C., Michael, I., Fatta-Kassinos, D. 2013. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. Science of the Total Environment, 447: 345-360.

Rosario, K., Symonds, E.M., Sinigalliano, C., Stewart, J., Breitbart, M. 2009. Pepper mild mottle virus as an indicator of fecal pollution. Applied and Environmental Microbiology, 75(22): 7261-7267.

Sanborn, M., Takoro, T. 2013. Recreational water–related illness: Office management and prevention. Canadian Family Physician, 59(5): 491-495.

Santiago-Rodriguez, T.M., Toranzos, G.A., Arce-Nazario, J.A. 2016. Assessing the microbial quality of a tropical watershed with an urbanization gradient using traditional and alternate fecal indicators. J Water Health. Volume 14(5):796-807.

Santiago-Rodriguez, T.M., Rivera, J.I., Coradin, M., Toranzos, G.A. 2013. Antibiotic-resistance and virulence genes in *Enterococcus* isolated from tropical recreational waters. Journal of Water and Health, 11(3): 387-396.

Santo Domingo, J.W., Lamendella, R., Ashbolt, N.J. 2011. Microbial Source Tracking: Current and future molecular tools in microbial water quality forensics. In Environmental Microbiology: Current Technology and Water Applications. Eds. K. Sen and N.J. Ashbolt, Chapter 10. Caister Academic Press, Norfolk, UK.

Sapkota, A.R., Curriero, F.C., Gibson, K.E., Schwab, K.J. 2007. Antibiotic-resistant enterococci and fecal indicators in surface water and groundwater impacted by a concentrated swine feeding operation. Environmental Health Perspectives, 115(7): 1040-1045.

Savichtcheva, O., Okabe, S. 2006. Alternative indicators of fecal pollution: Relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. Water Research, 40: 2463-2476.

Sayah, R.S., Kaneene, J.B., Johnson, Y., Miller, R. 2005. Patterns of antimicrobial resistance observed in *Escherichia coli* isolates obtained from domestic- and wild-animal fecal samples, human septage, and surface water. Applied and Environmental Microbiology, 71(3): 1394-1404.

Scheurer, M., Heß, S., Lüddeke, F., Sacher, F., Göde, H., Löffler, H., Gallert, C. 2015. Removal of micropollutants, facultative pathogenic and antibiotic resistant bacteria in a full-scale retention soil filter receiving combined sewer overflow. Environmental Science: Processes and Impacts, 17(1): 186-196.

Schijven, J.F., Blaak, H., Schets, F.M., de Roda Husman, A.M. 2015. Fate of extended-spectrum β-lactamase-producing *Escherichia coli* from faecal sources in surface water and probability of human exposure through swimming. Environmental Science and Technology, 49: 11825-11833.

Schoen, M.E., Ashbolt, N.J. 2010. Assessing pathogen risk to swimmers at non-sewage impacted recreational beaches. Environmental Science and Technology, 44(7): 2286-2291.

Schoen, M.E., Soller, J.A., Ashbolt, N.J. 2011. Evaluating the importance of faecal sources in human-impacted waters. Water Research, 45(8): 2670-2680.

Shanks, O.C., Atikovic, E., Blackwood, A.D., Lu, J., Noble, R.T., Domingo, J.S., Seifring, S., Sivaganesan, M., Haugland, R.A. 2008. Quantitative PCR for detection and enumeration of genetic markers of bovine fecal pollution. Applied and Environmental Microbiology, 74(3): 745-752.

Shanks, O.C., Kelty, C.A., Sivaganesan, M., Varma, M., Haugland, R.A. 2009 Quantitative PCR for genetic markers of human fecal pollution. Applied and Environmental Microbiology, 75(17): 5507-5513.

Shanks, O.C., White, K., Kelty, C.A., Sivaganesan, M., Blannon, J., Meckes, M., Varma, M., Haugland, R.A. 2010. Performance of PCR-based assays targeting Bacteroidales genetic markers of human fecal pollution in sewage and fecal samples. Environmental Science and Technology, 44: 6281-6288.

Shanks, O., Sivaganesan, M., Peed, L., Kelty, C., Blackwood, A., Greene, M., Noble, R., Bushon, R., Stelzer, E., Kinzelman, J., Anna'eva, T., Sinigalliano, C., Wanless, D. Griffith, J., Cao, Y., Weisberg, S., Harwood, V.J., Staley, C., Oshima, K., Varma, M., Haugland, R. 2012. Interlaboratory comparison of real-time PCR methods for quantification of general fecal indicator bacteria. Environmental Science and Technology, 46: 945-953.

Shanks, O.C., Newton, R.J., Kelty, C.A., Huse, S.M., Sogin, M.L., McLellan, S.L. 2013. Comparison of the microbial community structures of untreated wastewaters from different geographic locales. Applied and Environmental Microbiology, 79(9): 2906-2913.

Shanks, O.C., Kelty, C.A., Oshiro, R., Haugland, R.A., Madi, T., Brooks, L., Field, K.G., Sivaganesan, M. 2016. Data acceptance criteria for standardized human-associated fecal source identification quantitative real-time PCR methods. Applied and Environmental Microbiology, 82(9): 2773-2782.

Shively, D.A., Nevers, M.B., Breitenbach, C., Phanikumar, M., Przybyla-Kelly, K., Spoljaric, A., Whitman, R.L. 2016. Prototypic automated continuous recreational water quality monitoring of nine Chicago beaches. Journal of Environmental Management, 166: 285-293.

Simöes, R.R., Poirel, L., Da Costa, P.M., Nordmann, P. 2010. Seagulls and beaches as reservoirs for multidrug-resistant *Escherichia coli.* Emerging Infectious Diseases, 16: 110-112.

Sinclair, R., Jones, E., Gerba, C.P. 2009. Viruses in recreational water−borne disease outbreaks: A review. Journal of Applied Microbiology, 107(6): 1769-1780.

Sinigalliano, C.D., Fleisher, J.M., Gidley, M.L., Solo-Gabriele, H.M., Shibata, T., Plano, L.R., Elmir, S.M., Wanless, D., Bartkowiak, J., Boiteau, R., Withum, K., Abdelzaher, A.M., He, G., Ortega, C., Zhu, X., Wright, M.E., Kish, J., Hollenbeck, J., Scott, T., Backer, L.C., and Fleming, L.E., 2010. Traditional and molecular analyses for fecal indicator bacteria in non-point source subtropical recreational marine waters. Water Research, 44(13): 3763-3772.

Sivaganesan, M., Siefring, S., Varma, M., Haugland, R.A. 2014. Comparison of *Enterococcus* quantitative polymerase chain reaction analysis results from midwestern U.S. river samples using EPA Method 1611 and Method 1609 PCR reagents. Journal of Microbiological Methods, 101: 9-17. Corrigendum 115, 166.

Sjölund, M., Bonnedahl, J., Hernandez, J., Bengtsson, S., Cederbrant, G., Pinhassi, J., Kahlmeter, G., Olsen, B. 2008. Dissemination of multidrug resistant bacteria into the Arctic. Emerging Infectious Diseases, 14(1): 70-72.

Sobsey, M.D., Battigelli, D., Handzel, T., Schwab, K. 1995. Male-specific coliphages as indicators of viral contamination of drinking water, p. 1847-1862. In Proceedings of the 1994 Water Quality Technology Conference. American Water Works Association, Denver, CO.

Soller, J.A., Schoen, M.E., Bartrand, T., Ravenscroft, J., Wade, T.J. 2010a. Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. Water Research, 44(16): 4674-4691.

Soller, J.A., Bartrand, T., Ashbolt, N.J., Ravenscroft, J., Wade, T.J. 2010b. Estimating the primary etiologic agents in recreational fresh waters impacted by human sources of faecal contamination. Water Research, 44(16): 4736-4747.

Soller, J.A., Schoen, M.E., Varghese, A., Ichida, A.M., Boehm, A.B., Eftim, S., Ashbolt, N.J., Ravenscroft, J.E. 2014. Human health risk implications of multiple sources of faecal indicator bacteria in a recreational waterbody. Water Research, 66: 254-264.

Soller, J., Bartrand, T., Ravenscroft, J., Molina, M., Whelan, G., Schoen, M., Ashbolt, N. 2015. Estimated human health risks from recreational exposures to stormwater runoff containing animal faecal material. Environmental Modelling and Software, 72: 21-32.

Soller, JA, S Eftim, TJ Wade, AM Ichida, JL Clancy, TB Johnson, K Schwab, G Ramirez-Toro, S Nappier, JE Ravenscroft. 2016. Use of quantitative microbial risk assessment to improve interpretation of a recreational water epidemiological study. Microbial Risk Analysis. 1:2-11

Southern California Coastal Water Research Project (SCCWRP). 2016. The Surfer Health Study: A Three-Year Study Examining Illness Rates Associated with Surfing During Wet Weather. SCCWRP Technical Report 943

Stachler, E., Kelty, C., Sivaganesan, M., Li, X., Bibby, K., Shanks, O.C. 2017. Quantitative CrAssphage PCR assays for human fecal pollution measurement. Environmental Science and Technology Letters, 51: 9146-9154.

Staley, C., Gordon, K.V., Schoen, M.E., Harwood, V.J. 2012. Performance of two quantitative PCR methods for microbial source tracking of human sewage and implications for microbial risk assessment in recreational waters. Applied and Environmental Microbiology, 78(20): 7317-7326.

Stewart, J.R., Boehm, A.B., Dubinsky, E.A., Fong, T-T., Goodwin, K.D., Griffith, J.F., Noble, R.T., Shanks, O.C., Vijayavel, K., Weisberg, S.B. 2013. Recommendations following a multi-laboratory comparison of microbial source tracking methods. Water Research, 47: 6829-6838.

Stewart, I, Schluter PJ, and Shaw GR (2006). Cyanobacterial lipopolysaccharides and human health - a review. *BMC Public Health*, 5:7.

Sunger, N., Haas, C.N. 2015. Quantitative microbial risk assessment for recreational exposure to water bodies in Philadelphia. Water Environment Research, 87(3): 211-222.

Teunis, P.F., Moe, C.L., Liu, P., Miller, S.E., Lindesmith, L., Baric, R.S., Le Pendu, J., Calderon, R.L. 2008. Journal of Medical Virology, 80(8): 1468-1476.

Till, D., McBride, G., Ball, A., Taylor, K., Pyle, E. 2008. Large-scale freshwater microbiological study: Rationale, results and risks. Journal of Water and Health, 6(4): 443-460.

U.S. EPA (United States Environmental Protection Agency). 2001a. Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure. EPA 821-R-01-029. Retrieved November 14, 2017 from: http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/2008_11_25_methods_method_biological_1602.pdf.

U.S. EPA. 2001b. Method 1601: Detection of Male-specific (F+) and Somatic Coliphage in Water by two-step Enrichment Procedure. EPA 821-R-01-030 Office of Water, Engineering and Analysis Division, Washington, DC.

U.S. EPA. 2002a. Method 1600: Enterococci in Water by Membrane Filtration using Membrane-*Enterococcus* Indoxyl-β-D-glucoside agar (mEI). Retrieved November 14, 2017 from: http://www.epa.gov/microbes/1600sp02.pdf.

U.S. EPA. 2002b. Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified Membrane-thermotolerant *Escherichia coli* agar (Modified mTEC). Retrieved November 14, 2017 from: http://www.epa.gov/microbes/1603sp02.pdf.

U.S. EPA. 2004. Managing Manure Nutrients at Concentrated Animal Feeding Operations. Office of Water, Washington DC. Retrieved October 23, 2017 from: http://www.epa.gov/npdes/pubs/cafo_manure_guidance.pdf.

U.S. EPA. 2005. Microbial Source Tracking Guide Document. EPA-600-R-05-064. Office of Research and Development, Cincinnati, OH.

U.S. EPA. 2007a. Report of the Experts Scientific Workshop on Critical Research Needs for the Development of New or Revised Recreational Water Quality Criteria. EPA 823-R-07-006. Office of Water, Washington, DC. Retrieved October 24, 2017 from: https://nepis.epa.gov/Exe/ZyNET.exe/P1009KVG.TXT?ZyActionD=ZyDocument&Client=EPA&Index=2006+Thru+2010&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C06thru10%5CTxt%5C00000023%5CP1009KVG.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL.

U.S. EPA. 2007b. Critical Path Science Plan for the Development of New or Revised Recreational Water Quality Criteria. EPA 823-R-08-002. Office of Water, Washington, DC. Retrieved October 24, 2017 from: https://nepis.epa.gov/Exe/ZyNET.exe/P1009L0Z.TXT?ZyActionD=ZyDocument&Client=EPA&Index=2006+Thru+2010&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C06thru10%5CTxt%5C00000023%5CP1009L0Z.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL.

U.S. EPA. 2008. Great Lakes Beach Sanitary Survey, User Manual. EPA-823-B-06-001. Office of Water, Washington, DC. Retrieved October 23, 2017 from: https://nepis.epa.gov/Exe/ZyNET.exe/P1004SK9.TXT?ZyActionD=ZyDocument&Client=E

PA&Index=2006+Thru+2010&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C06thru10%5CTxt%5C00000009%5CP1004SK9.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL

U.S. EPA. 2010a. Predictive Modeling at Beaches Volume I: Review and Technical Proposal. EPA-823-R-10-003. Office of Water, Washington, DC. Retrieved October 23, 2017 from: https://www.epa.gov/sites/production/files/2015-11/documents/predictive-tools-volume1.pdf.

U.S. EPA. 2010b. Predictive Modeling at Beaches Volume II: Predictive Tools for Beach Notification. EPA-600-R-10-176. National Exposure Research Laboratory, Athens, Georgia. Retrieved October 23, 2017 from: https://nepis.epa.gov/Exe/ZyPDF.cgi/P1009IZT.PDF?Dockey=P1009IZT.PDF.

U.S. EPA. 2010c. Report on 2009 National Epidemiologic and Environmental Assessment of Recreational Water Epidemiology Studies (NEEAR 2010 - Surfside & Boquerón). EPA-600-R-10-168. Office of Water, Washington, DC.

U.S. EPA. 2011. Using Microbial Source Tracking to Support TMDL Development and Implementation. U.S. EPA Region 10.

U.S. EPA. 2012a. Recreational Water Quality Criteria. EPA 820-F-12-058. Office of Water, Washington, DC.

U.S. EPA. 2012b. Method 1611: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay. EPA-821-R-12-008. Office of Water, Washington, DC.

U.S. EPA. 2013a. Marine Beach Sanitary Survey, User Manual. EPA-820-F-13-001. Office of Water, Washington, DC. Retrieved October 23, 2017 from: https://nepis.epa.gov/Exe/ZyPDF.cgi/P100KM2C.PDF?Dockey=P100KM2C.PDF.

U.S. EPA. 2013b. Marine Beach Sanitary Survey, Routine On-Site Sanitary Survey. EPA-823-F-13-008. Office of Water, Washington, DC. Retrieved October 23, 2017 from: https://nepis.epa.gov/Exe/ZyPDF.cgi/P100KO2U.PDF?Dockey=P100KO2U.PDF.

U.S. EPA. 2013c. Marine Beach Sanitary Survey, Annual Sanitary Survey. EPA-823-F-13-007. Office of Water, Washington, DC. Retrieved October 23, 2017 from: https://nepis.epa.gov/Exe/ZyPDF.cgi/P100KO2H.PDF?Dockey=P100KO2H.PDF.

U.S. EPA. 2013d. Detection and Quantification Limits of EPA *Enterococcus* qPCR Methods. EPA 820-R-13-013. Office of Water, Washington, DC.

U.S. EPA. 2013e. Literature Review of Contaminants in Livestock and Poultry Manure and Implications for Water Quality. EPA 820-R-13-002. Office of Water, Washington, DC.

U.S. EPA. 2014a. National Beach Guidance and Required Performance Criteria for Grants. EPA-823-B-14-001. Office of Water, Washington, DC. Retrieved October 24, 2017 from: https://nepis.epa.gov/Exe/ZyPDF.cgi/P100KZDK.PDF?Dockey=P100KZDK.PDF.

U.S. EPA. 2014b. Overview of Technical Support Materials: A Guide to the Site-Specific Alternative Recreational Criteria TSM Document. EPA-820-R-14-010.

U.S. EPA. 2015a. Drinking Water Health Advisory for Cyanobacterial Microcystin Toxins. EPA 820R15100. Office of Water, Washington, DC. Retrieved October 23, 2017 from: https://www.epa.gov/sites/production/files/2017-06/documents/microcystins-report-2015.pdf.

U.S. EPA. 2015b. Drinking Water Health Advisory for Cyanobacterial Toxin Cylindrospermopsin. EPA 820R15101. Office of Water, Washington, DC. Retrieved October 23, 2017 from: https://www.epa.gov/sites/production/files/2017-06/documents/cylindrospermopsin-report-2015.pdf.

U.S. EPA. 2015c. Health Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin. EPA 820R15101. Office of Water, Washington, DC. Retrieved October 23, 2017 from: https://www.epa.gov/sites/production/files/2017-06/documents/cylindrospermopsin-support-report-2015.pdf.

U.S. EPA. 2015d. Health Effects Support Document for the Cyanobacterial Toxin Microcystin. EPA-820R1502. Office of Water, Washington, DC. Retrieved October 23, 2017 from: https://www.epa.gov/sites/production/files/2017-06/documents/microcystins-support-report-2015.pdf.

U.S. EPA. 2015e. Methods 1609.1 & 1611.1 Calculation Spreadsheet. Retrieved September 27, 2017 from: http://www2.epa.gov/cwa-methods/other-clean-water-act-test-methods-microbiological.

U.S. EPA. 2015f. Review of Coliphages as Possible Indicators of Fecal Contamination for Ambient Water Quality. EPA 820-R-15-098. Office of Water, Washington, DC.

U.S. EPA 2016a. Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin. EPA 822-P-16-002. Office of Water, Washington, DC.

U.S. EPA. 2016b. Six Key Steps to Developing and Using Predictive Tools at Your Beach. EPA-820-R-16-002. Office of Water, Washington, DC. Retrieved October 23, 2017 from: https://www.epa.gov/beach-tech/six-key-steps-developing-and-using-predictive-tools-your-beach.

U.S. EPA 2016c. U.S. Environmental Protection Agency. Office of Water and Office of Research and Development. *National Rivers and Streams Assessment 2008-2009: A Collaborative Survey* (EPA/841/R-16/007). Washington, DC. March 2016. http://www.epa.gov/national-aquatic-resource-surveys/nrsa

U.S. EPA. 2017a. 2016 Coliphage Workshop Meeting Proceedings. EPA 820-R-17-003. Office of Water, Washington, DC. Retrieved October 23, 2017 from: https://www.epa.gov/sites/production/files/2017-08/documents/coliphage-workshop-proceedings-2016-508.pdf.

U.S. EPA. 2017b. Polluted Runoff: Nonpoint Source Pollution. Retrieved October 23, 2017 from: https://www.epa.gov/nps/what-nonpoint-source.

U.S. EPA. Undated. Water Permitting 101. Office of Wastewater Management, Washington, DC. Retrieved October 23, 2017 from: https://www3.epa.gov/npdes/pubs/101pape.pdf.

van den Berg, H., Lodder, W., van der Poel, W., Vennema, H., de Roda Husman, A.M. 2005. Genetic diversity of noroviruses in raw and treated sewage water. Research Microbiology 156(4): 532-540540

Viau, E.J., Lee, D., Boehm, A.B. 2011. Swimmer risk of gastrointestinal illness from exposure to tropical coastal waters impacted by terrestrial dry-weather runoff. Environmental Science and Technology, 45: 7158-7165.

Vijayavel, K., Fujioka, R., Ebdon, J., Taylor, H. 2010. Isolation and characterization of Bacteroides host strain HB-73 used to detect sewage specific phages in Hawaii. Water Research, 44(12): 3714-3724.

Wade TJ, Augustine SAJ, Griffin SM, Sams EA, Oshima KH, Egorov AI, Simmons KJ, Eason TN, Dufour AP (2018). Asymptomatic norovirus infection associated with swimming at a tropical beach: A prospective cohort study. PloS one. 2018;13(3):e0195056.

Wade, T.J., Calderon, R.L., Brenner, K.P., Sams, E., Beach, M., Haugland, R., Wymer, L., Dufour, A.P. 2008. High sensitivity of children to swimming-associated gastrointestinal illness: Results using a rapid assay of recreational water quality. Epidemiology, 19(3): 375-383.

Wade, T.J., Sams, E.A., Haugland, R., Brenner, K.P., Li, Q., Wymer, L., Molina, M., Oshima, K., Dufour, A.P. 2010. Report on 2009 National Epidemiologic and Environmental Assessment of Recreational Water Epidemiology Studies. EPA 600-R-10-168. Office of Research and Development, Washington, DC.

Wade, T.J., Sams, E.A., Beach, M.J., Collier, S.A., Dufour, A.P. 2013. The incidence and health burden of earaches attributable to recreational swimming in natural waters: A prospective cohort study. Environmental Health, 12: 67.

Wade, T.J., Augustine, S., Griffin, S., Simmons, K., Easton, T., Oshima, K. 2016. Asymptomatic norovirus infection associated with swimming at a tropical beach. Society for Epidemiologic Research. *Poster presentation*. June 21-24 2016, Miami FL.

Wanjugi, P., Sivaganesan, M., Korajkic, A., Kelty, C.A., McMinn, B., Ulrich, R., Harwood, V.J., Shanks, O.C. 2016. Differential decomposition of bacterial and viral fecal indicators in common human pollution types. Water Research, 105: 591-601.

Wellington, E.M., Boxall, A.B., Cross, P., Feil, E.J., Gaze, W.H., Hawkey, P.M., Johnson-Rollings, A.S., Jones, D.L., Lee, N.M., Otten, W., Thomas, C.M., Williams, A.P. 2013. The role of the natural environment in the emergence of antibiotic resistance in gram-negative bacteria. The Lancet Infectious Diseases, 13(2): 155-165.

Wiedenmann, A., Krüger, P., Dietz, K., López-Pila, J.M., Szewzyk, R., Botzenhart, K. 2006. A randomized controlled trial assessing infectious disease risks from bathing in fresh recreational waters in relation to the concentration of *Escherichia coli*, intestinal enterococci, *Clostridium perfringens*, and somatic coliphages. Environmental Health Perspectives, 114(2): 228-236.

Whelan, G., Kim, K., Pelton, M.A., Soller, J.A., Castleton, K.J., Molina, M., Pachepsky, Y., Zepp, R. 2014. An integrated environmental modeling framework for performing quantitative microbial risk assessment. Environmental Modelling and Software, 55: 77-91.

Whelan, G., R. Parmar, and G. Laniak. Microbial Source Module (MSM): Documenting the Science and Software for Discovery, Evaluation, and Integration. US EPA Office of Research and Development, Washington, DC, USA, 2015a.

Whelan , G., M. Pelton, M. Molina , R. Zepp , J. Ravenscroft , and Y. Pachepsky. Microbial Properties Database Editor Tutorial. US EPA Office of Research and Development, Washington, DC, USA, 2015b.

Whelan, G., R. Parmar, K. Wolfe, M. Galvin, P. Duda, and M. Gray. Quantitative Microbial Risk Assessment Tutorial - SDMProjectBuilder: Import Local Data Files to Identify and Modify Contamination Sources and Input Parameters Updated 2017. US EPA Office of Research and Development, Washington, DC, USA, 2017a.

Whelan, G., K. Kim, R. Parmar, K. Wolfe, M. Galvin, M. Gray, P. Duda, M. Molina, and R. Zepp. Quantitative Microbial Risk Assessment Tutorial - Land-applied Microbial Loadings within a 12-Digit HUC - Updated 2017b.

Whelan, G., Kim, K., Parmar, R., Laniak, G.F., Wolfe, K., Galvin, M., Molina, M., Pachepsky, Y. A., Duda, P., Zepp, R., Prieto, L., Kinzelman, J.L., Kleinheinz, G. T., Borchardt, M. A. 2018. Capturing Microbial Sources Distributed in a Mixed-use Watershed within an Integrated Environmental Modeling Workflow. Environmental Software and Modeling. *99(2018)126-146.* http://dx.doi.org/10.1016/j.envsoft.2017.08.002 1

Whitman, R.I., Nevers, M.B. 2003. Foreshore sand as a source of *Escherichia coli* in nearshore water of a Lake Michigan Beach. Applied and Environmental Microbiology, 69: 5555-5562.

WHO (World Health Organization). 2003. Guidelines for Safe Recreational Water Environments: Volume 1: Coastal and Fresh Waters. World Health Organization.

Wilkes, G., Edge, T.A., Gannon, V.P., Jokinen, C., Lyautey, E., Neumann, N.F., Ruecker, N., Scott, A., Sunohara, M., Topp, E., Lapen, D.R. 2011. Associations among pathogenic bacteria, parasites, and environmental and land use factors in multiple mixed-use watersheds. Water Research, 45(18): 5807-5825.

Wong, M., Kumar, L., Jenkins, T.M., Xagoraraki, I., Phanikumar, M.S., Rose, J.B. 2009. Evaluation of public health risks at recreational beaches in Lake Michigan via detection of enteric viruses and a human-specific bacteriological marker. Water Research, 43: 1137-1149.

Wright, G.D. 2010. Antibiotic resistance in the environment: A link to the clinic? Current Opinion in Microbiology, 13: 589-594.

Wymer, Larry J., Timothy J. Wade, and Alfred P. Dufour. 2013. Equivalency of risk for a modified health endpoint: a case from recreational water epidemiology studies. *BMC public health* 13, no. 1 (2013): 459

Wu, J., Long, S.C., Das, D., Dorner, S.M. 2011. Are microbial indicators and pathogens correlated? A statistical analysis of 40 years of research. Journal of Water and Health, 9(2): 265-278.

Yan, T., Sadowsky, M.J. 2007. Determining sources of fecal bacteria in waterways. Environmental Monitoring and Assessment, 129: 97-106.

Yau, V., Wade, T.J., de Wilde, C.K., Colford Jr., J.M. 2009. Skin-related symptoms following exposure to recreational water: A systematic review and meta-analysis. Water Quality, Exposure, and Health, 1(2): 79-103.

Yau, V.M., Schiff, K.C., Arnold, B.F., Griffith, J.F., Gruber, J.S., Wright, C.C., Wade, T.J., Burns, S., Hayes, J.M., McGee, C. 2014. Effect of submarine groundwater discharge on bacterial indicators and swimmer health at Avalon Beach, CA, USA. Water Research, 59: 23-36.

# Appendix A. Advancements in Mitigating Interference in Quantitative Polymerase Chain Reaction (qPCR) Methods for Microbial Water Quality Monitoring

## A. Introduction

The U.S. EPA's 2012 Recreational Water Quality Criteria (2012 RWQC; U.S. EPA, 2012a) included qPCR Method 1611 (U.S. EPA, 2012b) as a supplemental indicator to detect and quantify *Enterococcus* spp. in ambient water on a site-specific basis. The qPCR methodology offers the advantage of providing rapid detection results (2-6 hours), allowing beach managers to make same-day decisions to protect families and their children. In contrast, water quality results for traditional culturable indicator methods are not available until 24-48 hours after sampling. In addition to providing rapid results, the EPA's *Enterococcus* spp. qPCR (Method A) was significantly associated with gastrointestinal (GI) illness in the human-impacted EPA NEEAR studies (Wade et al., 2006, 2008, 2010). At the time of the 2012 RWQC publication, however, the EPA still had limited experience with the method's performance across a broad range of environmental conditions. States were cautioned to be aware of the potential for qPCR interference in various waterbodies, which may vary on a site-specific basis. The EPA encouraged a site-specific analysis of the method's performance prior to use in a beach notification program or in the adoption of qPCR-based WQS (U.S. EPA, 2013a).

