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Applicability of Great Lakes NEEAR Dataset to Inland Recreational Water Criteria: Summary of Key Studies

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Abbreviations and Acronyms

AWQC	ambient water quality criteria
BMP	best management practice
BEACH Act	Beaches Environmental Assessment and Coastal Health Act
CFU	colony forming unit
CPSP	Critical Path Science Plan
CSO	combined sewer overflow
CWA	Clean Water Act
DNA	deoxyribonucleic acid
EPA	U.S. Environmental Protection Agency
EC	European Commission
FIB	fecal indicator bacteria
GI	gastrointestinal
MF	membrane filtration
MPN	most probable number
MST	microbial source tracking
NEEAR	National Epidemiological and Environmental Assessment of Recreational Water Study
NPDES	National Pollutant Discharge Elimination System
PC	prospective cohort
PCR	polymerase chain reaction
POTW	publicly-owned treatment works
QMRA	quantitative microbial risk assessment
qPCR	quantitative polymerase chain reaction
RCT	randomized control trial
TMDL	total maximum daily load(s)
UV	ultraviolet (light)
VBNC	viable but not culturable
WERF	Water Environment Research Foundation
WHO	World Health Organization
WWTP	wastewater treatment plant

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Executive Summary

This report evaluates the applicability of NEEAR Great Lakes data to inland waters and assesses the similarities and differences between coastal freshwaters and inland freshwaters to establish if there are significant differences to justify additional studies to support applicability of criteria to inland waters. Thirteen reports and peer-reviewed key publications pertaining to the establishment of new or revised recreational water quality criteria appropriate to U.S. inland waters were reviewed in this *Inland Waters Summary Report*. Focused observations and conclusions were extracted and organized into several finding categories in the table below. The reports and articles provide (1) overall assessments on whether criteria developed on the basis of studies of coastal waters (marine/estuarine and Great Lakes waters) are applicable to inland waters; (2) findings concerning differences in the microbial ecology, fate, and transport of indicators in inland and coastal waters; and (3) discussions of likely differences in implementing new or revised criteria to inland and coastal waters, including indicator detection and monitoring schemes. Current ongoing and future research EPA is pursuing and planning is also addressed in individual report sections, along with the suggestions provided by each report’s authors. Major findings as reported in the reports for each of the categories are summarized below (not in order of importance). Note that the table presents the major findings as proposed by the study authors. The findings differ in the degree to which they are supported either through data or citations of other studies. In some cases, the findings might reflect a conclusion drawn by the study authors based on best scientific judgment when data was insufficient to offer a complete scientific assessment.

Finding category	Conclusions and observations
Overall assessments pertaining to the extension of coastal water criteria to inland waters	Fecal pollution source is the main driver of health risk at inland and coastal sites, before specific setting (e.g. physical and biological processes). This observation is consistent with findings from quantitative microbial risk assessment (QMRA) studies and the limited number of epidemiology studies conducted in both inland and coastal settings and for sites with different fecal pollution sources.
	Application of coastal water-based criteria to inland recreational waters is expected to result in sporadic, mild illness at rates no higher and probably lower than those experienced in Great Lakes/coastal waters.
	Inland and Great Lakes/coastal waters might pose very different risks of severe diseases.
	Data and health effects relationships developed for Great Lakes waters that are primarily affected by publicly-owned treatment works (POTW) effluent are generally believed to be applicable to inland waters primarily affected by POTW effluent.
	Different studies interpret the state of the science differently from each other. All the studies directly assessing the extension of criteria on the basis of epidemiology studies of POTW-impacted Great Lakes waters to inland waters note that there are no definitive epidemiology studies to support or preclude making the extension.