As defined in this report, interference is any process that results in lower quantitative estimates than expected or actual values (Haugland et al., 2012). For qPCR-based enumeration methods, interference occurs when substances in the test sample inhibit polymerase function, or cause the DNA to be lost or unavailable for amplification. Examples of substances causing interference include humic acids, coral sands, calcium, and certain types of clay particles; however, there are likely many other unidentified substances that can also contribute to qPCR interference. From a public health standpoint, this interference can result in false negative results of the sample. However, several method controls have been created and refined over the past few years to estimate and control sample interferences (Table A-1).

**Table A-1. Common qPCR Interference Controls**

| Interference Controls | Abbreviation | Application | Common Types |
|---|---|---|---|
| Sample Processing Control<br>(EPA Method A, 1611, and 1609) | SPC | A non-target DNA sequence used to estimate recovery efficiency. The control involves spiking a known quantity of non-target DNA into the sample prior to processing. | Sketa 2<br>Sketa 22 |
| Internal Amplification Control<br>(EPA Method 1611 and 1609) | IAC | A non-target DNA sequence added to the reaction mix prior to the qPCR reaction. If the non-target DNA does not amplify as expected, a problem with the qPCR reaction is indicated (e.g., DNA polymerase inhibition). | IAC5 |
| Dilution<br>(Cao et al., 2012) | dilution | Dilution of the sample can result in dilution of other compounds that interfere with DNA amplification. Different dilutions can be compared (i.e., serial dilutions). | 5x<br>25x |
| Ratio spiked test matrix/spiked control matrix<br>(Haugland et al., 2016) | STM/SCM | The recovery of target DNA sequences (gene copies) from target organisms spiked into the water samples (STM) can be compared to the recovery of DNA from spiked target organisms in control samples (SCM). The STM/SCM ratio can provide an additional measure of interference caused by inhibitors in the water matrix. | Not applicable |
| Addition of higher salmon DNA concentrations to samples during extraction<br>(Haugland et al., 2012) | Not applicable | Demonstrated at one tropical site (PR) to reduce interference due to DNA loss during sample extraction (Haugland et al., 2012) | 25x increase in salmon DNA concentration |
| Calculation using delta-delta cycle threshold<br>(EPA Method A, 1611, and 1609) | ΔΔCt | A method to estimate *Enterococcus* spp. in a water sample, accounting for recovery and partial inhibition. The ΔΔCt is calculated from the ΔCt (*Enterococcus* assay Ct – Sketa SPC assay Ct value) for the water sample and for the calibrator/positive control sample and then subtracting the calibrator/positive control ΔCt from the water sample ΔCt. | Not applicable |

In the 2012 RWQC document, the EPA also noted other rapid qPCR methods, such as the draft EPA Bacteroidales qPCR Method B (U.S. EPA, 2010), which demonstrated a significant

association with illness at the NEEAR marine beaches (Wade et al., 2010), and emerging qPCR methods for *E. coli.* An example of the latter has been recently evaluated against culturable methods and demonstrated utility on a site-specific basis (Lavender and Kinzelman, 2009). As part of the five-year review of the 2012 RWQC, the EPA is interested in understanding information about the status of *Enterococcus* spp., *E. coli*, and *Bacteroidales* qPCR methods. The specific objectives of this work are to identify: 1) where qPCR methods have been applied since 2010; 2) the rate of interference when using molecular methods in those waterbodies; 3) method improvements that have reduced interference; and 4) method or water matrix attributes (e.g., turbidity) and dynamics of fecal contamination that may continue to contribute to poor performance or increased interference/inhibition. Additionally, we want to provide information on an upcoming enumeration tool, digital droplet PCR (ddPCR).

## B. Methods

### 1. Systematic Literature Search

We performed a systematic literature search of the peer-reviewed literature for articles reporting qPCR monitoring data in recreational water in PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and Web of Science. The search included the keywords shown in Table A-2. The literature search was limited to English language peer-reviewed citations published between 2010 and March 2017.

**Table A-2. Literature Search Terms**

| PubMed Set | Search Terms |
|---|---|
| Set 1 | (ambient-water[tiab] OR Beach[tiab] OR Beaches[tiab] OR estuaries[tiab] OR estuaries[mh] OR Estuarine[tiab] OR Estuary[tiab] OR freshwater[tiab] OR fresh-water[mh] OR fresh-water[tiab] OR Lake[tiab] OR lakes[tiab] OR lakes[mh] OR Marine[tiab] OR recreational-water[tiab] OR Reservoir[tiab] OR reservoirs[tiab] OR River[tiab] OR Rivers[tiab] OR rivers[mh] OR stormwater[tiab] OR storm-water[tiab] OR Stream[tiab] OR streams[tiab] OR surface-water[tiab]) |
| Set 2 | AND rapid-method[tiab]<br>OR molecular-method[tiab] OR qPCR[tiab] OR quantitative-PCR[tiab] OR quantitative-polymerase-chain-reaction[tiab] OR Real-Time-Polymerase-Chain Reaction[mh] OR RT-PCR[tiab] OR digital-droplet-PCR[tiab] OR Method-1609[tiab] OR Method-1611[tiab] OR Method-B[tiab] |
| Set 3 | AND fecal-indicator[tiab]<br>OR *Enterococcus*[tiab] OR *Escherichia-coli*[tiab] OR enterococci[tiab] OR *E. coli*[tiab] OR Bacteroidales[tiab] |
| Set 4 | AND detection[tiab] |
| Set 5 | AND PCR-inhibitory-compounds[tiab]<br>OR inhibition[tiab] OR inhibitor[tiab] OR inhibitory-effects[tiab] |

| | |
|---|---|
| NOT terms | NOT Pool[tiab]<br>OR Pools[tiab] OR hot-tub[tiab] OR hot-tubs[tiab] OR spa[tiab] OR spas[tiab] OR sauna[tiab] OR saunas[tiab] OR seeded[tiab] OR spiked[tiab] OR bench-top[tiab] |
| Limit: Language | AND (English[lang]) |
| Limit: Date | AND ("2010/01/01"[PDAT] : "3000/12/31"[PDAT]) |
| Web of Science Set | **Search Terms (searched in Title, Abstract, and Keywords)** |
| Set 1 | (ambient-water OR Beach OR Beaches OR estuaries OR Estuarine OR Estuary OR freshwater OR fresh-water OR Lake OR lakes OR Marine OR recreational-water OR Reservoir OR reservoirs OR River OR Rivers OR stormwater OR storm-water OR Stream OR streams OR surface-water) |
| Set 2 | AND rapid-method<br>OR molecular-method OR qPCR OR quantitative-PCR OR quantitative-polymerase-chain-reaction OR Real-Time-Polymerase-Chain Reaction OR RT-PCR OR digital-droplet-PCR OR Method-1609 OR Method-1611 OR Method-B |
| Set 3 | AND fecal-indicator<br>OR *Enterococcus* OR Escherichia-*coli* OR enterococci OR *E. coli* OR Bacteroidales |
| Set 4 | AND detection |
| Set 5 | AND PCR-inhibitory-compounds<br>OR inhibition OR inhibitor OR inhibitory-effects |
| NOT terms | NOT Pool<br>OR Pools OR hot-tub OR hot-tubs OR spa OR spas OR sauna OR saunas OR seeded OR spiked OR bench-top |

## 2. Systematic Literature Screening

Abstracts were screened for relevance to the scope, including papers using the following methods: *Enterococcus* spp. qPCR (Method 1609); *Enterococcus* spp. qPCR (Method 1611); Bacteroidales qPCR (Method B); *E. coli* qPCR, and digital droplet PCR. Following the abstract *screening*, the full text of articles passing scope was reviewed for specific information related to: study location, sampling time, waterbody type, analytical method(s) applied, how inhibition was controlled, contamination source(s) and dynamics (e.g., wet-weather driven), water quality results, percent of samples inhibited, limit of quantitation, and percent recovery. Studies had to

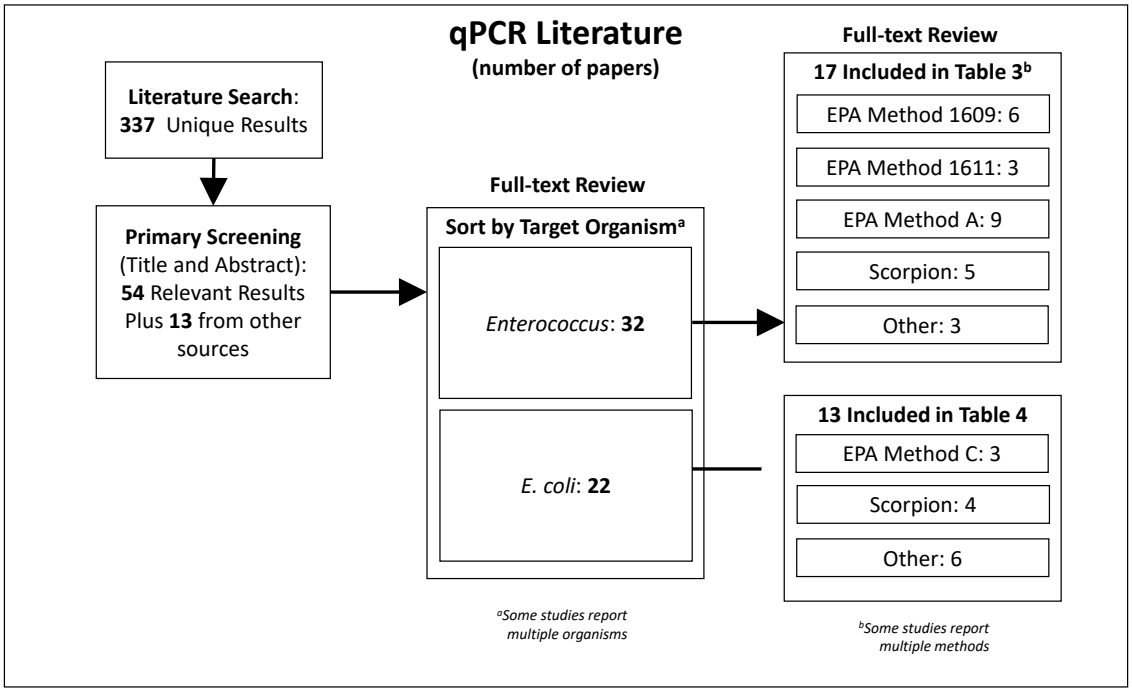
provide information on the occurrence and/or evaluation of inhibition to be included in the review.

## C. Results

### 1. Literature Screening and Review

The literature search returned 337 unique results, of which 54 were relevant based on the abstract screening (Figure 1). An additional 13 studies were identified through other sources (e.g., cited in another paper). A total of 32 studies included *Enterococcus* qPCR, 22 included *E. coli* qPCR, and 18 included *Bacteroides* qPCR. Tables 4 and 5 summarize the final subset of the *Enterococcus* and *E. coli* qPCR papers. No studies were found that evaluated the EPA Bacteroidales qPCR Method between 2010 and 2017 in fresh or marine waters. However, multiple studies were found that investigated *Bacteroides* for microbial source tracking (MST) purposes. Advancements in MST are discussed elsewhere in the 2017 Review.

**Figure A-1. Summary of Number of Articles Screened and Reviewed**




### 2. Advancements in *Enterococcus* spp. qPCR Methods

***EPA Methods:*** The EPA's first published qPCR method for *Enterococcus* spp. (Method A) was successfully applied to the EPA's NEEAR study (Haugland et al., 2005). Based on the literature review only, the freshwater sites in the Great Lakes and four temperate marine beaches demonstrated minimal to no interference, but the tropical marine beach site samples from Puerto Rico exhibited significant interference (U.S. EPA, 2010c; Haugland et al., 2012). Prior to the publication of the 2012 RWQC, The EPA updated Method A as the published Method 1611 (Table A-3). Updates included: 1) a requirement of the SPC assay to use Sketa 22; and 2) a

recommendation for using the IAC assay. As in EPA Method A, the method used a reagent called Universal Master Mix (UMM) (TaqMan; Applied Biosystems, Foster City, CA) (U.S. EPA, 2012b). However, EPA Method 1611 was found to result in high levels of interference in some waters, unless samples were diluted five-fold or more (Haugland et al., 2012, 2016; Sivaganesan et al., 2014). Dilution is a standard methodological approach to lessen interference or other negative amplification effects that can occur when utilizing undiluted extracts.

To address the potential for high interference levels, particularly due to inhibition, the EPA developed EPA Method 1609 (U.S. EPA, 2013b), which uses the Environmental Master Mix (EMM) reagent (TaqMan; Applied Biosystems, Foster City, CA), resulting in lower levels of interference in undiluted samples (Cao et al., 2012; Haugland et al., 2012, 2016; Sivaganesan et al., 2014). Like EPA Method 1611, EPA Method 1609 requires the SPC interference control using the Sketa 22 assay and recommends the IAC assay. Table A-3 summarizes analytical details related to reducing interference and the strategies for controlling for interference in the various qPCR methods.

***Non-EPA Methods:*** Most other qPCR methods for measuring *Enterococcus* spp. in ambient water have been applied by a single research laboratory. The exception is the Scorpion-based qPCR assay from Noble et al. (2010). The Scorpion qPCR technology uses a different master mix (OmniMix, Cepheid, Inc., Sunnyvale, CA) and processing controls (Smartbeads, Cepheid Inc., Sunnyvale, CA) and was designed to be faster than other qPCR chemistries. The Scorpion-based method was included in Tables A-3 and A-4 because multiple papers evaluated the method in ambient waters.

**Table A-3. *Enterococcus* spp. qPCR Methods**

| Method (reference) | Analytical Permutations Master Mix | Recommended Sample Extract Dilution | Performance/Interference Evaluation Analyses SPC: Acceptance range | IAC: Acceptance range | TSC or CE spike recovery: Acceptance range |
|---|---|---|---|---|---|
| EPA Method 1609 (Haugland et al., 2016) | EMM | Undiluted<br>(5x diluted optional) | Sketa 22: Test sample Ct within 3 units of calibrator samples (mandatory in method) | IAC 5: Test sample Ct within 1.5 units of negative control samples (recommended in method) | TSC: 50 – 200%[b] |
| EPA Method 1611 (Haugland et al., 2016) | UMM | 5x diluted | Sketa 22: Test sample Ct within 3 units of calibrator samples (mandatory in method) | IAC5: Test sample Ct within 1.5 units of negative control samples (recommended in method) | TSC: 50 – 200%[b] |
| EPA Method A<br>(EPA-821-R-10-004, 2012) | UMM | 5x or 25x diluted | Sketa 2[a]: Test sample Ct within 3 units of uninhibited reference samples | Not evaluated | CE: Acceptance ranges defined by study results[c]<br>Fresh water: detect - 333%<br>Marine water: detect - 1123% |
| Scorpion method<br>(Noble et al., 2010) | OmniMix | 10x diluted, if needed | *Lactococcus* (SmartBeads): 1.5 Ct shift | *Enterococcus* IC, *Lactococcus* IC (SmartBeads): 1.5 Ct shift | Not evaluated |

[a] Sketa 22 was also evaluated.

[b] Recovery ratio of spiked test matrix (filters and retentates from collected water samples spiked with *Enterococcus* cells) to spiked control matrix (clean filters spiked with *Enterococcus* cells).

[c] Recovery ratio of estimated qPCR cell equivalents in spiked test matrix to estimated CFU in the spikes. Spiking done with 550 CFU Bioballs™.
Acronyms and Abbreviations: CE = cell equivalent; EMM = Environmental MasterMix; IAC = Internal Amplification Control; IC = propriety PCR positive internal control template; UMM = Universal MasterMix; SPC = Sample processing control; TSC = target sequence copy

Table A-4 summarizes results from 16 papers that included information on the selected *Enterococcus* qPCR methods. In a recent national study focusing primarily on potentially

problematic sites, EPA Method 1609 showed an average qPCR interference rate of 10% (range 0-22%) and 11% (range 0-24%) in undiluted samples from 9 and 12 individual temperate marine and freshwater sites, respectively, based on the SPC and IAC controls (Haugland et al., 2016). Average interference rates from other studies were lower (Table A-4). A five-fold dilution of the water sample extracts from the national study reduced the average interference rates to 4% and 3% for temperate marine and freshwaters and reduced the interference rates at most sites (9/9 marine and 10/12 freshwater) to acceptable frequencies of <10% (U.S. EPA, 2013a; Haugland et al., 2016).

**Table A-4. Summary of Interference Rates for *Enterococcus* spp. qPCR Methods**

| Citation | Water Type | Location (# of sites) | Fecal Source | # of Samples Undiluted (% interference) | # of Samples Diluted 5X (% interference) | Strategies to Test Interference |
|---|---|---|---|---|---|---|
| ***EPA Method 1609 (EMM)*** | | | | | | |
| Dorevitch et al., 2017 | FW | MI (9) | WW, NPS | 1256 (1.1) | 540 (0.37) | SPC (Sketa 22) (Ct 3) |
| Haugland et al., 2016 | M | FL, CA, NC (9) | Not reported | 241 (10) | 356 (4) | SPC (Sketa 22) (Ct 3)[a]<br>IAC (IAC5) (Ct 1.5) |
| Haugland et al., 2016 | FW | WI, OH, FL (13) | Not reported | 491 (11) | 419 (3) | SPC (Sketa 22) (Ct 3)[a]<br>IAC (IAC5) (Ct 1.5) |
| Sivaganesan et al., 2014 | FW | OH, KY, IN, PA, IA (7) | NPS, SS, WW, AW, HW | 221 (5) | 221 (3) | SPC (Sketa 22) (Ct 3)<br>IAC (IAC5) (Ct 1.5) |
| Haugland et al., 2012 | FW | OH, KY (5) | NPS, SS, WW, AW, HW | 268[b] (0) | 268[b] (0.7) | SPC (Sketa 22) (Ct 3)[a]<br>IAC (IAC5) (Ct 1.5) |
| Cao et al., 2012 | M, FW | CA, IL (52) | NPS, HW | 133 (0) | Not reported | SPC (Sketa 2)[c] (Ct 3)[a] |
| Cao et al., 2012 | M, FW | CA, IL (52) | NPS, HW | 133 (1) | 133 (0) | IAC (IAC5) (Ct 1.7)[a] |

| Citation | Water Type | Location (# of sites) | Fecal Source | # of Samples Undiluted (% interference) | # of Samples Diluted 5X (% interference) | Strategies to Test Interference |
|---|---|---|---|---|---|---|
| ***EPA Method 1611 (UMM)*** | | | | | | |
| Haugland et al., 2016 | M | FL, CA, NC (9) | Not reported | 240[b] (>40)[d] | 359 (7) | SPC (Sketa 22) (Ct 3)[a]<br>IAC (IAC5) (Ct 1.5) |
| Haugland et al., 2016 | FW | WI, OH, FL (13) | Not reported | 490[b] (>40)[d] | 419 (6) | SPC (Sketa 22) (Ct 3)[a]<br>IAC (IAC5) (Ct 1.5) |
| Cao et al., 2013 | M | CA (9[b]) | HW, SS, WW | 12 (0) | Not reported | SPC (Sketa 22) (Ct threshold not indicated) |
| Converse et al., 2012a | FW | WI (1) | AW, | 80 (0) | Not reported | SPC (Sketa 22) (Ct 3) |
| Converse et al., 2012b | M | CA (3) | NPS | 1,200 (7) | Not reported | SPC (Sketa 22) (Ct 3) |
| Haugland et al., 2012 | FW | OH, KY (5) | NPS, SS, WW, AW, HW | 268[b] (18) | 268[b] (1.5) | SPC (Sketa 22) (Ct 3)[a]<br>IAC (IAC5) (Ct 1.5) |
| Haugland et al., 2012 | M | PR (6[b]) | Not reported | Not reported | 684 (32)[a] | SPC (Sketa 22) (Ct 3)<br>IAC (IAC5) (Ct 1.5) |
| ***EPA Method A (or Haugland et al., 2005)*** | | | | | | |
| Raith et al., 2014 | M | CA (9) | Not reported | Not reported | 306 (5)[j] | SPC (Sketa 2) (Ct 3)[e] |
| Zimmer-Faust et al., 2014 | M, FW | CA, Mexico (18) | AW, NPS, WW, SS | 82 (0) | Not reported | SPC |
| Cao et al., 2012 | M, FW | CA, IL (52) | NPS, HW | 133 (7) | 133 (1) | SPC (Sketa 2) (Ct 3)[a] |

| Citation | Water Type | Location (# of sites) | Fecal Source | # of Samples Undiluted (% interference) | # of Samples Diluted 5X (% interference) | Strategies to Test Interference |
|---|---|---|---|---|---|---|
| Cao et al., 2012 | M, FW | CA, IL (52) | NPS, HW | 133 (42) | 133 (7) | IAC (IAC5) (Ct 1.7)[a] |
| Haugland et al., 2012 | FW | Ohio River OH, KY (5) | NPS, SS, WW, AW, HW | 268[b] (30) | 268[b] (7) | SPC (Sketa 2) (Ct 3)[a]<br>IAC (IAC5) (Ct 1.5) |
| Haugland et al., 2012 | M | PR (6[b]) | Not reported | Not reported | 895 (36)[a] | SPC (Sketa 2) (Ct 3)<br>IAC (Ct 1.5) |
| Haugland et al., 2012 | FW | AZ, CA, GA,HI,IA, IN, LA, MD,MN, NC,NJ,NY, WA,WI (27) | Not reported | Not reported | 108 (7) | SPC (Sketa 2) (Ct 3) |
| Sauer et al., 2011 | FW | WI | NPS, WW | 214 (< 1) | Not reported | IAC |
| Abdelzaher et al., 2010 | M | FL (1) | NPS, HW | 12 (0) | Not reported | SPC |
| ***Scorpion*[f] (Noble et al. 2010)** | | | | | | |
| Raith et al., 2014 | M | CA (9) | Not reported | Not reported | 306 (5)[i] | SPC (Ct > 1.7) |
| Cao et al., 2013 | M | CA (9[b]) | HW, SS, WW | Not reported | 12 (0) | SPC (Sketa 22 |
| Converse et al., 2012b | M | CA (3) | NPS | 1,200 (16) | Not reported | SPC (Ct 1.6) |
| Cao et al., 2012 | M, FW | CA, IL (52) | NR, NPS, HW | 133 (42) | 133 (4) | SPC (Sketa 2) (Ct 3)[a] |
| Cao et al., 2012 | M, FW | CA, IL (52) | NR, NPS, HW | 133 (56) | 133 (18) | IAC (Ct 1.7)[a] |
| Noble et al., 2010 | M, FW | CA | WW, UR, SS | 238 (<5) | Not reported | SPC (*Lactococcus*) (Ct 1.5)<br>IAC (Ct 1.5) |

| Citation | Water Type | Location (# of sites) | Fecal Source | # of Samples Undiluted (% interference) | # of Samples Diluted 5X (% interference) | Strategies to Test Interference |
|---|---|---|---|---|---|---|
| ***Other*** | | | | | | |
| Cao et al., 2012 | M, FW | CA, IL (52) | NPS, HW | TF: 133 (9) | TF: 133 (1) | SPC (Sketa 2) (Ct 3)[a] |
| Cao et al., 2012 | M, FW | CA, IL (52) | NPS, HW | TF: 133 (53) | TF: 133 (8) | IAC (IAC5) (Ct 1.7)[a] |
| Cao et al., 2012 | M, FW | CA, IL (52) | NPS, HW | TFF: 133 (23) | TFF: 133 (2) | SPC (Sketa 2) (Ct 3)[a] |
| Cao et al., 2012 | M, FW | CA, IL (52) | NPS, HW | TFF: 133 (90) | TFF: 42 (37) | IAC (IAC5) (Ct 1.7)[a] |
| Bergeron et al., 2011 | M | Spain, France | HW, SS, WW | 85 (0) | Not reported | qPCR control |
| Santiago-Rodriguez et al., 2012[g] | FW | PR | NPS, AW, WW | 130 (0) | 130 (0)[h] | Not reported |
| Wang et al., 2016[g] | FW, M | CA (8) | Not reported | 24 (0) | Not reported | Compare to digital PCR results |

[a] Other interference controls evaluated (dilution and/or STM/SCM).
[b] Interference rates shown are based on SPC assay only, IAC assay results were generally in agreement when available.
[c] Deviated from Method 1609 by using Sketa2 rather than Sketa22 SPC assay.
[d] Average interference rate was not reported separately for M and FW in undiluted samples. However, separate rates are reported for 5x diluted samples.
[e] The SPC control was evaluated using Ct shift acceptance thresholds of 3.0 and 1.7. When using the 1.7 Ct acceptance threshold, 22% interference was found.
[f] Scorpion is a proprietary qPCR primer and probe chemistry.
[g] qPCR conducted with 1x TaqMan Universal PCR Master Mix and the Entero1 23S rRNA gene assay.
[h] 10-fold dilution
[i] Composite of undiluted and 5X dilution results. 5X dilutions analyzed only for undiluted samples that failed Sketa2 assay acceptance criterion.
Abbreviations and Acronyms: Master Mixes: EMM = environmental master mix; UMM = universal master mix; Waterbody Types: M = marine (Pacific Ocean, Atlantic Ocean, brackish stream); FW = Freshwater (river, stream, inland lake, Great Lakes); Fecal Sources: NPS = non-point source/urban runoff; HW = human waste; AW = animal waste; SS = spiked samples; WW = waste water; Interference Controls: SPC = sample processing control; IAC = internal amplification control; Ct shift = Difference in Cycle Threshold values for between control and environmental samples; STM/SCM = recovery ratio of spiked test matrix (STM) (filters and retentates from collected water samples spiked with *Enterococcus* cells) to spiked control matrix (SCM) (clean filters and buffers spiked with *Enterococcus* cells); Sketa 22 = primers for salmon sperm DNA; Sketa 2 = primers for salmon sperm DNA; TF = TaqFast method; TFF = Taq Fastfast method

In contrast, EPA Method 1611 exhibited a much higher average interference rate in undiluted samples, ranging from 18-53%, in studies of corresponding temperate marine and freshwater sites. A five-fold dilution of the water sample extracts again significantly reduced the interference rate in both freshwaters and marine waters to acceptable levels of <10% at most sites studied. For EPA's qPCR Method A, the interference rate was significantly higher when

using Sketa 2, as compared to using Sketa 22 in Method 1611 for analyses of Ohio River water samples (Table A-4).

Only one of the studies shown in Table A-4 addressed the potential reason for interference in the water samples tested (Haugland et al., 2012). Haugland et al. (2012) suggest that the predominance of polymerase inhibitory compounds (such as calcium, iron, iron containing compounds, and tannic acid) affecting amplification that would affect both IAC and SPC assay results in the Ohio River, and DNA binding compounds (such as humic acid and melanin) that would primarily affect the SPC assay results in Boquerón Bay could explain the discrepancy in failure rates observed for these two control assays among samples from the two locations. Kinzelman et al. (2011) speculated that runoff from land during precipitation events could have been a factor for interference in that particular study. Additionally, Wang et al. (2016) spiked qPCR reactions with organic (humic acid, 5 ng/μL) and inorganic (calcium, 2.0 mM) matter to test their inhibitory effects on PCR reactions. The study found that small concentrations of both caused significant inhibition. Additionally, too few studies provided adequate information on fecal source dynamics to draw any meaningful conclusions on how sources might impact the likelihood of interference (Table A-4).

Overall, EPA *Enterococcus* qPCR (Method 1609) resulted in fewer interfering samples, as compared to other methods (EPA Method A, Method 1611, and the Scorpion-based method). Use of the EMM and, when necessary, sample dilution addressed interference at the 9 marine and 23 of the 25 freshwater sites in 10 states that were comprehensively investigated in EPA studies (Haugland et al., 2012, 2016; Sivaganesan et al., 2014). Based on these results, use of EPA Method 1609, including the required and suggested controls, is appropriate on a site-specific basis.

### 3. Advancements in *E. coli* qPCR Methods

The EPA has developed a draft qPCR method for *E. coli* (Method C, using EC23S857 primers) (Chern et al., 2011). Three studies were found that referred to using the aforementioned *E. coli* primers (Table A-5). In addition to using Sketa 22 for an SPC, two of the studies (Peed et al., 2011; Molina et al., 2014) used the CowM2 plasmid as an IAC, which was originally developed by EPA researchers for bovine-specific microbial source tracking (Shanks et al., 2008). The method also employs the EMM reagent, which minimizes interference.

Over the past few years, other researchers have developed qPCR methods for *E. coli* and tested those methods in ambient waters, using a variety of available primers and probes specific to *E. coli* (Table A-5). These methods have not been directly compared to EPA's *E. coli* qPCR method in ambient waters, and thus advantages are unclear.

The 13 studies included in Table A-5 all illustrate low rates of interference (<10%). However, the number sites and samples reported is significantly smaller than for *Enterococcus* qPCR. EPA Method C shows promise to have similar performance characteristics as Method 1609 based on its current use of the same reagents (EMM), controls (Sketa22, SPC and IAC5, IAC assays) and

target genes (23S rRNA) for use on a site-specific basis. However, there are no peer-reviewed demonstrations of its use for routine monitoring..

**Table A-5. Summary of Interference Rates for *E. coli* qPCR Methods**

| Citation | Water Type | Location (# of sites) | Fecal Source | # of Samples Undiluted (% interference) | # of Samples 5x Diluted (% interference) | Strategies to Evaluate Inhibition |
|---|---|---|---|---|---|---|
| ***EPA draft Method C [EC23S857]***[a] | | | | | | |
| Chern et al., 2011 | M, FW | MA, PR (12) | NPS | 25 (0) | Not reported | SPC (Sketa 2) (Ct > 3) |
| Peed et al., 2011 | FW | OH (9) | NPS, WW | 215 (2.1) | Not reported | IAC (CowM2)[b] (Ct 35.1 ± 1.8) |
| Molina et al., 2014 | M | SC, FL (5) | NPS | 471[c] (7) | Not reported | IAC (CowM2)[b] (Ct 33.8 ± 1.6) |
| ***Scorpion (Noble et al., 2010)*** | | | | | | |
| Krometis et al., 2013 | FW | NC (4) | NPS | 94 (31) | 29 (20)[d] | SPC (Sketa 2) (Ct > 1.5) |
| Painter et al., 2013 | FW | TX (1) | HW, AW | Not reported | 102 (19)[e] | IAC |
| Converse et al., 2012b | FW | WI (1) | AW | 80 (0) | Not reported | SPC (Sketa 22) (Ct 3) |
| Noble et al., 2010 | M, FW | CA (6) | WW, UR, SS | 226 (<5) | Not reported | Ct shift (> 1.5) |
| ***Other*** | | | | | | |
| Cloutier and McLellan, 2017 | FW | WI (6) | Not reported | 124 | Not reported | |
| Byappanahalli et al., 2015 | FW | IN (1) | Not reported | 5[c] (0) | Not reported | SPC |
| Walker et al., 2013 | M, FW | PR, Trinidad (44) | NPS, WW | 210 (0) | Not reported | IAC (Ct <2) |
| Zhang et al., 2012a | FW | MO (1) | SS, NPS | 10[c] (8) | Not reported | IAC |
| Bergeron et al., 2011 | M | Spain, France (3) | HW, SS, WW | 80 | 0 | Ct shift (24.5 ± 0.5 cycles) |
| Sauer et al., 2011 | FW | WI (4) | NPS, WW | 220 (<1) | Not reported | IAC |

[a] Current draft method calls for the use of salmon DNA SPC with Sketa22 assay, IAC5 plasmid and assay for inhibition control, 56 degrees Celsius annealing temperature for thermal cycling, and EMM reagent. Some of these provisions were not followed in the reported studies.
[b] IAC using CowM2 plasmid DNA
[c] Estimated

[d] 10x dilution
[e] DNA not diluted but cleaned using post-extraction technique (Chelex®100 and solubilization/binding buffer QX1).
Abbreviations and Acronyms: Waterbody Type: M = marine (Pacific Ocean, Atlantic Ocean, brackish stream); FW = Freshwater (river, stream, inland lake, Great Lakes); Fecal Source: NPS = non-point source/urban runoff; HW = human waste; AW = animal waste; FC = fecal contamination; SS = spiked samples; WW = waste water; Interference Controls: SPC = sample processing control; Sketa 22 = primers for salmon sperm DNA; Sketa 2 = primers for salmon sperm DNA; IAC = internal amplification control

## 4. Digital PCR

Digital PCR (dPCR) is an emerging technology for determining the quantity of target DNA sequences in a sample. While traditional qPCR involves measuring DNA products in a single tube after each qPCR cycle, dPCR partitions the sample into thousands to millions of smaller reactions that are examined individually for binary endpoint results (presence/absence). The DNA density is then estimated from the fraction of positives using Poisson statistics. The dPCR method can be conducted in chambers or droplets, the latter is known as ddPCR. The discussion below does not differentiate between these types of dPCR. The dPCR methods may offer several possible advantages over qPCR discussed below. However, it should be noted that there are few publications to-date that have evaluated the method in ambient waters (Cao et al., 2015, 2016a, b; Wang et al., 2016). Thus, the method is not broadly recommended for routine monitoring, at this time.

First, dPCR does not require a standard curve, thus eliminating some of the labor and materials associated with regularly running batch standards and the biases associated with calibration model variability (Wang et al., 2016). However, it is important to note that a positive standard control is still recommended by dPCR experts (Bustin et al., 2009). As a result, practitioners will still need to create and maintain a standard reference material as a positive control for routine testing.

Second, dPCR may have improved repeatability and reproducibility compared to qPCR (Cao et al., 2016a) for some applications. Repeatability refers to the precision of an assay among replicates of the same sample over a short period of time (short-term precision). Reproducibility refers to the consistency in results among operators, runs, or laboratories (long-term precision). The higher precision associated with dPCR allows for the detection of a 1.25-fold difference in the DNA template, whereas qPCR can typically only detect a two-fold difference in clinical gene expression applications (Cao et al., 2016a). However, it remains unclear whether this small but important difference in precision will prove useful in environmental sample applications where additional variability in concentration estimates is possible and likely. Although dPCR may provide some advantages to qPCR with respect to repeatability and reproducibility, issues with accuracy may arise in samples requiring DNA extraction from a complex mix of biological materials (Huggett et al., 2013), such as environmental samples. Some ambient water samples may be characterized as complex and, in these instances, it is important that experimental replication and the number of replicates are appropriate for measurement of the desired target (Huggett et al., 2013). An increase in the associated error of a DNA target concentration estimate may occur in a given dPCR assay if replication includes variability from extractions. To date, it

remains unclear whether these issues potentially nullify any precision advantage of dPCR over traditional qPCR approaches for ambient water sample applications.

Third, because of sample partitioning, it has been reported that dPCR may be less prone to environmental amplification inhibition compared to qPCR applications although other types of interference may still occur (Cao et al., 2016a). In general, inhibitors within a sample matrix will either completely prevent or partially reduce amplification, the latter scenario resulting in an underestimation of the true target DNA concentration. Wang et al. (2016) found that humic acid caused a similar level of amplification inhibition in both dPCR and qPCR experiments, however inhibition of dPCR was partially relieved when the number of thermal cycles was increased. In addition, others have found that dPCR is able to tolerate PCR inhibitor concentrations that are one to two orders of magnitude higher than those in paired qPCR tests with conventional reagents (Cao et al., 2016a). However, the incidence of amplification inhibition in ambient surface water qPCR samples is reported to be extremely rare when using customized DNA polymerases such as Environmental Mastermix (Cao et al., 2012; Haugland et al., 2012, 2016). It is also important to note that both dPCR and qPCR are susceptible to partial amplification inhibition, where the presence of inhibitors could either reduce the percentage of positive partitions (dPCR) or lower the quantification cycle (qPCR) leading to an underestimation of the true DNA target concentration. As a result, it is useful to employ a quantitative control with each test sample to monitor for both complete or partial amplification inhibition to validate findings (Bustin et al., 2009; Huggett et al., 2013).

Finally, dPCR may be superior to qPCR for multiplex reaction applications, amplification of two or more different DNA templates in one reaction (Cao et al., 2016b). Multiplexing in traditional qPCR can lead to an underestimation of the less abundant target if not properly optimized, whereas dPCR may provide more robust quantification of multiple DNA targets. Using dPCR, Cao et al. (2016b) duplexed *Enterococcus* spp. and HF183 (EntHF183 dPCR assay), which provided accurate and repeatable information on both general and human-associated fecal contamination in environmental waters, without the need to run two separate qPCR tests.

There are also several potential limitations of dPCR as compared to qPCR. First, given this is a new technology, there would likely be additional costs associated with implementing it in a laboratory and obtaining the necessary instrumentation and supplies (Huggett et al., 2013). Secondly, the quantifiable range is smaller for dPCR, and currently the upper limit of quantitation of dPCR is four orders of magnitude lower than that of qPCR. Thus, sample dilution is required when measuring samples with a high concentration of a DNA target, like those potentially found in sewage spills (Cao et al., 2016b). Additionally, Poisson statistics require uniformity in the partitions for accurate results. Viscous DNA, due to high concentrations or long templates, may result in uneven distributions, biasing the partitions and leading to potentially inaccurate results. Finally, if double-stranded DNA is denatured into single strands, the template is effectively increased because single-strands can occupy different partitions, which could lead to up to a two-fold overestimation by dPCR (Cao et al., 2016b).

## D. References


Abdelzaher, A.M., Wright, M.E., Ortega, C., Solo-Gabriele, H.M., Miller, G., Elmir, S., Newman, X., Shih, P., Bonilla, A., Bonilla, T.D., Palmer, C.J., Scott, T., Lukasik, J., Harwood, V.J., McQuaig, S., Sinigalliano, C., Gidley, M., Plano, L.R.W., Zhu, X., Wang, J.D., Fleming, L.E. 2010. Presence of pathogens and indicator microbes at a non-point source subtropical recreational marine beach. Applied and Environmental Microbiology, 76(3): 724-732.

Bergeron, P., Oujati, H., Catalan Cuenca, V., Huguet Mestred, J.M., Courtoise, S. 2011. Rapid monitoring of *Escherichia coli* and *Enterococcus* spp. in bathing water using reverse transcription-quantitative PCR. International Journal of Hygiene and Environmental Health, 214(6): 478-484.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J., Wittwer, C.T. 2009. The MIQE Guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clinical Chemistry, 55(4): 611-622.

Byappanahalli, M.N., Nevers, M.B., Whitman, R.L., Ishii, S. 2015. Application of a microfluidic quantitative polymerase chain reaction technique to monitor bacterial pathogens in beach water and complex environmental matrices. Environmental Science and Technology Letters, 2(12): 347-351.

Cao, Y., Griffith, J.F., Dorevitch, S., Weisberg, S.B. 2012. Effectiveness of qPCR permutations, internal controls and dilution as means for minimizing the impact of inhibition while measuring *Enterococcus* in environmental waters. Journal of Applied Microbiology, 113(1): 66-75.

Cao, Y., Griffith, J.F., Weisberg, S.B., Sivaganesan, M., Haugland, R.A., Kinzelman, J., Blackwood, A.D., Noble, R.T. 2013. Effect of platform, reference material, and quantification model on enumeration of general fecal indicators by quantitative PCR methods. Water Research, 47(1): 233-241

Cao, Y., Raith, M.R., Griffith, J.F. 2015. Droplet digital PCR for simultaneous quantification of general and human-associated fecal indicators for water quality assessment. Water Research, 70: 337-349.

Cao, Y., Griffith, J.F., Weisberg, S.B. 2016a. The next-generation PCR-based quantification method for ambient waters: Digital PCR. Methods in Molecular Biology 1452:113-30.

Cao, Y., Raith, M.R., Griffith, J.F. 2016b. A duplex digital PCR assay for simultaneous quantification of the *Enterococcus* spp. and the human fecal-associated HF183 marker in waters. Journal of Visualized Experiments, 9(109).

Chern, E.C., Siefring, S., Paar, J., Doolittle, M., Haugland, R.A. 2011. Comparison of quantitative PCR assays for *Escherichia coli* targeting ribosomal RNA and single copy genes. Letters in Applied Microbiology, 52(3): 298-306.

Cloutier, D.D., McLellan, S.L. 2017. Distribution and Differential Survival of Traditional and Alternative Indicators of Fecal Pollution at Freshwater Beaches. Applied and Environmental Microbiology 83(4).

Converse, R.R., Kinzelman, J.L., Sams, E.A., Hudgens, E., Dufour, A.P., Ryu, H., Santo-Domingo, J.W., Kelty, C.A., Shanks, O.C., Siefring, S.D., Haugland, R.A., Wade, T.J. 2012a. Dramatic improvements in beach water quality following gull removal. Environmental Science and Technology 46(18): 10206-10213.

Converse RR, Griffith JF, Noble RT, Haugland RA, Schiff KC, Weisberg SB. 2012b. Correlation between quantitative PCR and culture-based methods for measuring *Enterococcus* spp. over various temporal scales at three California marine beaches. Applied and Environmental Microbiology 78(4): 1237-1242.

Dorevitch, S., Shrestha, A., DeFlorio-Barker, S., Breitenbach, C., Heimler, I. 2017. Monitoring urban beaches with qPCR vs. culture measures of fecal indicator bacteria: Implications for public notification. Environmental Health 16:45.

Haugland, R.A., Siefring, S.C., Wymer, L.J., Brenner, K.P., Dufour, A.P. 2005. Comparison of *Enterococcus* measurements in freshwater at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis. Water Research, 39: 559-568.

Haugland, R.A., Siefring, S., Lavender, J., Varma, M. 2012. Influences of sample interference and interference controls on quantification of enterococci fecal indicator bacteria in surface water by the qPCR method. Water Research, 46(18): 5989–6001.

Haugland, R.A., Siefring, S., Varma, M., Oshima, K. H., Sivaganesan, M., Cao, Y., Raith, M., Griffith, J., Weisberg, S.B., Noble, R.T., Blackwood, A. D., Kinzelman, J., Ananeva, T., Bushon, R.N., Harwood, V. J., Gordon, K.V., Sinigalliano, C. 2016. Multi-laboratory survey of qPCR enterococci analysis method performance in U.S. coastal and inland surface waters. Journal of Microbiological Methods, 123: 114-125.

Huggett, J.F., Foy, C.A., Benes, V., Emslie, K., Garson, J.A., Haynes, R., Hellmans, J., Kubista, M., Mueller, R.D., Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J., Wittwer, C.T., Bustin, S.A. 2013. The Digital MIQE guidelines: Minimum information for publication of quantitative digital PCR experiments. Clinical Chemistry, 59(6): 892-902.

Krometis, L.A., Noble, R.T., Characklis, G.W., Blackwood, A.D., Sobsey, M.D., 2013. Assessment of *E. coli* partitioning behavior via both culture-based and qPCR methods. Water Science and Technology 68(6): 1359-1369.

Lavender, J. S., Kinzelman, J.L. 2009. A cross comparison of qPCR to agar-based or defined substrate test methods for the determination of Escherichia coli and enterococci in municipal water quality monitoring programs. Water Research 43: 4967-4979.

Molina, M., Hunter, S., Cyterski, M., Peed, L.A., Kelty, C.A., Sivaganesan, M., Mooney, T., Prieto, L., Shanks, O.C. 2014. Factors affecting the presence of human-associated and fecal indicator real-time quantitative PCR genetic markers in urban-impacted recreational beaches. Water Research, 64: 196-208.

Noble, R.T., Blackwood, A.D., Griffith, J.F., McGee, C.D., Weisberg, S.B. 2010. Comparison of rapid quantitative PCR-based and conventional culture-based methods for enumeration of *Enterococcus* spp. and *Escherichia coli* in recreational waters. Applied Environmental Microbiology 76(22):7437-43.

Painter, S.M., Pfau, R.S., Brady, J.A., McFarland, A.M.S. 2013. Quantitative assessment of *Naegleria fowleri* and *Escherichia coli* concentrations within a Texas reservoir. Journal of Water and Health 11(2): 346-357.

Peed, L.A., Nietch, C.T., Kelty, C.A., Meckes, M., Mooney, T., Sivaganesan, M., Shanks, O.C. 2011. Combining land use information and small stream sampling with PCR-based methods for better characterization of diffuse sources of human fecal pollution. Environmental Science and Technology, 45: 5652-5659.

Raith, M., Ebentier, D., Cao, Y., Griffith, J., Weisberg, S. 2014. Factors affecting the relationship between quantitative polymerase chain reaction (qPCR) and culture-based enumeration of *Enterococcus* in environmental waters. Journal of Applied Microbiology 116:737-746.

Santiago-Rodriguez, T.M., Tremblay, R.L., Toledo-Hernandez, C., Gonzalez-Nieves, J.E., Ryu, H., Santo Domingo, J.W., Toranzos, G.A. 2012. Microbial quality of tropical inland waters and effects of rainfall events. Applied and Environmental Microbiology 78(15): 5160-5169.

Sauer, E.P., Vandewalle, J.L., Bootsma, M.J., McLellan, S.L. 2011. Detection of the human specific *Bacteroides* genetic marker provides evidence of widespread sewage contamination of stormwater in the urban environment. Water Research 45(14): 4081-4091.

Shanks, O.C., Atikovic, E., Blackwood, A.D., Lu, J., Noble, R.T., Domingo, J.S., Seifring, S., Sivaganesan, M., Haugland, R.A. 2008. Quantitative PCR for detection and enumeration of genetic markers of bovine fecal pollution. Applied and Environmental Microbiology, 74(3): 745-752.

Sivaganesan, M., Siefring, S., Varma, M., Haugland, R.A. 2014. Comparison of *Enterococcus* quantitative polymerase chain reaction analysis results from midwestern U.S. river samples using EPA Method 1611 and Method 1609 PCR reagents. Journal of Microbiological Methods, 101: 9-17. Corrigendum 115, 166

U.S. EPA 2010. Method B: Bacteroidales in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay. Office of Water. EPA-822-R-10-003.

U.S. EPA. 2012a. Recreational Water Quality Criteria. EPA 820-F-12-058. Office of Water, Washington, DC.

U.S. EPA. 2012b. Method 1611: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay. EPA-821-R-12-008. Office of Water, Washington, DC.

U.S. EPA 2013a. Acceptability of the EPA qPCR Test at Your Beach. Office of Water. EPA-820-R-13-012.

U.S. EPA 2013b. US Environmental Protection Agency: Method 1609: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) with Internal Amplification Control (IAC) Assay.

Wade, T.J., Calderon, R.L., Sams, E., Beach, M., Brenner, K.P., Williams, A.H., Dufour, A.P., 2006. Rapidly Measured Indicators of Recreational Water Quality Are Predictive of Swimming-Associated Gastrointestinal Illness. Environmental Health Perspectives 114(1): 24-28.

Wade, T.J., Calderon, R.L., Brenner, K.P., Sams, E., Beach, M., Haugland, R., Wymer, L., Dufour, A.P. 2008. High sensitivity of children to swimming-associated gastrointestinal illness: results using a rapid assay of recreational water quality. Epidemiology, 19(3): 375-383.

Wade, T.J., Sams, E.A., Haugland, R., Brenner, K.P., Li, Q., Wymer, L., Molina, M., Oshima, K., Dufour, A.P. 2010. Report on 2009 National Epidemiologic and Environmental Assessment of Recreational Water Epidemiology Studies. EPA 600-R-10-168. Office of Research and Development, Washington, DC.

Walker, T.J., Bachoon, D.S., Otero, E., Ramsubhag, A. 2013. Detection of verotoxin producing *Escherichia coli* in marine environments of the Caribbean. Marine Pollution Bulletin 76(1-2): 406-410.

Wang, D., Yamahara,K.M., Cao, Y., Boehm, A.B. 2016. Absolute Quantification of Enterococcal 23S rRNA Gene Using Digital PCR. Environmental Science & Technology 50: 3399−3408.

Zhang, Y.Y., Riley, L.K., Lin, M.S., Hu, Z. 2012. Determination of low-density Escherichia coli and Helicobacter pylori suspensions in water. Water Research 46(7): 2140-2148.

Zimmer-Faust, A.G., Thulsiraj, V., Ferguson, D., Jay, J.A. 2014. Performance and specificity of the covalently linked immunomagnetic separation-ATP method for rapid detection and enumeration of enterococci in coastal environments. Applied and Environmental Microbiology 80(9): 2705-2714.

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## Appendix B. Communication with Regional Coordinators on the Implementation of the RWQC

4/X/17

To: Regional Beach Coordinators

From: John Wathen & Samantha Fontenelle

Re: Barriers and experiences of states' implementation of 2012 RWQC and adoption of BAVs

Regional Beach Program folks:

This is not another call for an update on the status of states adoption of the criteria, although current information on that topic is always welcome. As we have discussed on Beach Program calls, OST is conducting a 5-year review of the 2012 RWQC as required by the BEACH Act. In addition to assessing the continued scientific currency of the RWQC, we are examining a range of issues pertinent to the RWQC.

One of those issues relates to barriers or other issues encountered by states that have adopted or are adopting the RWQC and BAVs and now have some experience with their application. For example, one exception that we heard from some states when the RWQC were issued was that the lower BAV would lead to more advisories, which would require more re-sampling to lift, and would lead to fewer beaches being monitored. We are interested in hearing from the states in your regions as to what their experiences have been to generally answer these questions:

1. What barriers to implementation of the 2012 RWQC, if any, has your state beach program encountered?
2. Have any adverse consequences to adoption been experienced?
3. Have there been positive experiences or outcomes as a result of adoption?
4. Without undoing any of the significant elements of the RWQC and implementation guidance, is there anything that could be addressed in the guidance that should be included in the review report and be subsequently changed in the guidance to improve the operation of the beach monitoring and advisory programs in the states.

We would like to schedule individual calls with each regional beach coordinator(s), HQ Beach Program staff, and state beach program leads in the region together on the phone during the month of April. Just to be clear, this is region by region and not with everyone on the line together, which would likely be unwieldy. This would be an informal opportunity for the states to be heard. We will be in touch with you the week of April 10 to schedule calls in the near future.

Thanks for your cooperation on this.

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**Appendix C. Review of the EPA's 2012 Recreational Water Quality Criteria Health Study Information Expert Consultation**

# Review of EPA's 2012 Recreational Water Quality Criteria Health Study Information Expert Consultation

Graham McBride (Graham.McBride@niwa.co.nz)
Principal Scientist, Water Quality–Aquatic Pollution
NIWA (National Institute of Water and Atmospheric Research,
Hamilton, New Zealand
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## List of Abbreviations

| | |
|---|---|
| AGI | Acute gastrointestinal illness |
| BAV | Beach Action Value |
| BEACH Act | Beaches Environmental Assessent and Coastal Health Act |
| CAT | *Catellicoccus marimammalium* |
| CAWS | Chicago Waterways System |
| EPA | U.S. Environmental Protection Agency |
| Epi | Epidemiological |
| ETEC | enterotoxigenic *E. coli* |
| FIB | Fecal indicator bacteria |
| FIO | Fecal indicator organism |
| GI | gastrointestinal |
| HAdV | human adenovirus |
| MST | Microbial Source Tracking |
| NEEAR | National Epidemiological and Environmental Assessment of Recreational Water |
| QMRA | Quantitative Microbial Risk Assessment |
| qPCR | quantitative Polymerase Chain Reaction |
| RWQC | Recreational Water Quality Criteria |
| STEC | Shigatoxigenic *E. coli* |
| WRP | Water Reclamation Plant |

## A. BACKGROUND

The 2012 U.S. Environmental Protection Agency (EPA) Recreational Water Quality Criteria (RWQC) are designed to protect the public from exposure to pathogens in waters designated for primary contact recreational uses. Criteria development included an analysis of research up to 2012, and an evaluation of the association between illness and extent of fecal contamination in these waters. The 2012 RWQC provide two sets of numeric concentration thresholds based on the use of two bacterial indicators, *E. coli* and enterococci. Illness rates upon which these recommendations are based include the outcomes from the National Epidemiological and Environmental Assessment of Recreational Water (NEEAR) study and earlier epidemiological studies used to support the 1986 Ambient Water Quality Criteria.

The Beaches Environmental Assessment and Coastal Health (BEACH) Act of 2000 requires that the EPA review and, as necessary, revise recreational water quality criteria within five years of publication. The EPA is currently doing that. The overall goal of this review is to develop an EPA report that describes available information and includes the Agency's assessment of whether revisions to the 2012 criteria are necessary to ensure the protection of recreational waters. The EPA is requesting expert consultation to facilitate two of the EPA's main objectives for this project:

- Inventory and evaluate health study information available since 2010 on public health impacts from exposure to fecal contamination in recreational waters.
- Assess new information regarding existing recommended and alternative indicator/methods combinations, and their relationship to health assessment for the general population and children

To assist this expert consultation, The EPA, via their consultants (ICF, www.icf.com) has provided the following charge questions.

## B. CHARGE QUESTIONS

1. Please provide a summary review of peer-reviewed health studies published since 2010 describing human health risks from exposure to recreational waters affected by fecal contamination. This can be presented in tabular form including the following headers: Reference, location, study type and design, contamination source(s), water quality metrics, health effects evaluated, health linkages reported, and conclusions. Epidemiological (including NEEAR), exposure, and microbial risk assessment studies should be considered.
2. Based on the above review of health studies, please separately summarize any new information as it pertains to children's health (e.g., exposure, ingestion rate, health risk studies). Regarding the quantitative polymerase chain reaction (qPCR)-health relationships (e.g., *Enterococcus* qPCR or Bacteroides qPCR), does the relationship between the molecular indicator and the health outcome (i.e. gastroenteritis) for adults/general population differ for children?
3. Based on the above review of health studies, please separately summarize any new information on relationships between health and the following currently-recommended indicators of water quality: culturable *E. coli*, culturable enterococci, and *Enterococcus* spp. qPCR. In what scenarios of fecal contamination are culture-based indicators and/or qPCR-based indicators predictive?

4. Based on the above review of health studies, please separately summarize information on relationships between health and alternative indicators (e.g., *Clostridium perfringens*, human or animal source markers, pepper mild mottle virus), with the exception of coliphage since the EPA has a separate ongoing effort with that specific indicator.
5. Based on the above review of health studies, describe the specific fecal sources and contamination dynamics (*e.g.*, differential effects between wet and dry weather) impacting waters in studies with a statistical relationship between health and water quality.
6. Please summarize any outbreak information during the last 10 years including, etiological agent, symptoms reported, and water quality information that may be relevant to this overall health study review.

## C. THE APPROACH TAKEN

A list of 98 potentially appropriate references was forwarded to the author by ICF for consideration. Selection of these references considered recent investigations using both epidemiological (Epi) and Quantitative Microbial Risk Assessment (QMRA) methods. A few of the papers selected were more in the nature of a microbiological experiment or survey – these were included to highlight issues arising from laboratory methodology.

Of these 98 documents, 15 were considered but not included in detailed analysis (judged to be not particularly relevant), and a further 23 were included, including nine papers published (or in press) in 2017. That is, 104 references in total were considered. A few of the included references predate 2010 (e.g., Harrington *et al.* (1993), Ferley *et al.* (1989)), because they are particularly relevant, but are not widely known.

Each reference was perused according to the items required by the charges. This entailed detailed review of texts to record responses to the charges, and checking of preliminary material provided by ICF. Full copies of all references are held by the author in electronic form.

To facilitate clarity in the following text, longer lists of references and detailed technical information in support of an inference are given in footnotes.