Finding category	Conclusions and observations
	<p>Experts participating in the 2009 Water Environment Research Foundation (WERF) inland waters workshop speculate that livestock and wildlife fecal pollution sources affect a greater proportion of inland waters than coastal waters. However, inland waters comprise a diverse set and individual sites might be affected by POTW, diffuse human pollution, livestock fecal pollution, or fecal pollution from wildlife.</p>
<p>Differences in occurrences, fate, and transport of indicators in inland and coastal waters</p>	<p>Fecal pollution sources discharge directly to inland waters, and fecal pollution undergoes less dilution in inland waters than in Great Lakes/coastal waters. Understanding that runoff and streamflow are highly variable, the result of less dilution capacity in inland waters results in higher densities of pathogens in inland waters under some flow conditions and higher risk of disease from exposure to inland waters.</p> <p>Reported ranges of indicator densities in inland and Great Lakes/coastal waters are comparable. While relatively few data are available for systematic comparison of response of coastal and inland sites to rain events, it is likely that indicator variability within storm events is greater for inland waters than for coastal waters.</p> <p>Indicator decay rates in Great Lakes and inland waters fall within comparable ranges.</p> <p><i>Escherichia coli</i> growth has been observed in water columns and sediments of both inland and coastal waters. <i>Enterococcus</i> growth has also been observed in soils and sediments, though fewer studies have assessed <i>Enterococcus</i> potential, and differences in growth in inland and coastal settings cannot be assessed. Growth might be more likely in sediments and soils of inland waters than in those of coastal waters.</p> <p>Resuspension mechanics of sediment and soil-associated indicators and pathogens differ for coastal and inland waters. Resuspension might be more important in inland waters because turbulent shear at the sediment-water interface results in large loads of suspended organisms and particles and because dilution is lower than that of resuspended indicator organisms at coastal sites.</p> <p>Quantitative polymerase chain reaction (qPCR) and cultural enumerations of indicator organisms tend to be better correlated for fresh fecal material and very poorly correlated for aged fecal pollution, indicators that have been subjected to sunlight, and chlorinated waters.</p>
<p>Findings related to implementation of criteria</p>	<p>Inland waters are more widely dispersed geographically and cover more territory overall (as stream miles) than do coastal waters (as coastal miles). Inland waters typically have use patterns different from developed coastal beaches. Monitoring for such inland waters likely entails assessing water quality on the basis of fewer samples.</p> <p>For inland waters, the ratio of qPCR counts of indicators to culture counts of indicators likely differs from that typical of coastal sites because of differences in age of fecal pollution, presence and concentration of chlorinated secondary effluent, and exposure of fecal pollution to solar radiation.</p> <p>Standardized sanitary survey tools should be developed for both inland and coastal waters. Completion of sanitary surveys should be an integral component in development of monitoring schemes.</p>

1. Introduction

1.1. Objective

New or revised ambient water quality criteria (AWQC) for the protection of primary contact recreation are likely to be based on health effects observed in epidemiology studies of coastal waters. A decision to extend or not extend those criteria to inland waters must be supported by analysis of the science of inland and coastal waters as well as analysis of practical concerns, such as likely differences in use and sampling of inland and coastal waters. Recognizing those needs, the U.S. Environmental Protection Agency's (EPA's) *Critical Path Science Plan for the Development of New or Revised Criteria for Recreational Waters* (the CPSP; USEPA 2007) proposed studies to develop the information necessary to inform extrapolation of AWQC.

This report summarizes the knowledge that EPA collected and produced in response to the CPSP based on a review of thirteen reports and key research articles. Each report and article is summarized, and the key concepts, results, or conclusions are highlighted. Three reports and five publications were pooled to condense all aspects of monitoring methodology relevant to this report's subject matter. Findings from these reports will be used to develop analyses that will support a decision of whether and how AWQC can be extended to inland waters on the basis of epidemiology studies conducted as part of EPA's National Epidemiological and Environmental Assessment of Recreational (NEEAR) Water Study, which include the Great Lakes epidemiology studies.

Stakeholder and scientific community concerns

In stakeholder meetings and in the scientific literature, concerns have been expressed regarding both the scientific and practical considerations of extending AWQC developed for coastal waters to inland waters. Concerns about the science of extending AWQC generally relate to perceptions or findings that indicator organisms perform differently in inland waters than in coastal waters. In this report, *performance* is taken as (1) the relationship between indicator density and the observed rates of illness in a particular type of water and (2) the fate and transport of indicators relative to pathogens and loads from specific fecal pollution sources. Practical concerns relate to the development of monitoring schemes and interpretation of sample results for inland waters. Paramount among those concerns is the interpretation for all waters of indicator densities from samples collected during and immediately after precipitation (especially rain) events (NRC 2004).

To develop context for this report, PowerPoint presentations from stakeholder meetings and correspondence between EPA and stakeholders were reviewed. Questions drawn from those communications are presented below. The questions provide a focus for reviewing the documents presented in this study. Some of these questions do not relate to differences between inland and coastal waters *per se*.

Questions related to the science of indicators

- How does the persistence of fecal indicator organisms in typical inland soils and sediments differ from that in coastal soils and sediments?
- How does the fecal pollution source affect the performance of indicators in inland and coastal waters? What are alternative criteria appropriate for fecal pollution sources other than publicly owned treatment works (POTW) effluent?