## D. RESPONSES

### Charge 1—Summary of peer-reviewed studies

The response, after addressing all the topics stated for this Charge, are summarized in Table 1**Error! Reference source not found.**. Use of a small font allows all the charges' components to be presented on the same page. Accordingly it appears after responding to Charge 6, with headers repeated on each page.

The Conclusions column (the last in the table) includes the health metrics item (the basis for the response to the third Charge). The content of cells in that column comprise materials derived from the document's abstract and conclusions. Information on water quality metrics and contaminant sources were obtained from the methods sections of the reviewed documents. Synthesis of those water quality metrics are included in the Conclusions column, in which the most important points taken from these information cells are underlined.

The main observations and inferences drawn, *other than those in response to Charges 2–6*, are guided by the Conclusions column in Table 1 and are as follows.

a) Epidemiological studies and QMRA are quite different approaches to the task of setting health-related water quality criteria, yet they are complementary. In particular, their relative strengths vary from one location to another. For example, Epi studies capture the actual water ingestion or inhalation volumes during exposure events at its site(s), whereas QMRA has to estimate those volumes. On the other hand, Epi studies are restricted to the location(s) and times at which they were carried out, whereas QMRA can be applied to many other situations for which Epi study results are scarce (such as locations not impacted by human wastes).
b) Epidemiological studies can gain an extra advantage by including in their monitoring a selection of fecal indicators *and* pathogens, as do some of the reviewed documents. This includes different ways of measuring a given indicator, e.g., enterococci by culture and by PCR. In contrast, including several pathogens in a QMRA is relatively straightforward and less expensive than in epidemiological studies.
c) It should always be recognised that environmental waters can contain a mix of pathogens, only some of which may be analysed. So the calculated risk for those for the selected pathogens may not capture the water's overall pathogenicity.
d) Many epidemiological studies report an increase in health risk for the exposed (e.g., swimmers) versus the non-exposed, but fail to find a relationship between some water quality variable and health risk, e.g., "Epidemiological studies show a generally elevated risk of gastrointestinal illness in bathers compared to non-bathers but often no clear association with water quality as measured by fecal indicator bacteria; this is especially true where study sites are impacted by non-point source pollution" (Fewtrell & Kay 2015). In contrast, QMRA models are built on pathogen dose-response curves which, in general, exhibit a monotonic increase in risk of infection or illness with increasing dose—so association of health risk and water quality is always evident.
e) The form of language used by Fewtrell & Kay (2015)–"no clear association"–is appropriate and is in common use in the studies reviewed. But it is common for science interpreters to make a stronger claim: That the absence of a statistically significant result admits a finding of "no association". However, failure to attain statistical significance is not the same as establishing the veracity of the tested hypothesis.[1] In the case of the phraseology used by Fewtrell & Kay (2015), that "failure" merely means that the relative strength of association is higher in one case ("exposed versus not-exposed") than in the other ("association of health risk with water quality"). It would be helpful to make this point in the revised criteria.
f) In general, risks posed by animal sources such as gull, chicken and pigs may pose a lesser risk compared to human fecal material, but not so for bovine cattle[2] and possibly for ovine ruminants. This inference has mostly been established using QMRA models.

[1]These hypotheses are all two-sided, positing that there is exactly zero change in some statistic of the population being sampled. So the lack of statistical significance merely means that the sample size was insufficient to obtain a finding of statistical significance because, in general, *P*-values for such tests decrease with increasing sample size, e.g., see McBride *et al.* (2014)—and many other statistical writings on this matter, such as those referenced therein. So failure to attain significance may merely reflect that the study size was simply not "big enough". In that regard it is notable that the earlier epidemiological studies that underpinned the "Ambient Water Quality for Bacteria–1986" were based on much larger sample sizes (on the order of 30,000) than many more recent studies have used: The "Dufour freshwater study" had 34,598 participants and the "Cabelli marine study" had 26,686.
[2]Schoen & Ashbolt 2010, Ehsan *et al.* 2015, Soller *et al.* 2015, Brown et al. 2017).

g) Dose-response models for Norovirus, as used in a number of QMRA models,[3] are remarkably dependent on the degree of aggregation of virions present in the low concentrations typical in environmental waters. For example, without loss of generality, take a simplified case where 10 people each ingest 100 mL of water from a container in which there are 10 Norovirus particles. If these pathogens are aggregated into one clump of ten, then only one of the ten people can be affected; the other nine are exposed to "no dose". At the other extreme, up to ten people could be affected if there is no aggregation—each particle is independent of the other. Many, if not all, of the ten subjects then receives a "low dose", thus increasing the average risk faced by these 10 people. Studies of the dynamics and effects of aggregation phenomena are therefore warranted (Soller *et al.* 2017 is an excellent start).
h) A meta-analysis of studies with two different gastrointestinal endpoints (NGI vs. HCGI) would be helpful, to enable comparisons between Epi studies to be “on common ground” (Wymer *et al.* 2013).
i) Future studies should consider including more emerging pathogens, especially anti-microbial pathogens (Leonard *et al*. 2015, Young 2016).
j) Eventually, MST (Microbial Source Tracking) markers may support source apportionment as well as risk assessment, given additional epidemiological data and/or empirical descriptions of pathogen-*Bacteroidales* relationships (Bambic *et al.* 2015).
k) Risks from mixed sources are driven predominantly by the proportion of the contamination source with the greatest ability to cause human infection (potency), which is not necessarily the greatest source(s) of fecal indicator bacteria (FIB) (Schoen & Ashbolt 2010, Soller *et al.* 2014).
l) Conditions in more tropical regions, especially Hawaii, may require more use of QMRA given the propensity for enterococci to be associated with contaminated soil (Vijayaval *et al.* 2010) and to exhibit higher decay rates in the environment (Kirs *et al.* 2016)–and the enhanced possibility of enterococci regrowth.

## Charge 2—Children's health

In response to Charges 2–6, epidemiological studies (Sanborn & Takoro 2013, de Man *et al.* 2014, Arnold *et al.* 2016) show that children appear to be at higher risk (cf. adults) when swimming/playing in water. There are two possible causes:

a) Children may have a higher rate of ingestion or inhalation of ambient water.
b) Children may be more susceptible to pathogen infection.

Regarding a), the innovative swimming pool studies reported by Dufour *et al.* (2017) show that children may ingest water at rates four times greater than adult rates. Increasingly, such data are being included in QMRA models. On the other hand, in some settings children may ingest at a lower rate (but still more than adults), depending on their swimming behaviour (Suppes *et al.*, 2014). The choice of exposure data, particularly in terms of duration, has a substantial effect on risk predicted by QMRA.

[3]Soller *et al.* (2010a&b), Schoen *et al.* (2011), Viau *et al.* (2011), Francy *et al.* (2013), McBride *et al.* (2013), de Man *et al.* (2014), Sales-Ortells & Medema (2014), Schijven *et al.* (2015), Zlot *et al.* (2015), Eregno *et al.* (2016), Soller *et al.* (2016), Soller *et al.* (2017).

Regarding b), it is commonly held in health risk modelling that children are born with inherent susceptibility that reduces over time[4] as some immunity is developed and maintained. Studies reported herein do not take explicit account of this aspect, yet it seems highly desirable to do so, especially as children appear to be the most at-risk group.

### Charge 3—New information on health and indicators

a) In general, Norovirus is likely to be the most important pathogen for humans in waters affected by discharges of treated sewage (Soller *et al.*, 2010a).
b) Prior-day *E. coli* culture testing was no better than chance in predicting the exceedance of the qPCR Beach Action Value (BAV). *E. coli* culture testing of beaches (on the same day) led to three times the number of BAV exceedance as did enterococci qPCR testing of beach water (Dorevich *et al.* 2017).
c) In French rivers (Ardèche Basin), fecal streptococci were best correlated to gastrointestinal morbidity, fecal coliforms less so. Swimmers suffer skin ailments much more frequently than non-swimmers.
d) Coupling QMRA with an epidemiological study at a single study site provides a unique ability to understand human health risk and illnesses, especially under conditions where water quality, as measured by traditional fecal indicator organisms (FIOs) is good and/or average illness rates are lower than can be quantified via epidemiological methods (Soller *et al.* 2016).
e) The fecal indicator bacteria *Enterococcus* spp., estimated by qPCR, is well-associated with gastrointestinal illness among swimmers (Wade *et al.* 2010, Wyer *et al.* 2013).
f) Statistically significant trends of increasing proportions of human adenovirus (HAdV)-positive results in categories of increasing FIO concentration were found in freshwater but not seawater samples (Wyer (2013).

These observations can be interpreted to imply that there is no strong case for changing the indicators currently recommended in the RWQC.

### Charge 4—Relationships between health and alternative indicators

a) A benchmark illness rate of 30 gatrointestinal (GI) illnesses per 1000 swimmers occurred at median concentrations of 4,200 copies of HF183 and 2,800 copies of HumM2 per 100 mL of recreational water (Boehm *et al.* 2015).
b) When the level of CAT (*Catellicoccus marimammalium*, a gull feces marker) exceeds $4 \times 10^6$ copies/100 mL of water, the median predicted illness exceeds 3 illnesses/100 swimmers (Brown *et al.* 2017).
c) Associations between GI and traditional and rapid methods for *Enterococcus* have been observed at marine beaches (Colford *et al.* 2012).
d) QMRA results reported by Corsi *et al.* (2016) highlight the importance of investigating multiple pathogens within multiple categories to avoid underestimating the prevalence and risk of waterborne pathogens.
e) Predictive models were not effective at estimating human health risks associated with recreation at all inland lake sites; however, their use at two lakes with high swimmer

[4]Pond, K. (2005). *Water Recreation and Disease*. World Health Organization, US Environmental Protection Agency, International Water Association, London, pp. 7, 29.

densities provided better estimates of public health risk than current methods, and will be a valuable resource for beach managers and the public (Francy *et al.* 2013).

f) Griffith *et al.* (2016) report that no indicator combinations consistently had a higher odds ratio[5] than EPA Method 1600, but one composite indicator, based on the number of pathogens detected at a beach, was significantly associated with gastrointestinal illness at both Avalon and Doheny when freshwater flow was high. These results suggest that site-specific conditions at each beach determine which indicator or indicators best predict GI illness.
g) Potential EPEC strains were readily isolated from contaminated marine recreational water and may represent a public health risk to swimmers and beach users. The frequency of detection of potential EPEC strains varied considerably by sample. Neither Shigatoxigenic *E. coli* (STEC) nor enterotoxigenic *E. coli* (ETEC) strains were detected (Hamilton *et al.* 2010).
h) Kirs *et al.* (2016) argue for the inclusion of HF183Taqman human fecal marker in future epidemiological studies.
i) Nnane *et al.* (2011) report a "very small" correlation coefficient between presumptive *E. coli* and phages of *Bacteroides* (GB-124).
j) Performance of a calibrated qPCR total enterococci indicator was compared to a culture-based assay to index infectious human enteric viruses released in treated human wastewater. Results illustrate that the pathogen source contributing the majority of risk in a mixture may be overlooked (when only assessing fecal indicators using a culture-based method (Schoen *et al.* 2011).
k) At a beach with no known point sources (e.g., discharge of treated sewage), a dose-response relationship was observed between skin infections and enterococci enumerated using membrane filtration methods. No other significant dose-response relationships between reports of human illness and any of the other FIB or environmental measures were observed (Sinigalliano *et al.* 2010).
l) Viau *et al.* (2011) report that GI illness risks from viral exposures were generally orders of magnitude greater than bacterial exposures in Hawaiian waters impacted by stream discharges. The median risk associated with each stream was positively, significantly correlated to the concentration of *Clostridium perfringens* in the stream water.
m) *Bacteroides* phages were considered potential markers of sewage because they also survived for three days in fresh stream water and two days in marine water (Vijayavel *et al.* 2010).
n) Yau *et al.* (2014) noted that associations between GI illness incidence and FIB levels (*Enterococcus* EPA Method 1600) among swimmers who swallowed water were not significant when not accounting for submarine groundwater discharge, but were strongly associated when submarine groundwater discharge was high compared to when it was low.

These observations show that development and use of alternative fecal indicators is a rich and evolving field. Given that, it seems premature to promulgate any form of directives on their selection and use.

[5]Odds ratios are a measure of relative risk and take the same values as the coefficients of a logistic regression statistical model (relating health outcomes to selected covariates), as used by Griffith *et al.* (2016).

### Charge 5—Fecal sources and contamination dynamics, wet/dry weather

a) Fecal indicator bacteria measured in seawater (*Enterococcus* spp., fecal coliforms, total coliforms) were strongly associated with incident illness only during wet weather. Urban coastal seawater exposure increases the incidence rates of many acute illnesses among surfers, with higher incidence rates after rainstorms (Arnold *et al*. 2017).
b) Ingestion of 1 mL of river water could lead to 0%–4% and 1%–74% probability of illness with *E. coli* during the dry and wet season, respectively. Activities that cause disturbance of sediments lead to elevated risk of infection to users of the river (Abia *et al*. 2016).
c) Swimming in natural swim environments and in pools following a recent fecal contamination event pose significant public health risks (Pintar 2010).
    - Wet weather conditions contribute to elevated pathogen loads in the Chicago Waterways System (CAWS) to such an extent that disinfecting the effluents of three major Water Reclamation Plants (WRPs) that discharge to the CAWS would reduce the aggregate recreation season risk to incidental contact recreators negligibly (Rijal *et al*. 2011).
d) Dry-weather risk estimates were found to be significantly lower than those predicted for wet-weather conditions (Sunger *et al.* 2016).

These observations highlight the importance of significant rainfall in determining the degree of water contamination. Note that contact recreation does occur during, and shortly after, rainfall events.

### Charge 6—Outbreak information during the last 10 years

The illnesses that may arise after contact with fecally-contaminated water are generally “mild”. As such they are usually substantially under-reported (unless the outbreak is “large”), even if the illness in question is “notifiable”. Also, contact with water is usually only one of several potential causes. Accordingly, it seems best to rely mostly on reports where careful investigations have identified the illness and its source.

a) An outbreak among white-water rafters provides evidence of the changing epidemiology of leptospirosis and suggests consideration of a wider range of risk exposures, including those related to recreational activities of more affluent urban populations, in addition to the well-recognized occupational hazards of rural farming (Agampodi *et al.* 2014).
b) During a seven-year period, illness outbreaks reported to the Australian OzFoodNet, were predominantly classified as being transmitted person-to-person or from an unknown source. Fifty-four (0.83%) outbreaks were classified as either ‘waterborne’ or ‘suspected waterborne’, of which 78% (42/54) were attributed to recreational water and 19% (10/54) to drinking water (Dale & Kirk, 2010).
c) The infection risks resulting from swimming in Belgian waters were above 50% for several days in waters near an accidental spillage of wild poliovirus type 3 (Duizer et al. 2016).
d) Approximately 5,700 outbreak-related cases were identified across the state of Utah in 2007. Of 1,506 interviewed patients with laboratory-confirmed cryptosporidiosis, 1,209 (80%) reported swimming in at least one of approximately 450 recreational water venues during their potential 14-day incubation period (Edwards *et al.* 2012).

e) Outbreaks, especially the largest ones, were most frequently associated with treated recreational water and characterized by acute gastrointestinal illness (AGI). *Cryptosporidium* remains the leading etiologic agent (Hlavsa 2011).

These observations show that while the endemic pattern of infectious disease generally accounts for the majority of illness cases, outbreaks cause public concern, especially for cases such as reported above for Belgian beaches. Outbreaks can serve as a warning against complacency.

## Table 1. Detailed response to the first charge

| Reference | Location (1) | Study type (1) | Contamination source(s) (1) | Health effects evaluated (1) | Water quality metrics (1) | Charges 2–6 | Conclusions (includes health linkages) |
|---|---|---|---|---|---|---|---|
| Abdelzaher *et al.* (2010) | Florida, Virginia Key | Epi: Randomized trial | Urban, dogs, stormwater | GI illness, skin illness, acute febrile respiratory illness | Enterococci (culture), *E.coli*, Fecal coliform, *C. perfringens*, Enterococci (qPCR), F-specific coliphage, somatic coliphage, *Cryptosporidium*, *Giardia*, Enterovirus, *V. vulnificus*, *S. aureus*, DogBac, BacHum-UCD, B. thetaiotaomicron, polyomavirus | 4 | No statistically significant correlations between health outcomes and any of the indicator organisms, including coliphages, were identified in this investigation. Average daily excess illness percentage rates (calculated by subtracting the daily illness rate for non-swimmers from that for swimmers) for gastrointestinal, skin, and acute febrile respiratory illness were 2.0% (standard deviation [SD] = 3.3), 5.6% (SD = 4.7), and 1.2% (SD = 2.9), respectively. |
| Abia, *et al.* (2016) | Apies River, South Africa | QMRA | Informal settlements, wastewater treatment plants, animal farms | Caused by measured pathogens | *E. coli*, *V. cholerae*, *Salmonella spp.*, *Shigella spp.* | – | Ingestion of 1 mL of river water could lead to 0%–4% and 1%–74% probability of illness uring the dry and wet season, respectively. Activities that cause disturbance of sediments would lead to elevated risk of infection to users of the river. |
| Agampodi *et al.* (2014) | Sri Lanka | Follow-up, exposures for white-water rafting | Rural runoff | Leptospirosis | None. Clinical | 6 | Exposure from white-water rafting. This outbreak provides evidence of the changing epidemiology of leptospirosis and suggests a wider range of risk exposures including those related to recreational activities of more affluent urban populations in addition to the well-recognized occupational hazards of rural farming**.** |
| Almeida *et al.* (2012) | Argentina | Epi | City wastewater treatment plant | General | Enterococci, *E. coli*, total coliforms, fecal coliforms | – | Following the RWQI values classification, most of the Potrero de los Funes water samples fell in the good quality range during the study period. Advocates conjoint use of microbial and physical/chemical components in a recreational water quality index. |
| Arnold *et al.* (2013) | USA, Malibu Beach, CA | Epi: prospective cohort | Dry weather runoff and non-point sources | Diarrhea and GI illness | Culturable *Enterococcus* | 3, 4 | *n* = 5,674. Diarrhea was more common among swimmers than non-swimmers (adjusted odds ratio = 1.88 [95% confidence interval = 1.09–3.24]) within 3 days of the beach visit. Water quality was generally good (fecal indicator bacteria levels exceeded water quality guidelines for only 7% of study samples). Fecal indicator bacteria levels were not consistently associated with swimmer illness. Sensitivity analyses demonstrated that |

| Reference | Location (1) | Study type (1) | Contamination source(s) (1) | Health effects evaluated (1) | Water quality metrics (1) | Charges 2–6 | Conclusions (includes health linkages) |
|---|---|---|---|---|---|---|---|
| | | | | | | | overall inference was not substantially affected by the choice of exposure and outcome definitions. |
| Arnold *et al*. (2016) | USA-wide | Epi; combined 13 prospective studies | Many | Diarrhea, gastrointestinal illness | Culturable *Enterococcus* | 2 | Combined data from 13 prospective cohort studies (n = 84,411). Water exposure accounted for 21% of diarrhea episodes and 9% of missed daily activities but was unassociated with gastroenteritis leading to medical consultation. Children aged 0 to 4 and 5 to 10 years had the most water exposure, exhibited stronger associations between levels of water quality and illness, and accounted for the largest attributable illness burden. Conclusions. The higher gastroenteritis risk and associated burden in young children presents important new information to inform future recreational water quality guidelines designed to protect public health. |
| Arnold *et al*. (2017) | USA, San Diego, two beaches (Tourmaline Surfing ark, Ocean Beach. | Epi: longitudinal study | Urban runoff after storms | Gastrointestinal and respiratory illness | Culturable *Enterococcus*, fecal coliforms, total coliforms | – | Study of surfers (*n* = 654). Fecal indicator bacteria measured in seawater (*Enterococcus* species, fecal coliforms, total coliforms) were strongly associated with incident illness only during wet weather. Urban coastal seawater exposure increases the incidence rates of many acute illnesses among surfers, with higher incidence rates after rainstorms. |
| Ashbolt *et al*. (2010) | USA | QMRA | Many | General | FIOs (generally) | – | Exploration of various scenarios with the aid of quantitative microbial risk assessment models has been shown to assist in identifying issues, research gaps and management goals. Major gaps that need to be filled before further real progress can be made with QMRA and predictive models include: defining the relationships between reference pathogens and a range of potential indicators, be they culture or PCR endpoint assays. |
| Bambic *et al.* (2015) | USA, Callegus Creek, CA | Data summaries, including Kaplan-Maier treatment for non-detect data. Monte Carlo model. | Municipal wastewater (dry conditions), agricultural and municipal stormwater (wet conditions) | – | *E. coli*. Real-time QPCR for surrogate PP7, Adenovirus and Enterovirus, and four fecal *Bacteroidales* assays (universal) BacUni, (human-associated) BacHum, (ruminant-associated) BacCow, and (dog-associated) BacCan. | 4 | Results demonstrate that MST based on *Bacteroidales* assays can inform watershed managers seeking to develop strategies to comply with criteria, but it is critical to handle non-detects with appropriate statistical methods and to acknowledge the underlying assumptions of qPCR-based MST. While MST shows promise for providing quantitative source apportionment, there are still data gaps including relative decay rates of FIB, *Bacteroidales* and pathogens in effluent-impacted surface waters and lack of qPCR assays for viruses that reflect viable/infective concentrations (e.g., using PMA). Eventually, MST markers may support not only source apportionment but also risk assessment, given additional epidemiological data and/or empirical descriptions of pathogen-*Bacteroidales* relationships. |
| Betancourt *et al*. (2014) | Venezuela | QMRA | Human sewage, | Gastrointestinal | *Cryptosporidium*, *Giardia*, | 2, 4 | Potential risks of *Cryptosporidium* and *Giardia* infection from recreational water exposure were estimated from the levels of viable (oo) cysts (DIC+, DAPI+, PI_) found |

| Reference | Location (1) | Study type (1) | Contamination source(s) (1) | Health effects evaluated (1) | Water quality metrics (1) | Charges 2–6 | Conclusions (includes health linkages) |
|---|---|---|---|---|---|---|---|
| | | | variously-treated | | enterococci, *C. Perfringens*, *Bacteroidales* marker (HF183) and *Clostridium coccides.* | | in near-shore swimming areas using an exponential dose response model. The study revealed the potential risk of parasite infections via primary contact with tropical marine waters contaminated with sewage; higher risk estimates for *Giardia* than for *Cryptosporidium* were found. Mean risks estimated by Monte Carlo methods were below the U.S. EPA upper bound on recreational risk of 0.036 for cryptosporidiosis and giardiasis for both children and adults. However, 95th percentile estimates for giardiasis for children exceeded the 0.036 level. |
| Boehm *et al.* (2015) | USA | QMRA | Human | GI | *Bacteroidales*: Human markers HumM2 and HF183Taqman | 4, 5 | Simulated GI risk increased with concentration of the human quantitative PCR markers in recreational waters. A benchmark illness rate of 30 GI illnesses per 1000 swimmers occurred at median concentrations of 4200 copies of HF183 and 2800 copies of HumM2 per 100 mL of recreational water. |
| Brown *et al.* (2017) | Six California beaches, | QMRA | Gulls | GI | "CAT": *Catellicoccus marimammalium* | 3,4 | Considered densities of CAT and infectious zoonotic pathogens *Salmonella* and *Campylobacter* in gull feces, volume of water ingested during bathing, and dose−response relationships. CAT densities measured in 37 fresh gull fecal droppings. $Log_{10}$ densities ranged from 4.6 to 9.8 log10 copies CAT/g of wet feces. When the level of CAT exceeds $4 \times 10^6$ copies/100 mL of water, the median predicted illness exceeds 3 illnesses/100 swimmers. |
| Colford *et al.* (2012) | United States (Doheny Beach, 2007–08) | Cohort, prospective | Small craft harbor, WWTP, San Juan Creek (when berm open), | 20 health outcomes, including GI and skin rash | 30 different microbial indicators, including rapid methods and new microbial indicators | 3, 4 | $n$ = 9,525. Significant increases in risk of diarrhoea (from swallowing water) and other outcomes in swimmers compared to non-swimmers. Exposure (body immersion, head immersion, swallowed water) was associated with increasing risk of GI illness. Daily GI illness incidence patterns differed: swimmers (2-day peak) and non-swimmers (no peak). With berm-open, associations between GI and traditional and rapid methods for *Enterococcus* were observed; fewer associations occurred when berm status was not considered. |
| Collier *et al.* (2015): NEEAR study | USA: five marine and four freshwater | Epi: prospective cohort | Human wastewaters | GI | – | 2 | Combined study: $n$ = 54,250. Overall, swimmers reported a higher unadjusted incidence of GI illness and earaches than non-swimmers. Current surveillance systems might not detect individual cases and outbreaks of illness associated with swimming in natural water. Water quality analysis not included. |
| Corsi *et al.* (2016) | Three Lake Michigan beaches | QMRA ($2^{nd}$-order, i.e., performing a set of iterations for each random sample from the dose-response curve) | Wastewater effluent, impervious runoff, agric. runoff, rural septic systems | GI | 22 pathogens (Human viruses, bovine viruses, protozoa, pathogenic bacteria | 4 | Detections of human and bovine viruses and pathogenic bacteria at all beaches, indicating influence of multiple contamination sources: occurrence 40 to 87% for human viruses, 65− 87% for pathogenic bacteria, and 13−35% for bovine viruses. Enterovirus, Adenovirus A, *Salmonella* spp., *Campylobacter jejuni*, bovine polyomavirus, and bovine Rotavirus A were present most frequently. Risk assessment done for *C. jejuni*, *Salmonella* spp., and Enteroviruses to estimate risk of infection and illness. Median infection risks for one-time swimming events were approximately $3 \times 10^{-5}$, $7 \times 10^{-9}$, and $3 \times 10^{-7}$ for *C. jejuni*, *Salmonella* spp., and Enteroviruses, respectively. Results highlight the importance of investigating multiple pathogens |

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| | | | | | | | within multiple categories to avoid underestimating the prevalence and risk of waterborne pathogens |
| Dale *et al.* (2009) | Australia, Melbourne | Survey | – | GI | – | 2, 6 | *n* = 2,811. The relationship between sporadic gastroenteritis and recreational swimming considered temporality between reported swimming (in public or private pools/spas and in marine or freshwater settings) and a highly credible gastroenteritis (HCG) event. Overall, HCG events were more likely in participants who had swum in a public pool/spa during the previous week or had swum in a public pool/spa during the previous 2 weeks. Sub-analysis by age showed that HCG episodes were also more likely in adults who had swum in a private pool/spa during the previous week or swum at an ocean/beach during the previous 2 weeks, demonstrating significant associations between all swimming locations and gastrointestinal symptoms. This study showed that although the incremental risk of recreational swimming is significant, it is relatively small. |
| Dale & Kirk (2010) | Australia | Survey | Pool water, public swimming pool | GI | Cryptosporidium, Salmonellae | 6 | *n* = 6,515. During seven years, outbreaks were reported to OzFoodNet, most of which were classified as being transmitted person-to-person or from an unknown source. Fifty-four (0.83%) outbreaks were classified as either 'waterborne' or 'suspected waterborne', of which 78% (42/54) were attributed to recreational water and 19% (10/54) to drinking water. ***Conclusions:*** There have been few waterborne outbreaks detected in Australia, and most of those reported have been associated with recreational exposure. However, there are difficulties in identifying and categorising gastroenteritis outbreaks, as well as in obtaining microbiological and epidemiological evidence, which is likely to result in misclassification or underestimation of water-associated events. |
| de Man *et al.* (2014) | Netherlands | QMRA | Urban floodwaters | | *E. coli*, intestinal enterococci, *Campylobacter*, *Cryptosporidium*, *Giardia*, *enteric viruses*, *Noroviruses (GI and GII)*, *Enterovirus* | 5 | 23 flood events (2011 & 2012). The water contained *Campylobacter jejuni* (prevalence 61%, range 14 to >103 MPN/L), *Giardia* spp. (35%, 0.1 – 142 cysts/L), *Cryptosporidium* (30%, 0.1 – 9.8 oocysts/L), Noroviruses (29%, $10^2 – 10^4$ pdu/L) and Enteroviruses (35%, $10^3 – 10^4$ pdu/L). The mean risk of infection per event for children was 33%, 23% and 3.5%, respectively, and for adults it was 3.9%, 0.58% and 0.039%. An exposure frequency of once every 10 years to flooding originating from combined sewers resulted in an annual risk of infection of 8%. |
| DeFlorio-Barker *et al.* (2017) | USA, NEEAR and CHEERS data | Epi | Mostly urban | GI | – | | The Cost-of-Illness (COI) provides more information than the frequency of illness, as it takes into account disease incidence, health care utilization, and lost productivity. Use of monetized disease severity information should be included in future studies of water quality and health. |
| Dorevitch *et al.* (2010) | USA | Epi (review) | Inland waters (IW) and | GI | – | 4, 5 | The distinction of IW versus CW is of less importance than more fundamental variables such as the scale of the body of water, the source of the pollutant, and the effects of sediment, which translate into differences in the densities, transport, and fate of |

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| | | | coastal waters (CW) | | | | indicators and pathogens. This may translate into weaker indicator–pathogen and indicator–health risk relationships for IW compared with CW. It remains an open question whether sediment in IW changes the relationship between enterococci qPCR measures and health risk, which has been described at coastal beaches impacted by human fecal pollution. In IW with limited dilution capacity and close proximity to sources, outbreaks of severe disease may be difficult to prevent by the application of coastal-derived criteria. |
| Dorevitch *et al*. (2011) | Chicago | Experiment | Urban | | (Amount of water ingested) | | The mean volume of water ingested during limited-contact recreation activities, about 3.5–4 mL is about 35-40% of that observed during swimming (about 10 mL). The frequency of swallowing at least one teaspoon amount of water during limited-contact recreation (about 1% of study participants) is about 1/50$^{th}$ the frequency observed during swimming in a pool (51% of participants). |
| Dorevitch *et al*. (2012) | Chicago | Epi | Urban | GI | – | | *n* = 11,297. Limited-contact recreation, both on effluent-dominated waters and on waters designated for general use, was associated with an elevated risk of gastrointestinal illness. |
| Dorevitch *et al*. (2015) | Chicago | Epi | Urban | GI | *E. coli*, enterococci, somatic coliphages, F+ coliphages, *Giardia* spp. and *Cryptosporidium* spp. (oo)cysts, turbidity | 3, 4 | *n* = 4,694. Gastrointestinal illness following incidental contact with water during recreation was not readily predicted by measures of water quality in the settings studied. Protozoan pathogens, while frequently detected, were not useful as predictors of illness. |
| Dorevitch *et al*. (2017) | Chicago | Epi | Urban | GI | *E. coli*, enterococci by qPCR | | Monitoring multiple beaches using qPCR methods can generate precise and accurate data for timely public notifications regarding beach water quality. Results of prior-day *E. coli* culture testing were no better than chance in predicting the exceedance of the qPCR BAV. *E. coli* culture testing of beaches (on the same day) led to three times the number of BAV exceedance as did enterococci qPCR testing of beach water. It is not known whether similar results would have been obtained at marine beaches or those significantly impacted by wastewater. |
| Dufour *et al*. (2012) | USA, Hong Kong, New Zealand | Epi | Birds, animals | GI | – | 6 | Reviewed epidemiological studies do not provide evidence for associations between swimming-associated gastrointestinal illness and exposures to bathing waters contaminated with feces from animals or birds. Other studies, such as outbreak investigations and case-control studies, have provided logical linkages to human infections with zoonotic pathogens and recreational or occupational exposures to water, but they have not established a definitive link between water contamination and specific animal sources. These conclusions do not completely answer the question whether exposure to animal-contaminated waters poses a health risk to swimmers. The exposure |

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| | | | | | | | to zoonotic pathogens is unlikely to have occurred at beaches meeting local beach water quality standards. |
| Dufour *et al.* (2017) | Columbus, Ohio | Exposure experiment | Swimming pools | – | chloroisocyanurate (cyanuric acid) | 2 | *n* = 549. Swimming pools disinfected by chloroisocyanurate used to determine the amount of water swallowed by swimmers. It is in equilibrium with chlorine and cyanuric acid in the pool water thus provides a biomarker: cyanuric acid that once swallowed passes through the body into the urine unchanged. The 549 participants, about evenly divided by gender, and young and adult swimmers, indicated that swimmers ingest about 32 mL per hour (arithmetic mean) and that children swallowed about four times as much water as adults during swimming activities. Males had a tendency to swallow more water than females. Children spent about twice as much time in the water than adults. |
| D'Ugo *et al.* (2016) | Six countries | Experiment | Open space, Grazing land, Urban | – | qPCR for Adenovirus 41, Mammalian Orthoreoviruses, Noroviruses | 4 | A 2-year survey showed that Norovirus, Mammalian Orthoreovirus and Adenoviruses were the most frequently identified enteric viruses in the sampled surface waters. Although it was not possible to establish viability and infectivity of the viruses considered, the detectable presence of pathogenic viruses may represent a potential risk for human health. The methodology developed may aid in rapid detection of these pathogens for monitoring quality of surface waters. |
| Duizer *et al.* (2016) | Belgium | QMRA | Accidental release of wild poliovirus type 3 | Infection risk from swimming and raw shellfish consumption | Wild poliovirus type 3 | 2, 6 | Accidental release of 1,013 infectious wild poliovirus type 3 particles by a vaccine production plant in Belgium into the sewage system and associated wastewater treatment plant (WWTP), and subsequently into rivers that flowed to the Western Scheldt and the North Sea. QMRA showed that the infection risks resulting from swimming in Belgium waters were above 50% for several days and that the infection risk by consuming shellfish harvested in the eastern part of the Western Scheldt warranted a shellfish cooking advice. Showed that relevant data on water flows were not readily available and that prior assumptions on dilution factors were overestimated. |
| Edwards *et al.* (2012) | Utah | Epi | Recreational water venues | GI | *Cryptosporidium* | 6 | During the summer of 2007, Utah experienced a state-wide outbreak of gastrointestinal illness caused by *Cryptosporidium*, Approximately 5,700 outbreak-related cases were identified across the state. Of 1,506 interviewed patients with laboratory-confirmed cryptosporidiosis, 1,209 (80%) reported swimming in at least one of approximately 450 recreational water venues during their potential 14-day incubation period. |
| Ehsan *et al.* (2015) | Belgium | QMRA | Swimming pools, lakes, splash parks, fountains | GI | *Giardia* and *Cryptosporidium* | 2 | *Cryptosporidium* oocysts and/or *Giardia* cysts were detected in swimming pools, recreational lakes, splash parks and water fountains in Belgium. Although in recreational lakes (oo)cysts were frequently present, most positive samples belonged to species/genotypes that were either animal-specific or predominantly found in animals, suggesting that the risk of infection during recreation is relatively low. Lower contamination rates were found in swimming pools, splash parks and water fountains, but assuming that humans are the most probable source of contamination for these waterbodies, these findings suggest a potential risk for human infection. |

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| Eregno *et al.* (2016) | Norway, Sandvika | QMRA | Sandvika recreational beaches | GI | *E. coli* | | Investigated the public health risk from exposure to infectious microorganisms at recreational beaches, Norway, and dose–response relationships by combining hydrodynamic modelling with QMRA. Meteorological and hydrological data were collected to produce a calibrated hydrodynamic model using *Escherichia coli*. Based on average concentrations of reference pathogens (Norovirus, *Campylobacter*, *Salmonella*, *Giardia* and *Cryptosporidium*) relative to *E. coli* in Norwegian sewage from previous studies, the hydrodynamic model was used for simulating the concentrations of pathogens at the local beaches during and after a heavy rainfall event. The simulated concentrations were used as input for QMRA and the public health risk was estimated as probability of infection from a single exposure of bathers during the three consecutive days after the rainfall event. The level of risk on the first day after the rainfall event was acceptable for the bacterial and parasitic reference pathogens, but high-to-severe for the viral reference pathogen at all beaches. |
| Ferley *et al.* (1989) | France Ardèche basin | Retrospective Epi | Rural summer camps | GI | Total coliforms, fecal coliforms, fecal streptococci, *Aeromonas*, *Pseudomonas* | – | *n* = 5,737. While dated, this often-overlooked paper is included because it focuses on rivers, not freshwater lakes. 5,737 tourists in eight holiday camps were questioned as to the occurrence of illness and their bathing habits during the week preceding the interviews. The rate-ratio contrasting swimmers and non-swimmers for total morbidity is 2.1. Fecal streptococci were best correlated to gastrointestinal morbidity, fecal coliforms less so. Swimmers suffer skin ailments much more frequently than non-swimmers (RR-= 3.7). This type of morbidity is well correlated with the concentration of fecal coliforms, *Aeromonas* and *Pseudomonas*. Produced relationship between person-days and fecal coliforms/streptococci. |
| Fewtrell & Kay (2015) | worldwide | Epi &QMRA review | – | – | – | | Epidemiological studies show a generally elevated risk of gastrointestinal illness in bathers compared to non-bathers but often no clear association with water quality as measured by fecal indicator bacteria (Wade *et al.* 2010 is an exception). This is especially true where study sites are impacted by non-point source pollution. Evidence from QMRAs support the lack of a consistent water quality association with health risk for non-point source-impacted beaches. Future Epi studies should include source attribution through quantified microbial source apportionment. |
| Francy *et al.* (2013) | 22 Eight Ohio inland recreational lakes | Experiment | Birds and other wildlife; septic tanks (1) and treated wastewater (1)d | – | **Culture**: *E. coli* & enterococci; **end point PCR**: *Shigella*, *Salmonella*, STEC, *C. Jejuni and coli* (PCR), Cryptosporidium, *Giardia* | 4 | Investigate using predictive models for *Escherichia coli* and to understand the links between *E. coli* concentrations, predictive variables, and pathogens. Based upon results from 21 beach sites, models were developed for 13 sites, and the most predictive variables were rainfall, wind direction and speed, turbidity, and water temperature. Models were not developed at sites where the *E. coli* standard was seldom exceeded. Models were validated at nine sites during an independent year. *Cryptosporidium*, Adenovirus, *eaeA* (*E. coli*), *ipaH* (*Shigella*), and *spvC* (*Salmonella*) were found in at least 20% of samples collected for pathogens at five sites. The presence or absence of the three bacterial genes was related to some of the model variables but was not consistently related to *E. coli* concentrations. Predictive models were not effective at all |

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| | | | | | **qPCR and qRT-PCR:** *Adenovirus*, *Enterovirus*, Norovirus | | inland lake sites; however, their use at two lakes with high swimmer densities will provide better estimates of public health risk than current methods and will be a valuable resource for beach managers and the public. |
| Galfi *et al.* (2016) | Sweden | – | Four urban sewers | – | Total coliforms, *E. coli*, enterococci, *C. perfringens* | 4, 5 | Covers baseflow and snowmelt periods in storm sewers of four urban catchments in a northern Swedish city. Catchment- and season- (i.e., rainy or snowmelt periods) specific variations were investigated. Compared to dry weather baseflow, concentrations of these three indicators in stormwater were 10 (snowmelt runoff) to $10^2$ (rain runoff) times higher. *C. perfringens* mean concentrations were practically constant regardless of season and catchment. The list of variables associated with bacteria included the flow rate, solids with associated inorganics (Fe and Al), and phosphorus, indicating similar sources of constituents regardless of the season. Although the study findings do not indicate any distinct surrogates to indicator bacteria, the inclusion of flow rate, solids and total phosphorus for all seasons, water temperature for rainfall runoff, and total nitrogen and pH for snowmelt only) in sanitary surveys of northern climate urban catchments would provide additional insight into indicator bacteria sources and their modeling. |
| Goodwin *et al.* (2012) | Three California beaches | Experiment | Urban | – | *Staphylococcus aureus*, MRSA, enterococci | 5 | The frequent detection (>50%) of *S. aureus* in seawater and beach sand samples and the correlation with water temperature supports the concern that bacterial pathogens exist and may persist in the environment, including at beaches. Although the correlation between *S. aureus* and the number of swimmers was weak and apparent only for *S. aureus* in seawater and not sand, the correlation held for data analysed by individual beach and combined across beaches. These data support the possibility that beach-goers are one source of this organism, but suggests that other sources not identified in this study are important as well. Although the prevalence of MRSA was much lower (<3% of samples) than for *S. aureus*, these data indicate the potential for virulent and antibiotic resistant strains to be encountered in this environment. *S. aureus* was correlated to enterococci, even though *S. aureus* is not considered a typical fecal organism. Perhaps the finding that *S. aureus* can sometimes be found in wastewater and in companion animal feces explains this observation. |
| Gorham& Lee (2016) | General | Literature | Various | – | – | 5, 6 | Pathogens of potential concern include *Campylobacter jejuni*, *Salmonella Typhimurium*, *Listeria monocytogenes*, *Helicobacter canadensis*, *Arcobacter* spp., *Enterohemorragic Escherichia coli* pathogenic strains, *Chlamydia psitacci*, *Cryptosporidium parvum* and *Giardia lamblia*. Scenarios presenting potential exposure to pathogens eluted from feces include bathers swimming in lakes, children playing with wet and dry sand impacted by geese droppings, and other common recreational activities associated with public beaches. Recent recreational water-associated disease outbreaks in the US support the |

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| | | | | | | | plausibility for some of these pathogens, including *Cryptosporidium* spp. and *C. jejuni*, to cause human illness. |
| Griffith *et al.* (2016) | Three beaches (CA): Doheny, Malibu, Avalon | Epi (prospective cohort) | Watershed runoff, point sources | GI | 41 target indicators using 6 different methodologies | 3, 4 | *n* = 10,785. $F^{\pm}$ coliphage (measured using EPA Method 1602) exhibited a stronger association with GI illness than did EPA Method 1600 at the two beaches where it was measured, while a molecular method, $F^{\pm}$ RNA Coliphage Genotype II, was the only indicator significantly associated with GI illness at Malibu. MRSA, a known pathogen, had the strongest association with GI illness of any microbe measured at Avalon. There were two methods targeting human-associated fecal anaerobic bacteria that were more strongly associated with GI illness than EPA Method 1600, but only at Avalon. No indicator combinations consistently had a higher odds ratio than EPA Method 1600, but one composite indicator, based on the number of pathogens detected at a beach, was significantly associated with gastrointestinal illness at both Avalon and Doheny when freshwater flow was high.<br>While EPA Method1600 performed adequately at two beaches based on its consistency of association with gastrointestinal illness and the precision of its estimated associations, $F^{+}$ coliphage measured by EPA Method 1602 had a stronger association with GI illness under high risk conditions at the two beaches where it was measured. One indicator, $F^{\pm}$ Coliphage Genotype II was the only indicator significantly associated with GI illness at Malibu. Several indicators, particularly those targeting human-associated bacteria, exhibited relationships with GI illness that were equal to or greater than that of EPA Method 1600 at Avalon, which has a focused human fecal source. Results suggest that site-specific conditions at each beach determine which indicator or indicators best predict GI illness. |
| Hall *et al.* (2017) | London, River Thames | Epi (retrospective cohort) | Urban | GI | – | 6 | GI surveyed for 1,100 swimmers in a Thames River event in London, UK, to describe the outbreak and identify risk factors. Associations were tested using robust Poisson regression. Survey response was 61%, and attack rate 53% (338 cases). Median incubation period was 34 h and median symptom duration 4 days. Microbiological diagnoses were confirmed in five cases (four Giardia, one Cryptosporidium). |
| Hamilton *et al.* (2010) | Avalon Bay, CA | Survey | Urban | – | Genomic composition and frequency of virulence genes present in *E. coli* isolated from beach water | 4 | Potential EPEC strains were readily isolated from contaminated marine recreational water and may represent a public health risk to swimmers and beach users. Neither STEC nor ETEC strains were detected. The frequency of detection of potential EPEC strains varied considerably by sample, suggesting a strong temporal component. Results indicate that potential EPEC strains in Avalon Bay were genetically diverse. Since genotypically identical EPEC strains were detected repeatedly, on successive dates and years, these data suggest that *E. coli* in Avalon Bay were likely due to continual deposition from an unknown reservoir or through persistence of *E. coli* in the environment. |

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| Harder-Lauridsen *et al*. (2013) | Copenhagen, Denmark | Epi (retrospective cohort) | Urban | GI | *E. coli* | 6 | *n* = 1,769. A triathlon was held shortly after rainfall. An established model of bacterial concentration in the water was used to examine the level of pollution in a spatiotemporal manner. Investigation was repeated after a triathlon competition held in non-polluted seawater in 2011. Results showed that the 3.8 kilometre open water swimming competition coincided with the peak of post-flooding bacterial contamination in 2010, with average concentrations of $1.5x10^4$ *E. coli* per 100 mL water. The attack rate of disease among 838 swimmers in 2010 was 42% compared to 8% among 931 swimmers in the 2011 competition (relative risk RR = 5.0). Confirmed aetiologies of infection included *Campylobacter*, *Giardia lamblia* and diarrhoeagenic *E. coli*. Results suggest a significant risk of disease in people ingesting small amounts of flood water following extreme rainfall in urban areas. |
| Harrington *et al*. (1993) | Sydney, Australia, six popular marine beaches. | Epi, longitudinal | Urban | GI, respiratory | Fecal coliforms, fecal streptococci, *C. perfingens* | | Beaches located north and south of Sydney Harbor: 2003 recruits were enrolled, recording 43,175 swimming events. Of these, 5,879 (14%) had possibly attributable illness. A rise in relative risks was noted for total illness and respiratory illness but not for gastrointestinal illness. Females showed an increase in reported illness when beach swimming was combined with non-ocean swimming. This study lends no support to the concept of correlating health risk in swimmers with threshold levels of currently used bacterial indicator organisms. The value of further exploring the role of *Clostridium perfringens* as an indicator organism is supported. |
| Helmi *et al*. (2011) | Reservoir in Luxembourg, used for recreation and drinking-water supply. | Survey, QMRA | Not stated | – | PCR *Giardia lambla*, *Cryposporidium parveum* | 5 | *Giardia lamblia* and *Cryptosporidium parvum* was monitored for 2 years in the largest drinking water reservoir in Luxembourg using microscopy and qPCR techniques. Parasite analyses were performed on water samples collected from three sites. Results show that both parasites are present in the reservoir throughout the year with a higher occurrence of *G. lamblia* cysts compared to *C. parvum* oocysts. Only 25% of the samples positive by microscopy were confirmed by qPCR. (Oo)cyst concentrations were 10 to 100 times higher between sites and they were positively correlated to the water turbidity and negatively correlated to the temperature. Highest (oo)cyst concentrations were observed in winter. No relationship between the concentrations of (oo)cysts in the reservoir and rain events could be established. In summer 2007, the maximal risk of parasite infection per exposure event for swimmers in the reservoir was estimated to be 0.0015% for *C. parvum* and 0.56% for *G. lamblia*. Finally, no (oo)cysts could be detected in large volumes of finished drinking water. |
| Hlavsa *et al*. (2011) | USA-wide | Survey | Various | Diseases | Numerous | 6 | Outbreaks, especially the largest ones, were most frequently associated with treated recreational water and characterized by AGI. *Cryptosporidium* remains the leading etiologic agent. Pool chemical–associated health events occur frequently but are preventable. Data on other select recreational water–associated health events further elucidate the epidemiology of U.S. waterborne disease by highlighting less frequently |

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| | | | | | | | implicated types of recreational water (e.g., oceans) and detected types of recreational water–associated illness (i.e., not AGI). |
| Hokajarvi *et al*. (2013) | Finland, freshwater, 17 locations | Survey | Land runoff | – | *Campylobacter*, Adenoviruses (qPCR), *E. coli*, intestinal enterococci | 4, 5 | 50 Finnish bathing water samples and 34 sewage effluent samples originating from 17 locations studied in of 2006 and 2007 summers. *Campylobacter* present in 58% and Adenoviruses in 12% of all bathing water samples; 53% of all sewage effluent samples were positive for *Campylobacter* spp. and 59% for Adenoviruses. *C. jejuni* was the most common *Campylobacter* species found and human Adenovirus serotype 41 was the most common identified Adenovirus type. Bathing water temperature displayed a significant negative relationship with the occurrence of *Campylobacter*. The counts of fecal indicator bacteria were not able to predict the presence of *Campylobacter* spp. or Adenoviruses in the bathing waters. |
| Kent & Bayne (2010) | Chattooga River, Southeastern USA | Epi | WWTPS, construction sites | Perceptions of skin infection | – | | Although bacterial skin infections are a chronic problem among whitewater rafters on the Chattooga River in the southeastern United States, little is known about the source of such infections. The Chattooga River is a federally designated ‘‘Wild and Scenic’’ river. Riverine water quality can be negatively impacted by tributaries that are not protected by federal guidelines. Water quality in Stekoa Creek, a major tributary of the Chattooga River, is degraded by sediment derived from construction sites near the creek, as well as fecal coliform contamination from wastewater treatment facilities. A survey of whitewater raft guides was conducted to collect data on incidence of skin infection, and to assess perceived health risk from recreation activities. Whitewater rafting guides working on the Chattooga River reported concerns about their personal health related to degraded water quality and microbial contamination from Stekoa Creek. Incidence of bacterial skin infection and perceived health risk was strongly correlated among the whitewater rafting guides. |
| Kirs *et al*. (2016) | Hawaiian waters | Experiment, epi-related | Treated wastewater, streams, marine | – | *Bacteroides* spp. (HF183TaqMan) and human polyomavirus (HPyV) markers, enterococci, *E. coli* | 4 | Evaluated human-associated *Bacteroides* spp. (HF183TaqMan) and human polyomavirus (HPyV) markers for host sensitivity and specificity. Both markers were strongly associated with sewage, although the cross-reactivity of the HF183TaqMan (also present in 82% of canine [n = 11], 30% of mongoose [*n* = 10], and 10% of feline [*n* = 10] samples) needs to be considered. Concentrations of HF183TaqMan in human fecal samples exceeded those in cross-reactive animals at least 1,000-fold. In the absence of sunlight, the decay rates of both markers were comparable to the die-off rates of enterococci in experimental freshwater and marine water microcosms. However, in sunlight, the decay rates of both markers were significantly lower than the decay rate of enterococci. Limitations can be mitigated by using both markers simultaneously; ergo, this study supports the concurrent use of HF183TaqMan and HPyV markers for the detection of sewage contamination in coastal and inland waters in Hawaii. Both markers are more conservative and more specific markers of sewage than fecal indicator bacteria (enterococci and *Escherichia coli*). |

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| | | | | | | | Analysis of HF183TaqMan (or newer derivatives) is recommended for inclusion in future epidemiological studies. |
| Kundu *et al.* (2013) | Calleguas Creek, Southern CAUSA | QMRA | Urban (upper areas), else agriculture. Three tertiary-treated effluents | GI | **PCR**: Human Adenovirus and Enterovirus: total and fecal coliforms, enterococci (tidal area only) | 3, 4 | Used site-specific QMRA to assess the probability of Adenovirus illness for three groups of swimmers: adults with primary contact, children with primary contact, and secondary contact regardless of age. Adenovirus type 40/41 was detected in 11% of 73 samples, ranging from 147 to 4117 genomes per liter. Enterovirus was detected only once (32 genomes per liter). Seven of eight virus detections occurred when *E. coli* concentrations were below the single sample maximum water quality criterion for contact recreation, and five of eight virus detections occurred when fecal coliforms were below the corresponding criterion. Dose-harmonization was employed to convert viral genome measurements to $TCID_{50}$ values needed for dose-response curves. The mean illness risk in children based on Adenovirus measurements obtained over 11 months was estimated to be 3.5%, which is below the 3.6% risk considered tolerable by the current United States EPA recreational criteria for GI. The mean risks of GI illness for adults and secondary contact were 1.9% and 1.0%, respectively. Risk was lowered considerably when a small proportion of Adenovirus 40/41 (3%) was assumed as infectious as Adenovirus type 4, compared to the assumption that all genomes were Adenovirus 4. |
| Lamparelli *et al*. (2015) | Brazil | Epi Prospective, cohort | Wastewater effluent-impacted waters | GI, diarrhoea, nausea, fever, vomiting | *E. coli* and enterococci | 2 | Swimming and sand contact associated with increased risk of GI illness in highly exposed swimmers. Increases in *E. coli* and enterococci associated with increased GI risk—more pronounced children age 0-10. |
| Lee *et al.* (2014) | Ohio | Experiment | 3 FW lake beaches. Non-point, crop culture, pasture | – | qPCR: Human Adenovirus, Enterovirus and Norovirus. *E. coli* and *Bacteroides*. | 4 | Human Adenovirus, Enterovirus and Norovirus were monitored using qPCR assays at freshwater beaches during the swimming season. Human Adenovirus (40%) and Enterovirus (17%) were detected, but Norovirus was not detected. Enteric virus densities exhibited no relationships with densities of fecal indicators or culture-independent genetic markers. Densities of human Enterovirus were correlated with water inflow rates into reservoirs of freshwater beaches. |
| Leonard *et al*. (2015) | England and Wales coastal waters | Survey–QMRA-related via estimates of doses. | Coastal discharges | – | *E. coli*, 3GCs (prevalence of 3GC-resistance) determined using culture-based methods. | | The role the natural environment plays in the spread of antibiotic resistant bacteria (ARB) and antibiotic resistance genes is not well understood. ARB have been detected in natural aquatic environments, and ingestion of seawater during water sports is one route whereby many people could be exposed directly. The aim was to estimate the prevalence of resistance to one clinically important class of antibiotics (third generation cephalosporins (3GCs)) amongst *E. coli* in coastal surface waters. Prevalence data were used to quantify ingestion of 3GC-resistant *E. coli* (3GCREC) by people participating in water sports. A further aim was to use this value to derive a population level estimate of exposure to these bacteria during recreational use of coastal waters in 2012. 0.12% of *E. coli* isolated from surface waters were resistant to 3GCs. This value was used to |

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| | | | | | | | estimate that in England and Wales over 6.3 million water sport sessions occurred in 2012 that resulted in the ingestion of at least one 3GCREC. Despite the low prevalence of resistance to 3GCs amongst *E. coli* in surface waters, there is an identifiable human exposure risk for water users, which varies with the type of water sport undertaken. The relative importance of this exposure is likely to be greater in areas where a large proportion of the population enjoys water sports. This is the first study to use volumes of water ingested during different water sports to estimate human exposure to ARB. |
| Lim *et al.* (2017) | Baby Beach, southern CA | QMRA | Undeveloped open space and urban. No obvious point sources of human waste. | GI infection | Enterococci | 5 | The utility of FIB as indicators of recreational water illness (RWI) risk has been questioned, particularly in coastal settings with no obvious sources of human sewage. The authors employed a source-apportionment QMRA (SA-QMRA) to assess RWI risk at a popular semi-enclosed recreational beach. SA-QMRA results suggest that, during dry weather, the median RWI risk at this beach is below the U.S. EPA recreational water quality criteria (RWQC) of 36 illness cases per 1000 bathers. During wet weather, the median RWI risk predicted by SA-QMRA depends on the assumed level of human waste associated with stormwater; the RWI risk is below the EPA RWQC illness risk benchmark 100% of the time provided that <2% of the FIB in stormwater are of human origin. However, these QMRA outcomes contrast strongly with the RWQC for 30-day geometric mean of enterococci bacteria. These results suggest that SA-QMRA is a useful framework for estimating robust RWI risk that takes into account local information about possible human and non-human sources of FIB. |
| Loge *et al.* (2009) | Worldwide | QMRA | Any | GI infection | – | 3 | Studies the relative significance of: (1) active shedding of microorganisms from bathers themselves, and (2) the type and concentration of etiological agent on the observed heterogeneity of the incidence of illness in epidemiological studies that have been used to develop ambient water quality criteria. The etiological agent and corresponding dose ingested during recreational contact was found to significantly impact the observed incidence of illness in an epidemiological study conducted in recreational water. In addition, the observed incidence of illness was found not to necessarily reflect background concentrations of indicator organisms, but rather microorganisms shed during recreational contact. Future revisions to ambient water quality criteria should address the etiological agent, dose, and the significance of microbial shedding relative to background concentrations of pathogens and indicator organisms in addition to the incidence of illness and concentration of indicator organisms. |
| Magill-Collins *et al.* (2015) | Colorado River, Grand Canyon rafting | Epi | Non-point | Norovirus illness | Norovirus by RT-qPCR | 6 | Confidential illness reports were completed by all individuals with symptoms of AGI, and samples of fecal matter and vomitus, surface swabs of rafting equipment, and environmental swabs at stops along the hiking corridor were collected and tested for the presence of Norovirus using reverse transcription–quantitative polymerase chain reaction (RT-qPCR). During the active outbreak 97 rafters (1.4%) from 10 trips (2.9% of all trips) declared AGI symptoms. AGI incidence within the 10 infected trips varied from 6% to 88%. Outbreaks occurred in 3 distinct temporal clusters that involved 2 |

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| | | | | | | | different genogroups of Norovirus. All available toilet fecal samples (5 samples) were positive for Norovirus RNA: 1 with genogroup I (GI) and 4 with GII. The vomitus sample tested positive for GI. None of the fomite samples from rafting equipment or from the hiking corridors were confirmed for Norovirus.<br>The results suggest that Norovirus may have been introduced by ill or asymptomatic individuals actively shedding the virus in their vomitus or feces, and spread within, or between, river trips by different modes of transmission. |
| Mallin *et al.* (2010) | Wrightsville beach, North Carolina. | Survey | Urban | – | Fecal coliforms, enterococci, 16S rDNA genes of *Bacteroides-Prevotella* markers | 4, 5, 6 | From 2007-2009 a study was undertaken to determine the sources of fecal bacteria contamination to the marine waters adjoining Wrightsville Beach. Sampling for optical brighteners was included, along with dye studies, and use of molecular bacterial source tracking techniques including polymerase chain reaction (PCR) and terminal restriction fragment polymorphism (T-RFLP) fingerprinting of the *Bacteroides-Prevotella* group. Of the 96 samples collected from nine locations during the study, the water contact standard for *Enterococcus* was exceeded on 13 occasions. The T-RFLP fingerprint analyses demonstrated that the most widespread source of fecal contamination was human, occurring in 38% of the samples, with secondary ruminant and avian sources also detected. Optical brightener concentrations were low, reflecting negligible sewer leakage or spills. A lack of sewer leaks and lack of septic systems in the town pointed toward discharge from boat heads into the marine waters as the major cause of fecal contamination; this was supported by dye studies. |
| Mannocci *et al.* (2016) | Worldwide. meta-analysis | Epi | – | Respiratory | – | | A meta-analysis conducted to assess the association between swimming in recreational water and the occurrence of respiratory illness. Fourteen independent studies that included 50,117 patients with significant heterogeneity were reviewed. The meta-analysis reports that people exposed to recreational water (swimmers/bathers) present a higher risk of respiratory illness compared to non-swimmers/non-bathers. This percentage increases if adjusted RR by age and gender are considered. A clear association between swimming in recreational water and the occurrence of respiratory illness was found. The surveillance of water quality monitoring systems is crucial not only for gastrointestinal illness, but also for respiratory ones. |
| Marion *et al.* (2010) | USA, East Fork Lake, Ohio | Epi, prospective cohort | Likely influenced by non-point source human fecal contamination; | GI Illness | *E. coli* | 2 | Examined relationships between water quality indicators and reported adverse health outcomes among users of a beach at an inland U.S. lake. Human health data were collected over 26 swimming days during the 2009 swimming season in conjunction with water quality measurements. Adverse health outcomes were reported 8–9 days post-exposure via a phone survey. Wading, playing or swimming in the water was observed to be a significant risk factor for GI illness (adjusted odds ratio (AOR) of 3.2). Among water users, *E. coli* density was significantly associated with elevated GI illness risk where the highest *E. coli* quartile was associated with an AOR of 7.0. GI illness associations are consistent with previous freshwater epidemiology studies. A unique finding was observations of positive associations with GI illness risk based upon a |

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| | | | | | | | single daily *E. coli* measurement. Lastly, this study focused on an understudied issue, illness risk at inland reservoirs. These results support the usefulness of *E. coli* as a health-relevant indicator of water quality for this inland U.S. beach. Includes an *E. coli* – HCGI regression. |
| Marion *et al.* (2014) | USA, East Fork Lake, Ohio | Epi: prospective cohort | likely influenced by non-point source human fecal contamination; | GI illness, diarrhoea, vomiting | HAdV, Enterovirus,Norovirus, *E. coli*, enterococci, *Bacteroides* | | Data pertaining to genetic marker exposure and 8 or 9-day health outcomes were available for a total of 600 healthy susceptible swimmers, and with this population we observed a significant positive association between human Adenovirus (HAdV) exposure and diarrhea (odds ratio = 1.6) as well as gastrointestinal illness (OR = 1.5) upon adjusting for culturable *E. coli* densities in multivariable models. No significant associations between bacterial genetic markers and swimming-associated illness were observed. Positive association between increasing densities of HAdV and *E. coli*; increased odds of GI and HCGI among swimmers. |
| McBride *et al.* (2013) | USA-wide | QMRA | Stormwater | GI, Respiratory | 'Reference pathogens': *Giardia Cryptosporidium*, Adenovirus, Enterovirus and *Salmonella* | 2, 5, 6 | Data were collected from 12 sites representative of seven discharge types (including residential, commercial/industrial runoff, agricultural runoff, combined sewer overflows, and forested land), mainly during wet weather conditions during which times human health risks can be substantially elevated. Using an example waterbody and mixed source, pathogen concentrations were used in QMRA models to generate risk profiles for primary and secondary water contact (or inhalation) by adults and children. A number of critical assumptions and considerations around the QMRA analysis are highlighted, particularly the harmonization of the pathogen concentrations measured in discharges during this project with those measured (using different methods) during the published dose-response clinical trials. Norovirus was the most dominant predicted health risk, though further research on its dose-response for illness (cf. infection) is needed. Even if the example mixed-source concentrations of pathogens had been reduced 30 times (by inactivation and mixing), the predicted swimming-associated illness rates (largely driven by Norovirus infections) can still be appreciable. Rotavirus generally induced the second-highest incidence of risk among the tested pathogens while risks for the other reference pathogens were considerably lower. Secondary contact or inhalation resulted in considerable reductions in risk compared to primary contact. |
| Mika *et al.* (2014) | Southern California; Santa Monica Channel (SMC); Ventura Harbor, Keys and marina | Experiment | Natural state (SMC); mixed use (residential, commercial) | – | Total coliforms, *E. coli*, enterococci, *Bacteroides* 16s gene marker (HF183), by qPCR. | 4 | The variability of levels of FIB and a human-associated genetic marker (HF183) during wet and dry weather conditions was investigated. Seventy-eight to 86% of the samples collected from SMC sites exceeded standard water quality standards for FIB (n=59 to 76). At SMC, HF183 was present in 58%of the samples ($n$ = 78) and was detected at least once at every sample site. No individual site at SMC appeared as a hotspot for the measured indicators, pointing to a likely chronic issue stemming from urban runoff in wet and dry weather. In Ventura, the Arundell Barranca, which drains into Ventura Harbor and Marina, was a source of FIB, and HF183 was most frequently detected off a dock in the Marina. Rainfall significantly increased FIB levels at both SMC and |

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| | | | | | | | Ventura. Sample locations with elevated FIB were geographically distinct from the sites with elevated HF183 in Ventura, which supports the importance of measuring host-associated parameters along with FIB in chronically impaired watersheds to guide water quality managers in pollution remediation efforts. |
| Ming *et al.* (2014) | China (Bohai Bay) | QMRA | Domestic sewage, aquaculture industry, domestic sewage, non-point source runoff, besides, ship waste holding tanks | GI | ICC-qPCR Rotavirus | 4 | Dose-response data indicate that Rotavirus (RV) may be one of the more infective agents among enteric viruses. The major limitation at present in the assessment of infection from Rotavirus is lack of quantitative data on viral infectivity. In this work, an integrated cell culture and real-time quantitative polymerase chain reaction (ICC-qPCR) method and a Beta-Poisson model for risk assessment were employed. A set of 28 surface seawater samples was collected from December 2010 to September 2011 in Bohai Bay, China, to enable a seasonal risk assessment of infective RV at recreational beaches. Thirty-two percent of the samples were positive for Rotavirus, and the estimated concentration range of infectious human Rotavirus was 1 to 279 PFU/L. Contamination of seawater with Rotavirus was higher in autumn and winter, in reasonable agreement with the trend observed in a prior epidemiological study. A preliminary risk assessment indicated the daily risk of illness at almost all the contaminated sites exceeded an acceptable threshold of marine recreational water quality (19 illnesses per 1000 swimmers). |
| Nevers & Whitman (2011) | USA, Lake Michigan, 50 beaches | Survey | Various | – | – | 3 | Examined whether re-evaluation of the U.S. EPA ambient water quality criteria (AWQC) and the epidemiological studies on which they are based could increase public beach access without affecting presumed health risk. Single-sample maxima were calculated using historic monitoring data for 50 beaches along coastal Lake Michigan on various temporal and spatial groupings to assess flexibility in the application of the AWQC. No calculation on either scale was as low as the default maximum (235 CFU/100 mL) that managers typically use, indicating that current applications may be more conservative than the outlined AWQC. It was notable that beaches subject to point source FIB contamination had lower variation, highlighting the bias in the standards for these beaches. Until new water quality standards are promulgated, more site-specific application of the AWQC may benefit beach managers by allowing swimmers greater access to beaches. |
| Nnane *et al.* (2011) | UK, River Ouse | Survey | Predominantly rural (agriculture), 7% urban., | *E. coli*, intestinal enterococci;phages of *Bacteroides* GB-124, *Clostridium perfringens*, Heterotrophic | – | 4 | Investigated the integration and application of a novel and simple MST approach to monitor microbial water quality over one calendar year, thereby encompassing a range of meteorological conditions. A key objective of the work was to develop simple low-cost protocols that could be easily replicated. Bacteriophages (viruses) capable of infecting a human specific strain of *Bacteroides* GB-124, and their correlation with presumptive *Escherichia coli*, were used to distinguish sources of fecal pollution. The results reported here suggest that in this river catchment, non-human sources of fecal pollution predominate. During storm events, presumptive *E. coli* and presumptive intestinal enterococci levels were 1.1–1.2 logs higher than during dry weather |

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| | | | | plate count, somatic coliphage | | | conditions, and levels of the fecal indicator organisms (FIOs) were closely associated with increased turbidity levels (presumptive *E. coli* and turbidity, r = 0.43). The correlation coefficient between presumptive *E. coli* and phages of *Bacteroides* GB-124 was very small (r= 0.05), whilst that between turbidity and suspended solids was high (r = 0.62). Variations in climate, animal and anthropogenic interferences were all, either directly or indirectly, related to fecal contamination. |
| Papastergiou *et al*. (2011) | Greece, three marine beaches | Epi | Septic tanks, river, | GI, respiratory | *E. coli*, fecal coliforms, total coliforms, enterococci, *S. aureus* | | *n* = 4293 incl. 3,805 bathers;149 samples from the beaches. Despite all beaches being characterized as of high quality, the levels of bacterial indicators differed among them. Health effects among bathers were not associated with bacterial indicators. A statistically significant increased risk for symptoms related to respiratory illness, gastroenteritis, medical consultation and use of medication was observed among bathers at beaches with higher bather density. |
| Pintar *et al.* (2010) | Canada, Ontario community level, public swimming pool, river, lake. | QMRA | Various | Cryptosporidiosis | *Cryptosporidium* | 2 | Simulated the role of recreational water contact in the transmission of cryptosporidiosis. Stochastic simulations were based on plausible modes of contamination of a pool (literature derived), river (site-specific), and recreational lakes (literature derived). The highest estimated risks of infection were derived from the (highly contaminated) recreational lake scenario, considered the upper end for risk of infection for both children (10 infections per 1,000 swims and adults (four infections per 1,000 swims. Simulating the likely *Cryptosporidium* oocyst concentration in a lane pool that a child would be exposed to following a diarrheal fecal release event resulted in the third highest mean risk of infection (four infections per 10,000 swims [5‰: three infections per 100,000; 95‰: 10 infections per 10,000 swims]). Findings illustrate the need for systematic and standardized research to quantify *Cryptosporidium* oocyst levels in Canadian public pools and recreational beaches. There is also a need to capture the swimming practices of the Canadian public, including most common forms and frequency measures. The study findings suggest that swimming in natural swim environments and in pools following a recent fecal contamination event pose significant public health risks. When considering these risks relative to other modes of cryptosporidiosis transmission, they are significant. |
| Pintar *et al.* (2017) | Canada, Ontario community level, public swimming pool, river, lake. | QMRA | Various | Campylobacteriosis | *Campylobacter* | 2 | A comparative exposure assessment was developed to estimate the relative exposure to *Campylobacter*, the leading bacterial gastrointestinal disease in Canada, for 13 different transmission routes within Ontario, Canada, during the summer. Exposure was quantified with stochastic models at the population level, which incorporated measures of frequency, quantity ingested, prevalence, and concentration, using data from FoodNet Canada surveillance, the peer-reviewed and gray literature, other Ontario data, and data specifically collected for this study. The mean number of cells of *Campylobacter* ingested per Ontarian per day during the summer, ranked from highest to lowest is as follows: household pets, chicken, living on a farm, raw milk, visiting a farm, recreational water, beef, drinking water, pork, vegetables, seafood, petting zoos, |

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| | | | | | | | and fruits. The study results identify knowledge gaps for some transmission routes, and indicate that some transmission routes for *Campylobacter* are underestimated in the current literature, such as household pets and raw milk. Many data gaps were identified for future data collection consideration, especially for the concentration of *Campylobacter* in all transmission routes. |
| Reddy *et al*. (2011) | UK (Cornwall) | Epi | various | "Surfer's ear" | – | | ($n$ = 92. 78 males and 14 females, mean age 27 years, standard deviation 7.9 years). Participants were grouped according to their awareness of the preventability of surfer's ear (55 aware, 37 unaware). These groups were comparable in age, surfing history and gender mix. Surfers aware of the preventability of exostoses (66 per cent) were more likely to use water precautions than those who were not (38 per cent) ($p < 0.01$). Two surfers used water precautions regularly and 48 used them occasionally. Sixty-one of the 76 surfers who did not use water precautions (ear plugs) suggested they would consider doing so in the future. |
| Rijal *et al*. (2011) | Chicago Area Waterway | QMRA | Urban, treated wastewater, land runoff | GI | pathogenic *E. coli* [estimated], *Giardia*, *Cryptosporidium*, Adenovirus, Norovirus, enteric virus | 5 | A microbial risk assessment was conducted to estimate the human health risks from incidental contact recreational activities such as canoeing, boating and fishing in the Chicago Area Waterway System (CAWS) receiving secondary treated, but non-disinfected, effluent from three municipal water reclamation plants. Results under the current treatment scheme with no disinfection indicated that the total expected gastrointestinal illness (GI) rate per 1000 incidental contact recreational exposure events during combined weather (dry and wet) conditions ranged from 0.10 to 2.78 in the CAWS. Wet weather conditions contribute to elevated pathogen load in the CAWS; this study determined that disinfecting the effluents of three major WRPs that discharge to the CAWS would result in an extremely small reduction in the aggregate recreation season risk to incidental contact recreators. |
| Sales-Ortells & Medema. (2014) | Watergraafsmeer, Amsterdam, 20 waterbodies/ features | QMRA | Urban | GI, Legionnaires' disease | *Cryptosporidium*, *Campylobacter*, Norovirus, and *L. pneumophila* | 2 | Event and annual GI probability and Legionnaires' disease were analysed in QMRA models using selected literature data. Highest mean event probabilities of GI were found for playing in pluvial flood from a combined sewer overflow (34%), swimming (18%), and rowing (13%) in the river, swimming (8.7%) and rowing (4.5%) in the lake, and playing in a water playground (3.7%) and in the pluvial flood from stormwater sewers (4.7%). At these locations, the GI probability was above the EU Bathing Water Directive threshold for excellent water quality (3%). All the annual risk medians were below the national incidence of legionellosis of 0.002%. The illness probability was most sensitive to the pathogens concentration (particularly *Campylobacter*, Norovirus, and *Legionella*) and exposure frequency. |
| Sanborn & Takoro (2013) | Canada | Epi | PubMed | Acute GI | – | 2, 5 | There is a 3% to 8% risk of acute gastrointestinal illness (AGI) after swimming. The high-risk groups for AGI are children younger than 5 years, especially if they have not been vaccinated for Rotavirus, and elderly and immunocompromised patients. Children are at higher risk because they swallow more water when swimming, stay in the water |

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| | | | | | | | longer, and play in the shallow water and sand, which are more contaminated. Participants in sports with a lot of water contact such as triathlon and kite surfing are also at high risk, and even activities involving partial water contact such as boating and fishing carry a 40% to 50% increase in risk of AGI compared with non-water recreational activities. |
| Sánchez-Nazario *et al.* (2014) | Puerto Rico, Three beaches | Epi, prospective cohort | Creeks, wastewater treatment plants, septic tanks, animal feces | GI, Respiratory, Skin, Earache, Headache, Fever | Coliphage, *E. coli* (two methods), *Staphylococcus spp., enterococci* | | *n* = 641. Increased risk of illness in swimmers as compared to non-swimmers, even when waters met current microbial standards for recreational water quality. Illnesses included GI, skin and respiratory symptoms, earache and fever. Odds ratios (ORs) ranged from 0.32 to 42.35 (GI illness), 0.69 to 3.12 (skin infections), 0.71 to 3.21 (respiratory symptoms), 0.52 to 15.32 (earache) and 0.80 to 1.68 (fever). The indicators that better predicted the risks of symptoms (respiratory) in tropical recreational waters were total (somatic and male-specific) coliphages (OR = 1.56, $p<0.10$, $R^2 = 3.79\%$) and *E. coli* (OR = 1.38, $p<0.10$, $R^2 = 1.97\%$). Study indicates that coliphages are potentially good predictors of risks of respiratory illness in tropical recreational waters. |
| Schets *et al.* (2011a) | Netherlands | Epi | – | – | – | 6 | The Netherlands revealed 742 outbreaks during 1991–2007 mainly comprising of skin conditions (48%) and gastroenteritis (31%), involving at least 5,623 patients. The number of outbreaks per bathing season correlated with the number of days with temperatures over 25 °C (r=0.8–0.9), but was not reduced through compliance with European bathing-water legislation (r=0.1), suggesting that monitoring of fecal indicator parameters and striving for compliance with water-quality standards may not sufficiently protect bathers. Bathing sites were prone to incidental fecal contamination that favoured the growth of naturally occurring pathogens. |
| Schets *et al.* (2011b) | Netherlands | EPI (exposure study) | – | – | – | 2 | *n* = 8,000 of whom 1924 additionally answered the questions for their eldest child (<15 years). Differences between men and women were small, but children behaved differently: they swam more often, stayed in the water longer, submerged their heads more often and swallowed more water. Swimming pools were visited most frequently (on average 13–24 times/year) with longest duration of swimming (on average 67–81 min). On average, fresh and seawater sites were visited 6–8 times/year and visits lasted 41–79 min. Dependent on the water type, men swallowed on average 27–34 mL per swimming event, women 18–23 mL, and children 31–51 mL. |
| Schijven *et al.* (2015) | Netherlands | QMRA | – | GI infection | *E. coli*, a human-associated *Bacteroidetes*) marker, Enterovirus, Norovirus, *Campylobacter*, | 2 | QMRAcatch, was developed to simulate pathogen concentrations in water. The model domain encompasses a main river with wastewater discharges and a floodplain with a floodplain river. Diffuse agricultural sources not yet included. The floodplain river is fed by the main river and may flood the plain. Fecal deposits from wildlife, birds, and visitors in the floodplain are resuspended in flood water, runoff to the floodplain river, or infiltrate groundwater. Fecal indicator and MST marker data facilitate calibration. Infection risks from exposure to the pathogens by swimming or drinking water consumption are calculated, and the required pathogen removal by treatment to meet a |

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| | | | | | and *Cryptosporidium* | | health-based quality target can be determined. Applicability is demonstrated by calibrating the tool for a study site at the River Danube near Vienna, Austria, using field data, including a sensitivity analysis and evaluation of the model outcomes. |
| Schoen & Ashbolt (2010) | Worldwide | QMRA | Seagulls, treated sewage | GI | Seagulls: *Campylobacter jejuni* and *Salmonella enterica* Sewage: Norovirus, *Giardia intestinalis*, *Cryptosporidium* spp., *Salmonella enterica* | – | QMRA estimated probability of illness for accidental ingestion of recreational water with a specific concentration of fecal indicator bacteria–the geometric mean enterococci limit of 35 cfu/100 mL, from either a mixture of sources or an individual source. Using seagulls as a non-sewage fecal source example, the predicted median probability of illness was less than the illness benchmark of 0.01. When the fecal source was changed to poorly treated sewage, a relativity small difference between the median probability of illness and the illness benchmark was predicted. For waters impacted by a mixture of seagull and sewage waste, the dominant source of fecal indicator was not always the predicted dominant source of risk. |
| Schoen *et al.* (2011) | World-wide | QMRA | Human sewage variously-treated | GI | Norovirus, enterococci (culture and PCR) | | Evaluated the relative contribution of fecal indicators and pathogens when a mixture of human sources impacts a recreational waterbody. Used Norovirus as the reference pathogen and enterococci as the reference fecal indicator. Contribution made by each source to the total waterbody volume, indicator density, pathogen density, and illness risk was estimated for a number of scenarios that accounted for pathogen and indicator inactivation based on the age of the effluent (source to-receptor), possible sedimentation of microorganisms, and the addition of a non-pathogenic source of fecal indicators (such as old sediments or an animal population with low occurrence of human-infectious pathogens). Enterococci was held constant at 35 cfu/100 mL to compare results across scenarios. For the combinations evaluated, either the untreated sewage or the non-pathogenic source of fecal indicators dominated the recreational waterbody enterococci density assuming a culture method. In contrast. The results support the use of a calibrated qPCR total enterococci indicator, compared to a culture-based assay, to index infectious human enteric viruses released in treated human wastewater, and illustrate that the source contributing the majority of risk in a mixture may be overlooked when only assessing fecal indicators by a culture-based method. |
| Seto *et al.* (2016) | Oakland, CA | QMRA | Urban treated wastewater, wet-weather flows | GI | Fecal coliform, *E. coli*, *Enterococcus*, male specific coliphage, Adenovirus, Enterovirus, *Giardia* spp., | | A static QMRA was used to estimate the incremental risk to public health from recreational exposure to Adenovirus and the protozoan *Giardia* spp. in San Francisco Bay for wet season (generally between October and March) blending and non-blending events. The mean risks of infection per recreational exposure event during the wet season for all of the modeled scenarios were more than an order-of-magnitude below the USEPA's illness level (36 illnesses per 1000 contact events) associated with recreational water quality. While the QMRA results showed discernible differences in per event estimated risks between blending and non-blending scenarios, the estimated incremental increase in the annual number of infections due to blending (based on |

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| | | | | | *Cryptosporidium* spp. | | median estimates) resulted in an estimated combined increase of less than one infection annually. These estimates are subject to various uncertainties, including the potential for secondary transmission, assumptions on the extent of exposures, and the number of blending days required in the future due to climate change. |
| Sidhu *et al*. (2012) | Brisbane, Australia, urban stormwater runoff | Survey | Broad range of urban | – | **Culture:** *E. coli*, *Enterococcus*, **PCR**: Adenovirus, polyomavirus, *S. enterica*, *Campylobacter* spp. and *Bacteroides* HF183 gene detected with published primer and probe sets. | 4, 5 | Water samples (20 L) were collected after storm events and during the dry weather from six sites in Brisbane, Australia. Samples were analyzed for FIB, and then concentrated using hollow fiber ultrafiltration followed by molecular detection of selected enteric pathogens. The levels of FIB were found to frequently exceed the upper limit of Australian guidelines for managing risks in recreational water, during the dry periods and by further several orders of magnitude in the stormwater runoff. *Enterococcus* spp. numbers as high as $3x10^4$ /100 mL were detected in the stormwater runoff. Human Adenovirus and polyomavirus were frequently detected from all six sites during wet and dry weather conditions suggesting their wide spread presence in the urban aquatic environments. *Campylobacter jejuni*, *Campylobacter coli* and *S. enterica* were also detected during both dry and wet weather conditions. Presence of human-specific HF183 *Bacteroides* marker in most of the samples tested suggests ubiquitous sewage contamination. Since stormwater runoff routinely contains high numbers of FIB and other enteric pathogens, some degree of treatment of captured stormwater would be required if it were to be used for non-potable purposes. |
| Sinigalliano *et al*. (2010) | United States | EPI: Prospective randomized exposure | Recreational marine waters with no known point source of sewage | Included GI, skin illness, acute febrile respiratory illness | *Enterococcus* (culture methods and qPCR), *Bacteroidales* (qPCR, human and dog markers), gull *Catellicoccus* marker, *S. aureus* | | *n* = 1,341, 15 study days. No known point source (e.g., discharge of treated sewage). The study reported symptoms between one set of human subjects randomly assigned to marine water exposure with intensive environmental monitoring compared with other subjects who did not have exposure. Among the bathers, a positive dose-response relationship was observed for skin illness and enterococci enumeration by membrane filtration. Skin illness was positively related to 24 hour antecedent rainfall, while acute febrile respiratory illness was inversely related to water temperature. There were no significant dose-response relationships between report of human illness and any of the other FIB or environmental measures. |
| Soldanova *et al*. (2013) | Europe | Review | Snails and birds | Schistosomiatis | – | 6 | Summarizes current knowledge about the occurrence and distribution of swimmer's itch, with a focus on Europe. Relevant studies from the past decade are analyzed to reveal an almost complete set of ecological factors as a prerequisite for establishing the life cycle of bird schistosomes. Based on records of the occurrence of the parasite infective agents, and epidemiological studies that investigate outbreaks of swimmer's itch, this review concentrates on the risk factors for humans engaged in recreational water activities. |
| Soller *et al.* (2010a) | World-wide | QMRA | Various | GI | Norovirus, Rotavirus, Adenovirus, | 2, 6) | Epidemiology studies of recreational waters have demonstrated that swimmers exposed to fecally-contaminated recreational waters are at risk of excess gastrointestinal illness. Epidemiology studies provide valuable information on the nature and extent of health |

| Reference | Location (1) | Study type (1) | Contamination source(s) (1) | Health effects evaluated (1) | Water quality metrics (1) | Charges 2–6 | Conclusions (includes health linkages) |
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| | | | | | *Cryptosporidium* spp., *Giardia lamblia*, *Campylobacter jejuni*, *Salmonella enterica*, and *E. coli* O157:H7. | | effects, the magnitude of risks, and how these risks are modified or associated with levels of fecal contamination and other measures of pollution. However, such studies have not provided information about the specific microbial agents that are responsible for the observed illnesses in swimmers. Considers epidemiological results from studies conducted on the Great Lakes in the US during 2003 and 2004 by identifying pathogens that could have caused the illnesses observed. Two QMRA-based approaches were used to estimate the pathogen combinations that would be consistent with observed illness rates: in the first, swimming-associated gastrointestinal (GI) illnesses were assumed to occur in the same proportion as known illnesses in the US due to all non-foodborne sources, and in the second, pathogens were assumed to occur in the recreational waters in the same proportion as in disinfected secondary effluent. The results indicate that human enteric viruses and in particular, Norovirus, could have caused the vast majority of the observed swimming associated GI illnesses during the 2003/2004 water epidemiology studies. Evaluation of the time-to-onset of illness strongly supports the principal finding and sensitivity analyses support the overall trends of the analyses even given their substantial uncertainties. |
| Soller *et al.* (2010b) | World-wide | QMRA | gull, pig, chicken, cattle | GI | Norovirus, Rotavirus, *Cryptosporidium* spp., *Giardia lamblia*, *Campylobacter jejuni*, *Salmonella enterica*, and *E. coli* O157:H7 | | This work was conducted to determine whether estimated risks following exposure to recreational waters impacted by gull, chicken, pig, or cattle fecal contamination are substantially different than those associated with waters impacted by human sources such as treated wastewater. Published QMRA methods were employed and extended to meet these objectives w.r.t. GI. Illness risks from these pathogens were calculated for exposure to fecally contaminated recreational water at the U.S. regulatory limits of 35 cfu/100 mL enterococci and 126 cfu/100 mL *E. coli*. Three scenarios were simulated, representing a range of feasible interpretations of the available data. The primary findings are that: 1) GI illness risks associated with exposure to recreational waters impacted by fresh cattle feces may not be substantially different from waters impacted by human sources; and 2) the risks associated with exposure to recreational waters impacted by fresh gull, chicken, or pig feces appear substantially lower than waters impacted by human sources. These results suggest that careful consideration may be needed in the future for the management of recreational waters not impacted by human sources. |
| Soller *et al.* (2014) | World-wide | QMRA | Human and animal | GI | enterococci | | Simulated the influence of multiple sources of enterococci (ENT) by considering waters impacted by human and animal sources, human and non-pathogenic sources, and animal and non-pathogenic sources. They illustrate that risks vary with the proportion of culturable ENT in waterbodies derived from these sources and estimate corresponding ENT densities that yield the same level of health protection that the recreational water quality criteria in the United States seeks (benchmark risk). The benchmark risk is based on epidemiological studies conducted in waterbodies predominantly impacted by human fecal sources. The key result is that the risks from mixed sources are driven |

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| | | | | | | | predominantly by the proportion of the contamination source with the greatest ability to cause human infection (potency), not necessarily the greatest source(s) of FIB. Predicted risks from exposures to mixtures comprising approximately 30% ENT from human sources were up to 50% lower than the risks expected from purely human sources when contamination is recent and ENT levels are at the current water quality criteria levels (35 cfu/100 mL). For human/non-pathogenic, human/gull, human/pig, and human/ chicken fecal mixtures with relatively low human contribution, the predicted culturable enterococci densities that correspond to the benchmark risk are substantially greater than the current water quality criteria values. These findings are important because they highlight the potential applicability of site-specific water quality criteria for waters that are predominantly un-impacted by human sources |
| Soller *et al.* (2015) | World-wide | QMRA | animals (cattle, pigs, chicken) | GI | Literature values of:<br>*E. coli* O157, *Campylobacter*, *Salmonella*, *Cryptosporidium*, *Giardia* spp. | 5 | Epidemiological studies conducted at locations impacted by non-human fecal sources have provided ambiguous and inconsistent estimates of risk. QMRA is another tool. The potential risk differential between human and selected non-human fecal sources was characterized previously for direct deposition of animal feces to water. In this evaluation, the human illness potential from recreational exposure to freshwater impacted by rainfall-induced runoff containing agricultural animal fecal material was examined. Risks associated with these sources would be at least an order of magnitude lower than the benchmark level of public health protection associated with current US recreational water quality criteria, which are based on contamination from human sewage sources. |
| Soller *et al.* (2016) | Boquerón beach in Puerto Rico | QMRA/EPI (prospective cohort study, Puerto Rico) | GI | Various | **Indicators**<br>*Bacteroidales*, *C. perfingens*, Coliphage (male-specific), *E. coli*, *Enterococcus* spp. (CFU, CCE)<br>**Pathogens**<br>Norovirus, Adenovirus, *Cryptosporidium*, *Giardia*, *Salmonella* | 4 | Estimated the GI illness levels associated with recreational water exposures. The previously reported epidemiological study had sufficient statistical power to detect an average illness rate of approximately 17 swimming associated GI illnesses per 1000 recreation events or greater, and found no consistent relationships between water quality measured by fecal indicator organisms (FIO) and swimming-associated illnesses. The QMRA incorporated monitoring data for pathogens and fecal indicators collected during the epidemiological study period and calculated average swimming-associated illness levels that were approximately two GI illnesses per 1000 recreation events. To the authors' knowledge, this is the first time that a comprehensive water quality monitoring program and QMRA analysis has been conducted in parallel with a recreational water epidemiological study. The QMRA results were consistent with the low rate of reported illnesses during the 2009 epidemiological study (i.e. < 17 GI illnesses per 1000 recreation events), and provide additional context for understanding the epidemiological results. The results illustrate that coupling QMRA with an epidemiological study at a single study site provides a unique ability to understand human health illnesses especially under conditions where water quality, as measured by traditional FIO is good and/or average illness rates are lower than can be quantified via epidemiological methods alone. |

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| Soller *et al.* (2017, in press) | San Diego | QMRA | Various | GI (Norovirus illness) | **Indicators** total coliform, *E. coli* and enterococci **Pathogens** Norovirus G1, Norovirus G2, Enterovirus, Adenovirus, Campylobacter, Salmonella invA, Salmonella ttr | 4, 5 | Modeled GI risk associated with recreational exposures to marine water following storm events. Estimated GI illness risks via QMRA techniques by consolidating site specific pathogen monitoring data of stormwater, site specific dilution estimates, literature-based water ingestion data, and literature based pathogen dose-response and morbidity information. The authors' water quality results indicated that human sources of contamination contribute viral and bacterial pathogens to streams draining an urban watershed during wet weather that then enter the ocean and affect nearshore water quality. A series of approaches to account for uncertainty in the Norovirus dose-response model selection and compared our model results to those from a concurrently conducted epidemiological study that provided empirical estimates for illness risk following ocean exposure were evaluated. The preferred Norovirus dose-response approach yielded median risk estimates for water recreation associated illness (15 GI illnesses per 1000 recreation events) that closely matched the reported epidemiological results (12 excess GI illnesses per 1000 wet weather recreation events). The results are consistent with Norovirus, or other pathogens associated with Norovirus, as an important cause of GI among surfers in this setting. This study demonstrates the applicability of QMRA for recreational water risk estimation, even under wet weather conditions and describes a process that might be useful in developing site-specific water quality criteria in this and other locations. |
| Sunger & Haas (2015) | Philadelphia | QMRA | Urban | GI | – | 5 | QMRA under dry and wet weather conditions. Stochastic exposure models were generated for each exposure scenario and Monte Carlo techniques were applied to characterize uncertainty in final risk estimates. The dry-weather risk estimates were significantly lower than those predicted for wet-weather conditions. Moreover, the predicted risk, calculated in proportion of the frequency of use, was elevated at 6 out of 10 sites (ranging from 9 to 52 illnesses/1000 users/day). Activities contributing most to the risk of GI illness at creeks were identified as wading and playing (81%), while fishing was the potential risk contributor (65%) at rivers. |
| Suppes *et al.* (2014) | Four pool sites in Tuscon Alabama | Experiment | Drinking water supply | – | – | | n = 126. Less frequent head submersion appears to be associated with greater pool water ingestion rates, which may conflict with one's intuition: These swimmers were younger and likely less skilled than adults. Video observations suggest children are 'bobbing' with mouths open at water surface to stay above water, and intentionally spitting and spouting water. Outbreak tools should assess leisure activity engagement and number of splashes received to the face among cases and non-cases, as both activities were associated with increased pool water ingestion. Assessing skill as an ingestion predictor is recommended for future swimming exposure assessments to clarify why children ingest more pool water than adults. Quantifying and comparing pool water ingestion with frequency of spitting. |

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| Tong *et al.* (2011) | Treated and untreated wastewater; 16 sites around O'ahu | Experiment | Sewage, land runoff | – | – | 4 | The current recreational water quality criteria using growth-based measurements of FIB concentration have their limitations for swimmer protection. Human Norovirus (huNoV) was tested as a model in this study. To establish a highly sensitive protocol for effective huNoV detection in waters, 16 published and newly designed reverse transcription polymerase chain reaction (RT-PCR) primer pairs specific for huNoV genogroup I (GI) and genogroup II (GII) were comparatively evaluated side-by-side using single sources of huNoV RNA stock extracted from local clinical isolates. Under optimized conditions, these RT-PCR protocols shared a very different pattern of detection sensitivity for huNoV. The primer sets COG2F/COG2R and QNIF4/NV1LCR were determined to be the most sensitive ones for huNoV GII and GI, respectively, with up to $10^5$ and $10^6$-fold more sensitive as compared to other sets tested. These two sensitive protocols were validated by positive detection of huNoV in untreated and treated urban wastewater samples. In addition, these RT-PCR protocols enabled detection of the prevalence of huNoV in 5 (GI) and 10 (GII) of 16 recreational water samples, confirmed by cDNA sequencing and sequence analysis. Findings from this study support the possible use of enteric viral pathogens for environmental monitoring and argue the importance and essentiality for such monitoring activity to ensure a safe use of recreational waters. |
| Tseng & Jiang (2012) | Southern California | QMRA | Mostly urban beaches | GI | FIO data obtained from monitoring results by a number of agencies | | QMRAs were applied to eight popular Southern California beaches using readily available *Enterococcus* and fecal coliform data and dose-response models to compare health risks associated with surfing during dry weather and storm conditions. The results showed that the level of gastrointestinal illness risks from surfing post-storm events was elevated, with the probability of exceeding the US EPA health risk guideline up to 28% of the time. The surfing risk was also elevated in comparison with swimming at the same beach due to ingestion of greater volume of water. The study suggests that refinement of dose-response model, improving monitoring practice and better surfer behavior surveillance will improve the risk estimation. |
| Viau *et al.* (2011) | O'ahu, Hawaii | QMRA | Land runoff, septic tanks | GI | **Viruses** Adenovirus, Enterovirus, Norovirus GI, and Norovirus GII; **Markers** human, ruminant, and pig *Bacteroidales* | 4 | This study used molecular methods to measure concentrations of four enteric viruses (Adenovirus, Enterovirus, Norovirus GI, and Norovirus GII) and fecal source tracking markers (human, ruminant, and pig *Bacteroidales*) in land-based runoff from 22 tropical streams on O'ahu, Hawai'i. Each stream was sampled twice in the morning and afternoon during dry weather. Viruses and human *Bacteroidales* were widespread in the streams. Watershed septic tank densities were positively associated with higher occurrence of human *Bacteroidales* and Norovirus. There were no associations between occurrence of viruses and fecal indicator concentrations. Virus concentrations and previously reported culturable *Salmonella* and *Campylobacter* were used as inputs to a QMRA model to estimate the risk of acquiring GI illness from swimming in tropical marine waters adjacent to discharging streams. Monte Carlo methods were used to incorporate uncertainties in the dilution of stream discharge with seawater, swimmer |

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| | | | | | | | ingestion volumes, pathogen concentrations, and dose-response parameters into the model. Median GI illness risk to swimmers from exposure to coastal waters adjacent to the 22 streams ranged from 0 to 21/1000. GI illness risks from viral exposures were generally orders of magnitude greater than bacterial exposures. The median risk adjacent to each stream was positively, significantly correlated to the concentration of *Clostridium perfringens* in the stream. |
| Vijayavel *et al.* (2010) | O'ahu, 3 STPs to represent sewage (variously-treated), three coastal sites and a harbor | Survey | Land runoff, treated sewage | – | *Bacteroides*, HB-73 phage | 3, 4 | Previous studies have shown *E. coli* and enterococci to be unreliable indicators of fecal contamination in Hawaii because of their ability to multiply in soils. In this study, the method of detecting *Bacteroides* phages as specific markers of sewage contamination in Hawaii's recreational waters was evaluated because these sewage-specific phages cannot multiply under environmental conditions. *Bacteroides* hosts (GB-124, GA-17), were recovered from sewage samples in Europe and were reported to be effective in detecting phages from sewage samples obtained in certain geographical areas. However, GB-124 and GA-17 hosts were ineffective in detecting phages from sewage samples obtained in Hawaii. *Bacteroides* host HB-73 was isolated from a sewage sample in Hawaii, confirmed as a *Bacteroides* sp. and shown to recover phages from multiple sources of sewage produced in Hawaii at high concentrations (5.2-7.3 x $10^5$ PFU/100 mL). These *Bacteroides* phages were considered to be potential markers of sewage because they also survived for three days in fresh stream water and two days in marine water. Water samples from Hawaii's coastal swimming beaches and harbors, which were known to be contaminated with discharges from streams, were shown to contain moderate (20-187 cfu/100 mL) to elevated (173-816 cfu/100 mL) concentrations of enterococci. These same samples contained undetectable levels (<10 PFU/100 mL) of $F^+$ coliphage and *Bacteroides* phages and provided evidence to suggest that these enterococci may not necessarily be associated with the presence of raw sewage. These results support previous conclusions that discharges from streams are the major sources of enterococci in coastal waters of Hawaii and the most likely source of these enterococci is from environmental soil rather than from sewage. |
| Wade *et al.* (2006) | Two Great Lakes beaches | Epi: Prospective cohort | Wastewater treatment plants | GI | *Enterococcus* (qPCR), *Bacteroides* | | $n$ = 5,717. Methods to measure recreational water quality in ≤ 2 hr have been developed. We conducted a prospective study of beachgoers at two Great Lakes beaches to examine the association between recreational water quality, obtained using rapid methods, and GI illness after swimming. We tested water samples for *Enterococcus* and *Bacteroides* species using the quantitative polymerase chain reaction (PCR) method. We observed significant trends between increased GI illness and *Enterococcus* at the Lake Michigan beach and a positive trend for *Enterococcus* at the Lake Erie beach. The association remained significant for *Enterococcus* when the two beaches were combined. We observed a positive trend for *Bacteroides* at the Lake Erie beach, but no trend was observed at the Lake Michigan beach. *Enterococcus* samples collected at |

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| | | | | | | | 0800 hr were predictive of GI illness that day. The association between *Enterococcus* and illness strengthened as time spent swimming in the water increased. |
| Wade *et al.* (2008) | Four Great Lakes beaches | Epi: , prospective | Wastewater effluent-impacted waters | GI, respiratory, rash, eye ailments, earache | Enterococci (qPCR) and *Bacteroides* measured via qPCR | 2, 3 | *n* = 5,717. Swimmers at two beaches had a higher incidence of GI illness when compared to non-swimmers. A statistically significant relationship was observed between an increased rate of GI illness and enterococci at the Lake Michigan beach, and a positive trend for enterococci at the Lake Erie beach was noted. Association between enterococci (qPCR) and increased risk of GI illness was significant when results for the two beaches were combined. Positive trend was observed at the Lake Erie beach for *Bacteroides*, but no trend was observed at the Lake Michigan beach. |
| Wade *et al.* (2010) | United States (Mississippi. Rhode Island, Alabama | Epi: Cohort, prospective | Wastewater effluent-impacted waters | GI | enterococci (qPCR) and *Bacteroides* measured via qPCR | 4 | *n* = 6,350. Swimmers at two beaches had a higher incidence of GI illness when compared to non-swimmers. A statistically significant relationship was observed between an increased rate of GI illness and enterococci at the Lake Michigan beach, and a positive trend for enterococci at the Lake Erie beach was noted. The association between enterococci and increased risk of GI illness was significant when results for the two beaches were combined. A positive trend was observed at the Lake Erie beach for *Bacteroides*, but no trend was observed at the Lake Michigan beach. |
| Wade *et al.* (2013a) | South Carolina | EPI: Prospective cohort | Stormwater runoff | diarrhea | – | 5 | *n* = 11,159. The association between diarrhea among swimmers and rain events at a beach in South Carolina impacted by stormwater runoff was investigated. During the summer of 2009, 11,159 beachgoers were enrolled and interviewed. Information about swimming exposures was obtained, followed by telephone contact 10-12 days later to ascertain the incidence of diarrhea (3 or more loose stools in a 24 hour period), and other symptoms. Rainfall was classified as none; low-moderate (≤0.39 inches); or high (>0.4 inches, 90th percentile). Unadjusted incidence of diarrhea was 3.0%, 4.0%, 4.4%, and 6.5% among non-swimmers; swimmers (body-immersion) following no rainfall in the previous 24 hours; swimmers following low-moderate rainfall and swimmers following high rainfall, respectively. Adjusted Odds Ratios and 95% Confidence Intervals compared to non-swimmers were: 1.33 (0.95-1.86); 1.55 (1.07-2.25); and 2.14 (1.32-3.48) for swimmers with no rainfall, low, and high rainfall in the prior 24 hours, respectively. There was also a significant trend across categories among swimmers ($p$ = 0.003). Rainfall the day of swimming and during the 24-48 hour lag were not as consistently associated with diarrhea. In conclusion, diarrhea among swimmers was associated with rainfall in the 24 hours prior to swimming at a beach impacted by urban runoff. |
| Wade *et al.* (2013b) | 9 USA beaches (four freshwater, 5 marine, incl. Puerto Rico) | Epi: Prospective cohort | Various | earache | FIO (not identified) | 5 | *n* = 50,000. Excess risk and health burden of earaches due to swimming in natural fresh and marine waters was estimated using for nine beaches across the United States. Economic and physical burdens were also obtained. Model results were used to calculate excess risk for earaches attributable to swimming. The overall incidence of self-reported earache was 1.6% in the 10–12 days after the beach visit. Earaches were |

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| | | | | | | | more frequent in head immersion swimmers compared to non-swimmers for all beach sites and age groups. Earaches were un-associated with water sample measures of fecal contamination and turbidity. After adjustment for covariates, we calculated 7.12 excess earaches among head immersion swimmers per 1,000 swimming events. |
| Wyer *et al*. (2012) | Europe-wide | Experiment | Various | – | *Escherichia coli*, intestinal enterococci and somatic coliphage | 4 | During the EU FP6 Project VIROBATHE a database of over 290 HAdV analyses with corresponding fecal indicator organism (FIO) determinations was gathered and used to explore statistical associations between HAdV and FIO results. The FIOs measured were *E. coli*, intestinal enterococci and somatic coliphage. Statistically significant trends of increasing proportions of HAdV-positive results in categories of increasing FIO concentration were found in freshwater but not seawater samples. The analysis of these trends in freshwater samples was refined, the trends remaining statistically significant when using categories of 0.5 log10 intervals of FIO concentration. Logistic regression models were then developed to predict the probability of a HAdV-positive outcome from FIO concentration. |
| Wymer *et al*. (2013) | NEEAR sites, USA | EPI (combining previous studies) | Various | NGI (vs. HCGI) | total coliforms, fecal coliforms, *E. coli*, *Enterococcus* | 4 | The US EPA and its predecessors have conducted three distinct series of epidemiological studies beginning in 1948 on the relationship between bathing water quality and swimmers' illnesses. Keeping pace with advances in microbial technologies, these studies differed in their respective microbial indicators of water quality. Another difference, however, has been their specific health endpoints. The latest round of studies, the National Epidemiological Assessment of Recreational (NEEAR) Water studies initiated in 2002, used a case definition, termed "NEEAR GI illness" (NGI), for gastrointestinal illness corresponding closely to classifications employed by contemporary researchers, and to that proposed by the World Health Organization. NGI differed from the previous definition of "highly credible gastrointestinal illness" (HCGI) upon which the USEPA's 1986 bathing water criteria had been based, primarily by excluding fever as a prerequisite.<br>Incidence of NGI from the NEEAR studies was compared to that of HCGI from earlier studies. The ratio of NGI risk to that of HCGI is estimated to be 4.5 with a credible interval 3.2 to 7.7. Conclusions: A risk level of 8 HCGI illnesses per 1000 swimmers, as in the 1986 freshwater criteria, would correspond to 36 NGI illnesses per 1000 swimmers. Given a microbial DNA-based (qPCR) water quality vs. risk relationship developed from the NEEAR studies, 36 NGI per 1000 corresponds to a geometric mean of 475 qPCR cell-equivalents per 100 ml. Figure 1 shows marine and freshwater relationships combined. |
| Xiao *et al*. (2013) | Three Gorges Reservoir, China (TGR) | QMRA | City wastes, stormwater, land runoff | GI | **Culture**<br>*E. coli*, total coliform, fecal | 4 | During two successive 1-year study periods (July 2009 to July 2011), the water quality in Wanzhou watershed of the TGR was tested with regard to the presence of fecal indicators and pathogens. *Salmonella*, Enterohemorrhagic *E. coli* (EHEC), *Giardia* and *Cryptosporidium* were detected in the watershed. Prevalence and concentrations of the pathogens in the mainstream were lower than those in backwater areas. The estimated |

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| | | | | | coliform, fecal streptococci<br>**MPN-PCR**<br>*Salmonella*,, EHEC, enterococci | | risk of infection with *Salmonella*, EHEC, *Cryptosporidium*, and *Giardia* per exposure event ranged from $2.9 \times 10^{-7}$ to $1.68 \times 10^{-5}$, $7.04 \times 10^{-10}$ to $2.36 \times 10^{-7}$, $5.39 \times 10^{-6}$ to $1.25 \times 10^{-4}$ and 0 to $1.2 \times 10^{-3}$, respectively, for occupational divers and recreational swimmers. The estimated risk of infection at exposure to the 95% upper confidence limit concentrations of *Salmonella*, *Cryptosporidium* and *Giardia* may be up to $2.62 \times 10^{-5}$, $2.55 \times 10^{-4}$ and $2.86 \times 10^{-3}$, respectively. |
| Yau *et al*. (2014) | United States (Avalon) | Epi, prospective cohort | Wastewater effluent-impacted waters, groundwater | GI, skin infection, eye infection, ear infection | *Enterococcus*, total coliforms, fecal coliforms, *E. coli*; incl three rapid methods for *Enterococcus* | 4 | *n* = 7,317. Swimmers who swallowed water were more likely to experience GI illness within 3 days of a beach visit than non-swimmers. Risk elevated when either submarine groundwater discharge was high or solar radiation was low. The risk of GI illness was not significantly elevated for swimmers who swallowed water when groundwater discharge was low or solar radiation was high. Associations between GI illness incidence and FIB levels (*Enterococcus* EPA Method 1600) among swimmers who swallowed water were not significant when not accounting for groundwater discharge, but were strongly associated when groundwater discharge was high compared to when it was low. |
| Young (2016) | Marine, Worldwide | Survey | Point vs. non-point | GI, respiratory, skin | enterococci | | Numerous studies have demonstrated increased GI risk with marine swimming – typically defined as head immersion: Potential emerging marine threats include Shewanella and Vibrio bacteria, and the presence of human pathogens in the marine environment that are resistant to antimicrobials. |
| Zeigler *et al*. (2014) | Las Vega, Nellis Air Force Base | Epi: case-control | Cattle ranch | fever, vomiting, hemorrhagic diarrhea | – | 6 | On October 12, 2012, the Nellis Air Force Base Public Health Flight (Nellis Public Health), near Las Vegas, Nevada, was notified by the Mike O'Callaghan Federal Medical Center (MOFMC) emergency department (ED) of three active duty military patients who went to the ED during October 10–12 with fever, vomiting, and hemorrhagic diarrhea. Initial interviews by clinical staff members indicated that all three patients had participated October 6–7 in a long distance obstacle adventure race on a cattle ranch in Beatty, Nevada, in which competitors frequently fell face first into mud or had their heads submerged in surface water. There were 22 cases (18 probable and four confirmed) of *Campylobacter coli* infection among active duty service members and civilians. A case control study using data provided by patients and healthy persons who also had participated in the race showed a statistically significant association between inadvertent swallowing of muddy surface water during the race and *Campylobacter* infection (odds ratio = 19.4; $p<0.001$). |

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| Zlot *et al*. (2015) | Oregon, Lake Regional Park | Epi, retrospective cohort | Lake, cause unknown | Norovirus illness | – | 6 | In July 2014, Multnomah County public health officials investigated a Norovirus outbreak among persons visiting Blue Lake Regional Park in Oregon. During the weekend of the reported illnesses (Friday, July 11-Sunday, July 13) approximately 15,400 persons visited the park. The investigation identified 65 probable and five laboratory-confirmed cases of Norovirus infection (70 total cases). No hospitalizations or deaths were reported. Analyses from a retrospective cohort study revealed that swimming at Blue Lake during July 12-13 was significantly associated with illness during July 13-14 (adjusted relative risk = 2.3; 95% confidence interval [CI] = 1.1-64.9). Persons who swam were more than twice as likely to become ill compared with those who did not swim in the lake. |

## E. ACKNOWLEGEMENT

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## F. REFERENCES

Abbott, B.; Lugg, R.; Devine, B.; Cook, A.; Weinstein, P. (2011). Microbial risk classifications for recreational waters and applications to the Swan and Canning Rivers in Western Australia. *Journal of Water and Health 9*: 70-79.

Abdelzaher, A.M., *et al.* (2010). Presence of pathogens and indicator microbes at a non-point source subtropical recreational marine beach. *Applied and Environmental Microbiology 76*: 724-732.

Abia, A.L.K.; Ubomba-Jaswa, E.; Genthe, B.; Momba, M.N.B. (2016). Quantitative microbial risk assessment (QMRA) shows increased public health risk associated with exposure to river water under conditions of riverbed sediment resuspension. *Science of the Total Environment 566*: 1143-1151.

Agampodi, S.B.; Karunarathna, D.; Jayathilala, N.; Rathnayaka, H.; Agampodi, T.C.; Karunanayaka, L. (2014). Outbreak of leptospirosis after white-water rafting: sign of a shift from rural to recreational leptospirosis in Sri Lanka? *Epidemiology and Infection 142*: 843-846.

Almeida, C.; Gonzalez, S.O.; Mallea, M.; Gonzalez, P. (2012). A recreational water quality index using chemical, physical and microbiological parameters. *Environmental Science and Pollution Research 19*: 3400-3411.

Arnold, B.F.; Schiff, K.C.; Griffith, J.F.; Gruber, J.S.; Yau, V.; Wright, C.C.; Wade, T.J.; Burns, S.; Hayes, J.M.; McGee, C.; Gold, M.; Cao, Y.P.; Weisberg, S.B.; Colford, J.M. (2013). Swimmer illness associated with marine water exposure and water quality indicators impact of widely used assumptions. *Epidemiology 24*: 845-853.

Arnold, B.F.; Wade, T.J.; Benjamin-Chung, J.; Schiff, K.C.; Griffith, J.F.; Dufour, A.P.; Weisberg, S.B.; Colford, J.M. (2016). Acute gastroenteritis and recreational water: Highest burden among young US children. *American Journal of Public Health 106*: 1690-1697.

Arnold, B.F.; Schiff, K.C.; Ercumenv, A.; Benjamin-Chung, J.; Steele, J.A.; Griffith, J.F.; Steinberg, S.J.; Smith, P.; McGee, C.D.; Wilson, R.; Nelsen, C.; Weisberg, S.B.; Jr., C.J.M. (2017). Acute illness among surfers after exposure to seawarer in dry- and wet-weather conditions. *American Journal of Epidemiology* doi: 10.1093/aje/kwx019.

Ashbolt, N.J.; Schoen, M.E.; Soller, J.A.; Rose, D.J. (2010). Predicting pathogen risks to aid beach management: The real value of quantitative microbial risk assessment (QMRA). *Water Research 44*: 4692-4703.

Bambic, D.G.; Kildare-Hann, B.J.; Rajal, V.B.; Sturm, B.S.M.; Minton, C.B.; Schriewer, A.; Wuertz, S. (2015). Spatial and hydrologic variation of *Bacteroidales*, Adenovirus and Enterovirus in a semi-arid, wastewater effluent-impacted watershed. *Water Research 75*: 83-94.

Betancourt, W.Q.; Duarte, D.C.; Vasquez, R.C.; Gurian, P.L. (2014). Cryptosporidium and Giardia in tropical recreational marine waters contaminated with domestic sewage: Estimation of bathing-associated disease risks. *Marine Pollution Bulletin 85*: 268-273.

Boehm, A.B.; Soller, J.A.; Shanks, O.C. (2015). Human-associated fecal quantitative polymerase chain reaction measurements and simulated risk of gastrointestinal illness in recreational waters contaminated with raw sewage. *Environmental Science & Technology Letters 2*: 270-275.

Brown, K.I.; Graham, K.E.; Boehm, A.B. (2017). Risk-based threshold of gull-associated fecal marker concentrations for recreational water. *Environmental Science & Technology Letters 4*: 44-48.

Colford, J.M.; Schiff, K.C.; Griffith, J.F.; Yau, V.; Arnold, B.F.; Wright, C.C.; Gruber, J.S.; Wade, T.J.; Burns, S.; Hayes, J.; McGee, C.; Gold, M.; Cao, Y.P.; Noble, R.T.; Haugland, R.; Weisberg, S.B. (2012). Using rapid indicators for *Enterococcus* to assess the risk of illness after exposure to urban runoff contaminated marine water. *Water Research 46*: 2176-2186.

Collier, S.A.; Wade, T.J.; Sams, E.A.; Hlavsa, M.C.; Dufour, A.P.; Beach, M.J. (2015). Swimming in the USA: beachgoer characteristics and health outcomes at US marine and freshwater beaches. *Journal of Water and Health 13*: 531-543.

Corsi, S.R.; Borchardt, M.A.; Carvin, R.B.; Burch, T.R.; Spencer, S.K.; Lutz, M.A.; McDermott, C.M.; Busse, K.M.; Kleinheinz, G.T.; Feng, X.P.; Zhu, J. (2016). Human and bovine viruses and bacteria at three Great Lakes Beaches: Environmental variable associations and health risk. *Environmental Science & Technology 50*: 987-995.

Dale, K.; Kirk, M.; Sinclair, M.; Hall, R.; Leder, K. (2010). Reported waterborne outbreaks of gastrointestinal disease in Australia are predominantly associated with recreational exposure. *Australian and New Zealand Journal of Public Health 34*: 527-530.

Dale, K.; Wolfe, R.; Sinclair, M.; Hellard, M.; Leder, K. (2009). Sporadic gastroenteritis and recreational swimming in a longitudinal community cohort study in Melbourne, Australia. *American Journal of Epidemiology 170*: 1469-1477.

de Man, H.; van den Berg, H.H.J.L.; Leenen, E.J.T.M.; Schijven, J.F.; Schets, F.M.; van der Vliet, J.C.; van Knapen, F.; Husman, A.M.D. (2014). Quantitative assessment of infection risk from exposure to waterborne pathogens in urban floodwater. *Water Research 48*: 90-99.

DeFlorio-Barker, S.; Wade, T.J.; Jones, R.M.; Friedman, L.S.; Wing, C.; Dorevitch, S. (2017). Estimated costs of sporadic gastrointestinal illness associated with surface water recreation: A combined analysis of data from neear and cheers studies. *Environmental Health Perspectives 125*: 215-222.

Dorevitch, S.; Ashbolt, N.J.; Ferguson, C.M.; Fujioka, R.; McGee, C.D.; Soller, J.A.; Whitman, R.L. (2010). Meeting report: Knowledge and gaps in developing microbial criteria for inland recreational waters. *Environmental Health Perspectives 118*: 871-876.

Dorevitch, S.; Panthi, S.; Huang, Y.; Li, H.; Michalek, A.M.; Pratap, P.; Wroblewski, M.; Liu, L.; Scheff, P.A.; Li, A. (2011). Water ingestion during water recreation. *Water Research 45*: 2020-2028.

Dorevitch, S.; Pratap, P.; Wroblewski, M.; Hryhorczuk, D.O.; Li, H.; Liu, L.C.; Scheff, P.A. (2012). Health risks of limited-contact water recreation. *Environmental Health Perspectives 120*: 192-197.

Dorevitch, S.; DeFlorio-Barker, S.; Jones, R.M.; Liu, L. (2015). Water quality as a predictor of gastrointestinal illness following incidental contact water recreation. *Water Research 83*: 94-103.

Dorevitch, S.; Shrestha, A.; DeFlorio-Barker, S.; Breitenbach, C.; Heimler, I. (2017). Monitoring urban beaches with qPCR vs. culture measures of fecal indicator bacteria: Implications for public notification. *Environmental Health 16*: 45.

Dufour, A.P.; Wade, T.J.; Kay, D. (2012). Epidemiological studies on swimmer health effects associated with potential exposure to zoonotic pathogens in bathing beach water - a review In: *Animal waste, water quality and human health, Chapter 11*. A. Dufour, J. Bartram, R. Bos; V. Gannon, ed. IWA Publishing, London. Vol. Chapter 11, pp.

Dufour, A.P.; Behymer, T.D.; Cantu, R.; Magnusin, M.; Wymer, L.J. (2017). Ingestion of swimming pool water by recreational swimmers. *Journal of Water and Health in press*:

D'ugo, E.; Marcheggiani, S.; Fioramonti, I.; Giuseppetti, R.; Spurio, R.; Helmi, K.; Guillebault, D.; Medlin, L.K.; Simeonovski, I.; Boots, B.; Breitenbach, U.; Koker, L.; Albay, M.; Mancini, L. (2016). Detection of human enteric viruses in freshwater from european countries. *Food and Environmental Virology 8*: 206-214.

Duizer, E.; Rutjes, S.; Husman, A.M.D.; Schijven, J. (2016). Risk assessment, risk management and risk-based monitoring following a reported accidental release of poliovirus in Belgium, September to November 2014. *Eurosurveillance 21*: 16-26.

Edwards, G.; Bogdanow, L.; Rolfs, R.T.; Wrathall, J.; Collier, S.A.; Hlavsa, M.C.; Beach, M.J.; Prue, C.E.; Hough, C.O.; Shevach, A.L.; Calanan, R.M. (2012). Promotion of healthy swimming after a statewide outbreak of cryptosporidiosis associated with recreational water venues - utah, 2008-2009. *American Journal of Transplantation 12*: 2247-2251.

Ehsan, M.A.; Casaert, S.; Levecke, B.; Van Rooy, L.; Pelicaen, J.; Smis, A.; De Backer, J.; Vervaeke, B.; De Smedt, S.; Schoonbaert, F.; Lammens, S.; Warmoes, T.; Geurden, T.; Claerebout, E. (2015). *Cryptosporidium* and *Giardia* in recreational water in Belgium. *Journal of Water and Health 13*: 870-878.

Eregno, F.E.; Tryland, I.; Tjomsland, T.; Myrmel, M.; Robertson, L.; Heistad, A. (2016). Quantitative Microbial Risk Assessment combined with hydrodynamic modelling to estimate the public health risk associated with bathing after rainfall events. *Science of the Total Environment 548*: 270-279.

Ferley, J.P.; Zmirou, D.; Balducci, F.; Baleux, B.; Fera, P.; Larbaigt, G.; Jacq, E.; Moissonnier, B.; Blineau, A.; Boudot, J. (1989). Epidemiological significance of microbiological pollution criteria for river recreational waters. *International Journal of Epidemiology 18*: 198-205.

Fewtrell, L.; Kay, D. (2015). Recreational water and infection: A review of recent findings. *Curr Environ Health Rep 2*: 85-94.

Francy, D.S.; Stelzer, E.A.; Duris, J.W.; Brady, A.M.G.; Harrison, J.H.; Johnson, H.E.; Ware, M.W. (2013). Predictive models for *Escherichia coli* concentrations at inland lake beaches and relationship of model variables to pathogen detection. *Applied and Environmental Microbiology 79*: 1676-1688.

Galfi, H.; Osterlund, H.; Marsalek, J.; Viklander, M. (2016). Indicator bacteria and associated water quality constituents in stormwater and snowmelt from four urban catchments. *Journal of Hydrology 539*: 125-140.

Goodwin, K.D.; Mcnay, M.; Cao, Y.P.; Ebentier, D.; Madison, M.; Griffith, J.F. (2012). A multi-beach study of *Staphylococcus aureus*, MRSA, and enterococci in seawater and beach sand. *Water Research 46*: 4195-4207.

Gorham, T.J.; Lee, J. (2016). Pathogen loading from Canada geese faeces in freshwater: Potential risks to human health through recreational water exposure. *Zoonoses and Public Health 63*: 177-190.

Griffith, J.F.; Weisberg, S.B.; Arnold, B.F.; Cao, Y.P.; Schiff, K.C.; Colford, J.M. (2016). Epidemiologic evaluation of multiple alternate microbial water quality monitoring indicators at three California beaches. *Water Research 94*: 371-381.

Hall, V.; Taye, A.; Walsh, B.; Maguire, H.; Dave, J.; Wright, A.; Anderson, C.; Crook, P. (2017). A large outbreak of gastrointestinal illness at an open-water swimming event in the River Thames, London. *Epidemiology and Infection 145*: 1246-1255.

Hamilton, M.J.; Hadi, A.Z.; Griffith, J.F.; Ishii, S.; Sadowsky, M.J. (2010). Large scale analysis of virulence genes in *Escherichia coli* strains isolated from Avalon Bay, CA. *Water Research 44*: 5463-5473.

Harder-Lauridsen, N.M.; Kuhn, K.G.; Erichsen, A.C.; Molbak, K.; Ethelberg, S. (2013). Gastrointestinal illness among triathletes swimming in non-polluted versus polluted seawater affected by heavy rainfall, Denmark, 2010-2011. *Plos One 8*: ARTN e78371: 10.1371/journal.pone.0078371.

Harrington, J.F.; Wilcox, D.N.; Giles, P.S.; Ashbolt, N.J.; Evans, J.C.; Kirton, H.C. (1993). The health of Sydney surfers - an epidemiologic-study. *Water Science and Technology 27*: 175-181.

Helmi, K.; Skraber, S.; Burnet, J.B.; Leblanc, L.; Hoffmann, L.; Cauchie, H.M. (2011). Two-year monitoring of *Cryptosporidium parvum* and *Giardia lamblia* occurrence in a recreational and drinking water reservoir using standard microscopic and molecular biology techniques. *Environmental Monitoring and Assessment 179*: 163-175.

Hlavsa, M.C.; Roberts, V.A.; Anderson, A.R.; Hill, V.R.; Kahler, A.M.; Orr, M.; Garrison, L.E.; Hicks, L.A.; Newton, A.; Hilborn, E.D.; Wade, T.J.; Beach, M.J.; Yoder, J.S.; Cdc (2011). Surveillance for waterborne disease outbreaks and other health events associated with recreational water - United States, 2007-2008. *MWWR (Morbidity and Mortality Weekly Report) Surveillance Summaries: Centres for Disease Control and Prevention 60*: 1-32.

Hokajarvi, A.M.; Pitkanen, T.; Siljanen, H.M.P.; Nakari, U.M.; Torvinen, E.; Siitonen, A.; Miettinen, I.T. (2013). Occurrence of thermotolerant *Campylobacter* spp. and Adenoviruses in Finnish bathing waters and purified sewage effluents. *Journal of Water and Health 11*: 120-134.

Kent, A.D.; Bayne, Z.L. (2010). Degraded water quality influences microbial community composition and perception of health risks in the Chattooga river. *DNA and Cell Biology 29*: 509-517.

Kirs, M.; Caffaro, R.A.; Wong, M.; Harwood, V.J.; Moravcik, P.; Fujioka, R.S. (2016). Human-associated *Bacteroides* spp. and human polyomaviruses as microbial source tracking markers in Hawaii. *Applied and Environmental Microbiology 82*: 6757-6767.

Kundu, A.; McBride, G.; Wuertz, S. (2013). Adenovirus-associated health risks for recreational activities in a multi-use coastal watershed based on site-specific quantitative microbial risk assessment. *Water Research 47*: 6309-6325.

Lamparelli, C.C.; Pogreba-Brown, K.; Verhougstraete, M.; Sato, M.I.Z.; Bruni, A.D.; Wade, T.J.; Eisenberg, J.N.S. (2015). Are fecal indicator bacteria appropriate measures of recreational water risks in the tropics: A cohort study of beach goers in Brazil? *Water Research 87*: 59-68.

Lee, C.S.; Lee, C.; Marion, J.; Wang, Q.H.; Saif, L.; Lee, J. (2014). Occurrence of human enteric viruses at freshwater beaches during swimming season and its link to water inflow. *Science of the Total Environment 472*: 757-766.

Leonard, A.F.C.; Zhang, L.H.; Balfour, A.; Garside, R.; Gaze, W.H. (2015). Human recreational exposure to antibiotic resistant bacteria in coastal bathing waters. *Environment International 82*: 92-100.

Lim, K.Y.; Shao, S.; Peng, J.; Grant, S.B.; Jiang, S.C. (2017). Evaluation of the dry and wet weather recreational health risks in a semi-enclosed marine embayment in Southern California. *Water Research 111*: 318-329.

Loge, F.J.; Lambertini, E.; Borchardt, M.A.; Basagaoglu, H.; Ginn, T.R. (2009). Effects of etiological agent and bather shedding of pathogens on interpretation of epidemiological data used to establish recreational water quality standards. *Risk Analysis 29*: 257-266.

Magill-Collins, A.; Gaither, M.; Gerba, C.P.; Kitajima, M.; Iker, B.C.; Stoehr, J.D. (2015). Norovirus outbreaks among Colorado river rafters in the Grand Canyon, summer 2012. *Wilderness & Environmental Medicine 26*: 312-318.

Mallin, M.A.; Haltom, M.I.; Song, B.; Tavares, M.E.; Dellies, S.P. (2010). Bacterial source tracking guides management of boat head waste in a coastal resort area. *Journal of Environmental Management 91*: 2748-2753.

Mannocci, A.; La Torre, G.; Spagnoli, A.; Solimini, A.G.; Palazzo, C.; De Giusti, M. (2016). Is swimming in recreational water associated with the occurrence of respiratory illness? A systematic review and meta-analysis. *Journal of Water and Health 14*: 590-599.

Marion, J.W.; Lee, J.; Lemeshow, S.; Buckley, T.J. (2010). Association of gastrointestinal illness and recreational water exposure at an inland U.S. beach. *Water Research 44*: 4796-4804.

Marion, J.W.; Lee, C.; Lee, C.S.; Wang, Q.H.; Lemeshow, S.; Buckley, T.J.; Saif, L.J.; Lee, J. (2014). Integrating bacterial and viral water quality assessment to predict swimming-associated illness at a freshwater beach: A cohort study. *Plos One: ARTN e112029 10.1371/journal.pone.0112029 9*:

McBride, G.B.; Stott, R.; Miller, W.; Bambic, D.; Wuertz, S. (2013). Discharge-based QMRA for estimation of public health risks from exposure to stormwater-borne pathogens in recreational waters in the United States. *Water Research 47*: 5282-5297.

McBride, G.; Cole, R.G.; Westbrooke, I.; Jowett, I. (2014). Assessing environmentally significant effects: a better strength-of-evidence than a single P value? *Environmental Monitoring and Assessment 186*: 2729-2740.

Mika, K.B.; Ginsburg, D.W.; Lee, C.M.; Thulsiraj, V.; Jay, J.A. (2014). Fecal indicator bacteria levels do not correspond with incidence of human-associated HF183 *bacteroides* 16sRMA genetic marker in two urban Southern California watersheds. *Water Air and Soil Pollution 225*: doi: 10.1007/s11270-014-1960-7.

Ming, H.X.; Zhu, L.; Feng, J.F.; Yang, G.; Fan, J.F. (2014). Risk assessment of Rotavirus infection in surface seawater from Bohai bay, China. *Human and Ecological Risk Assessment 20*: 929-940.

Nevers, M.B.; Whitman, R.L. (2011). Beach monitoring criteria: Reading the fine print. *Environmental Science & Technology 45*: 10315-10321.

Nnane, D.E.; Ebdon, J.E.; Taylor, H.D. (2011). Integrated analysis of water quality parameters for cost-effective fecal pollution management in river catchments. *Water Research 45*: 2235-2246.

Papastergiou, P.; Mouchtouri, V.A.; Rachiotis, G.; Pinaka, O.; Katsiaflaka, A.; Hadjichristodoulou, C. (2011). Bather density as a predominant factor for health effects related to recreational bathing: Results from the Greek bathers cohort study. *Marine Pollution Bulletin 62*: 590-595.

Pintar, K.D.; Fazil, A.; Pollari, F.; Charron, D.F.; Waltner-Toews, D.; McEwen, S.A. (2010). A risk assessment model to evaluate the role of fecal contamination in recreational water on the incidence of cryptosporidiosis at the community level in Ontario. *Risk Anal 30*: 49-64.

Pintar, K.D.M.; Thomas, K.M.; Christidis, T.; Otten, A.; Nesbitt, A.; Marshall, B.; Pollari, F.; Hurst, M.; Ravel, A. (2017). A comparative exposure assessment of *Campylobacter* in Ontario, Canada. *Risk Anal 37*: 677-715.

Reddy, V.M.; Abdelrahman, T.; Lau, A.; Flanagan, P.M. (2011). Surfers' awareness of the preventability of 'surfer's ear' and use of water precautions. *Journal of Laryngology and Otology 125*: 551-553.

Rijal, G.; Tolson, J.K.; Petropoulou, C.; Granato, T.C.; Glymph, A.; Gerba, C.; Deflaun, M.F.; O'Connor, C.; Kollias, L.; Lanyon, R. (2011). Microbial risk assessment for recreational use of the Chicago Area Waterway System. *Journal of Water and Health 9*: 169-186.

Sales-Ortells, H.; Medema, G. (2014). Screening-level microbial risk assessment of urban water locations: A tool for prioritization. *Environmental Science & Technology 48*: 9780-9789.

Sanborn, M.; Takaro, T. (2013). Recreational water-related illness office management and prevention. *Canadian Family Physician 59*: 491-495.

Sanchez-Nazario, E.E.; Santiago-Rodriguez, T.M.; Toranzos, G.A. (2014). Prospective epidemiological pilot study on the morbidity of bathers exposed to tropical recreational waters and sand. *Journal of Water and Health 12*: 220-229.

Schets, F.M.; Husman, A.M.D.; Havelaar, A.H. (2011a). Disease outbreaks associated with untreated recreational water use. *Epidemiology and Infection 139*: 1114-1125.

Schets, F.M.; Schijven, J.F.; Husman, A.M.D. (2011b). Exposure assessment for swimmers in bathing waters and swimming pools. *Water Research 45*: 2392-2400.

Schijven, J.; Derx, J.; Husman, A.M.D.; Blaschke, A.P.; Farnleitner, A.H. (2015). QRMAcatch: Microbial quality simulation of water resources including infection risk assessment. *Journal of Environmental Quality 44*: 1491-1502.

Schoen, M.E.; Ashbolt, N.J. (2010). Assessing pathogen risk to swimmers at non-sewage impacted recreational beaches. *Environmental Science & Technology 44*: 2286-2291.

Schoen, M.E.; Soller, J.A.; Ashbolt, N.J. (2011). Evaluating the importance of fecal sources in human-impacted waters. *Water Research 45*: 2670-2680.

Seto, E.Y.; Konnan, J.; Olivieri, A.W.; Danielson, R.E.; Gray, D.M.D. (2016). A quantitative microbial risk assessment of wastewater treatment plant blending: case study in San Francisco Bay. *Environmental Science-Water Research & Technology 2*: 134-145.

Sidhu, J.P.S.; Hodgers, L.; Ahmed, W.; Chong, M.N.; Toze, S. (2012). Prevalence of human pathogens and indicators in stormwater runoff in Brisbane, Australia. *Water Research 46*: 6652-6660.

Sinigalliano, C.D., *et al.* (2010). Traditional and molecular analyses for fecal indicator bacteria in non-point source subtropical recreational marine waters. *Water Research 44*: 3763-3772.

Soldanova, M.; Selbach, C.; Kalbe, M.; Kostadinova, A.; Sures, B. (2013). Swimmer's itch: etiology, impact, and risk factors in Europe. *Trends in Parasitology 29*: 65-74.

Soller, J.A.; Bartrand, T.; Ashbolt, N.J.; Ravenscroft, J.; Wade, T.J. (2010a). Estimating the primary etiologic agents in recreational freshwaters impacted by human sources of fecal contamination. *Water Research 44*: 4736-4747.

Soller, J.A.; Schoen, M.E.; Bartrand, T.; Ravenscroft, J.E.; Ashbolt, N.J. (2010b). Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of fecal contamination. *Water Research 44*: 4674-4691.

Soller, J.A.; Schoen, M.E.; Varghese, A.; Ichida, A.M.; Boehm, A.B.; Eftim, S.; Ashbolt, N.J.; Ravenscroft, J.E. (2014). Human health risk implications of multiple sources of fecal indicator bacteria in a recreational waterbody. *Water Research 66*: 254-264.

Soller, J.; Bartrand, T.; Ravenscroft, J.; Molina, M.; Whelan, G.; Schoen, M.; Ashbolt, N. (2015). Estimated human health risks from recreational exposures to stormwater runoff containing animal fecal material. *Environmental Modelling & Software 72*: 21-32.

Soller, J.; Eftim, S.; Wade, T.; Ichida, A.M.; Clancy, J.L.; Johnson, T.B.; Schwab, K.; Ramirez-Toro, G.; Nappier, S.; Bartrand, T.; Ravenscroft, J.E. (2016). Use of quantitative microbial risk assessment to improve interpreatation of a recreational water epidemiological study. *Microbial Risk Analysis 1*: 2-11.

Soller, J.A.; Schoen, M.E.; Steele, J.A.; Griffith, J.F.; Schiff, K.C. (2017). Incidence of gastrointestinal illness following wet weather recreational exposures: Harmonization of quantitative microbial risk assessment with an epidemiologic investigation of surfers. *Water Research in press*:

Sunger, N.; Haas, C.N. (2015). Quantitative microbial risk assessment for recreational exposure to water bodies in Philadelphia. *Water Environment Research 87*: 211-222.

Suppes, L.M.; Abrell, L.; Dufour, A.P.; Reynolds, K.A. (2014). Assessment of swimmer behaviors on pool water ingestion. *Journal of Water and Health 12*: 269-279.

Tong, H.I.; Connell, C.; Boehm, A.B.; Lu, Y.N. (2011). Effective detection of human Noroviruses in Hawaiian waters using enhanced RT-PCR methods. *Water Research 45*: 5837-5848.

Tseng, L.Y.; Jiang, S.C. (2012). Comparison of recreational health risks associated with surfing and swimming in dry weather and post-storm conditions at Southern California beaches using Quantitative Microbial Risk Assessment (QMRA). *Marine Pollution Bulletin 64*: 912-918.

Viau, E.J.; Lee, D.; Boehm, A.B. (2011). Swimmer risk of gastrointestinal illness from exposure to tropical coastal waters impacted by terrestrial dry-weather runoff. *Environmental Science & Technology 45*: 7158-7165.

Vijayavel, K.; Fujioka, R.; Ebdon, J.; Taylor, H. (2010). Isolation and characterization of *Bacteroides* host strain HB-73 used to detect sewage specific phages in Hawaii. *Water Research 44*: 3714-3724.

Wade, T.J.; Calderon, R.L.; Sams, E.; Beach, M.; Brenner, K.P.; Williams, A.H.; Dufour, A.P. (2006). Rapidly measured indicators of recreational water quality are predictive of swimming-associated gastrointestional illness. *Environmental Health Perspectives 114*: 24-28.

Wade, T.J.; Calderon, R.; Brenner, C.; Sams, E.; Beach, M.; Brenner, K.P.; Haugland, R.; Wymer, L.; Dufour, A.P. (2008). High sensitivity of children to swimming-associated gastrointestinal illness response. *Epidemiology 19*: 375-383.

Wade, T.J.; Sams, E.; Brenner, K.P.; Haugland, R.; Chern, E.; Beach, M.; Wymer, L.; Rankin, C.C.; Love, D.; Li, Q.L.; Noble, R.; Dufour, A.P. (2010). Rapidly measured indicators of recreational water quality and swimming-associated illness at marine beaches: a prospective cohort study. *Environmental Health 9*: doi: 10.1186/1476-069X-9-66.

Wade, T.J.; Converse, R.R.; Sams, E.A.; Williams, A.H.; Hudgens, E.; Dufour, A.P. (2013a). Gastrointestinal symptoms among swimmers following rain events at a beach impacted by urban runoff. *American Journal of Epidemiology 177*: S157-S157.

Wade, T.J.; Sams, E.A.; Beach, M.J.; Collier, S.A.; Dufour, A.P. (2013b). The incidence and health burden of earaches attributable to recreational swimming in natural waters: a prospective cohort study. *Environmental Health 12*: Artn 67: 10.1186/1476-069x-12-67.

Wyer, M.D.; Wyn-Jones, A.P.; Kay, D.; Au-Yeung, H.K.C.; Girones, R.; Lopez-Pila, J.; Husman, A.M.D.; Rutjes, S.; Schneider, O. (2012). Relationships between human Adenoviruses and fecal indicator organisms in European recreational waters. *Water Research 46*: 4130-4141.

Wymer, L.J.; Wade, T.J.; Dufour, A.P. (2013). Equivalency of risk for a modified health endpoint: a case from recreational water epidemiology studies. *Bmc Public Health 13*: Artn 459 doi: 10.1186/1471-2458-13-459

Xiao, G.S.; Wang, Z.D.; Chen, J.; Qiu, Z.Q.; Li, Y.J.; Qi, J.S.; Liu, W.Y.; Shu, W.Q. (2013). Occurrence and infection risk of waterborne pathogens in Wanzhou watershed of the Three Gorges Reservoir, China. *Journal of Environmental Sciences 25*: 1913-1924.

Yau, V.M.; Schiff, K.C.; Arnold, B.F.; Griffith, J.F.; Gruber, J.S.; Wright, C.C.; Wade, T.J.; Burns, S.; Hayes, J.M.; McGee, C.; Gold, M.; Cao, Y.P.; Boehm, A.B.; Weisberg, S.B.; Colford, J.M. (2014). Effect of submarine groundwater discharge on bacterial indicators and swimmer health at Avalon Beach, CA, USA. *Water Research 59*: 23-36.

Young, N. (2016). The association between marine bathing and infectious diseases - a review. *Journal of the Marine Biological Association of the United Kingdom 96*: 93-100.

Zeigler, M.; Claar, C.; Rice, D.; Davis, J.; Frazier, T.; Turner, A.; Kelley, C.; Capps, J.; Kent, A.; Hubbard, V.; Ritenour, C.; Tuscano, C.; Qiu-Shultz, Z.; Leaumont, C.F.; Centers for Disease, C.; Prevention (2014). Outbreak of campylobacteriosis associated with a long-distance obstacle adventure race--Nevada, October 2012. *MWWR (Morbidity and Mortality Weekly Report) Surveillance Summaries: Centres for Disease Control and Prevention 63*: 375-378.

Zlot, A.; Simckes, M.; Vines, J.; Reynolds, L.; Sullivan, A.; Scott, M.K.; McLuckie, J.M.; Kromer, D.; Hill, V.R.; Yoder, J.S.; Hlavsa, M.C. (2015). Norovirus outbreak associated with a natural lake used for recreation - Oregon, 2014. *MWWR (Morbidity and Mortality Weekly Report) Surveillance Summaries: Centres for Disease Control and Prevention 64*: 485-490.