- Does indicator performance differ in flowing waters, impounded inland and coastal waters?
- Does climate (i.e., tropical, temperate, and subtropical) affect indicator performance?
- If *E. coli* is a better indicator of fecal pollution in freshwaters, could *Enterococcus* criteria be equally protective of human health for inland and coastal waters?

Questions related to implementing indicator-based criteria to inland waters

- How do indicator-based AWQC for beaches, where monitoring occurs frequently, apply to inland waters, where recreational use is likely intermittent and where water quality assessments are based on much less frequent sampling?
- What criterion relates to long-term water quality and eliminates undue influence of spikes in fecal pollution and indicator density?
- Can/should criteria be relaxed during extreme-flow events?
- How will culture methods be integrated with new or revised criteria? Is a rapid method needed for inland waters, particularly those with low use?

1.2. Methodology

1.2.1. Documents reviewed

Eight studies were originally proposed for review for this report. Of those, seven were available for review during preparation of the report, and one further study was added. The added study (number 2 below) is a condensed version of the Water Environmental Research Foundation (WERF) experts workshop report (number 1 below). The condensed version of the workshop proceedings was reviewed separately because it has been distributed widely in the scientific community (it is a peer-reviewed publication) and because it makes more definitive statements than the full workshop report. Key documents reviewed for this report were as follows:

1. *Final Report on the Experts Scientific Workshop on Critical Research and Science Needs for the Development of Recreational Water Quality Criteria for Inland Waters* (WERF 2009)
2. *Meeting Report: Knowledge Gaps in Developing Microbial Criteria for Inland Recreational Waters* (Dorevitch et al. 2010)
3. Final report *Literature Review of Assessment of the Applicability of Existing Epidemiology Data to Inland Waters* (USEPA 2010a)
4. Final report *Sampling and Consideration of Variability (Temporal and Spatial) for Monitoring of Recreational Waters* (USEPA 2010b)
5. Interim draft report *Comparison of Different Methodologies for the Enumeration of Fecal Indicator Organisms* (USEPA 2010c)
6. Draft final report *Quantification of Pathogens and Sources of Microbial Indicators for QMRA in Recreational Waters* (WERF 2010a)
7. Final report *Comparative Evaluation of Molecular and Culture Methods for Fecal Indicator Bacteria for use in Inland Recreational Waters* (WERF 2010b)

8. *Results of the Single-Laboratory Validation of EPA Method A for Enterococci and Method B for Bacteroidales in Waters by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay* (USEPA 2010d)

In addition, the following five key articles were selected and reviewed for their direct relevance to the subject matter:

9. “Covariation and Photoinactivation of Traditional and Novel Indicator Organisms and Human Viruses at a Sewage-Impacted Marine Beach” (Boehm et al. 2009)
10. “Persistence of Nucleic Acid Markers of Health-Relevant Organisms in Seawater Microcosms” (Walters et al. 2009)
11. “Discrimination of Viable and Dead Fecal *Bacteroidales* Bacteria by Quantitative PCR with Propidium Monoazide” (Bae and Wuertz 2009)
12. “Linking Non-Culturable (qPCR) and Culturable Enterococci Densities with Hydrometeorological Conditions” (Byappanahalli et al. 2010)
13. “A Cross Comparison of QPCR to Agar-Based or Defined Substrate Methods for Determination of *Escherichia coli* and Enterococci in Municipal Water Quality Monitoring Programs” (Lavender and Kinzelman 2009)

Note that five out of eight reports (#1 to 4 and 6) are discussed individually in this report, while the other reports (#5, 7, and 8), as well as the five published research articles (#9 to 13), are discussed together in Section 2.3 to condense all aspects of methodology performance with respect to applicability of criteria to inland waters.

1.2.2. Topics featured in document review

The stakeholder questions presented in Section 1.1.2 and the physical differences between inland and coastal waters presented in Section 1.3 provide topic areas that were emphasized for review and synthesis from the key reports and articles. Those areas can be divided into topics that inform the performance of indicators and topics related to differences in implementing criteria in inland and coastal waters.

Topics related to the performance of indicators in inland and coastal waters include findings on the intrinsic physical, biological, and hydrologic differences between inland and coastal waters; findings related to the incidence of pathogens or the association of illness in inland and coastal waters; and findings on differences in method performance for inland and coastal waters.

Topics related to implementing criteria relate to differences in the content of sanitary surveys for inland and coastal waters, practical constraints causing inland water monitoring plans to differ from those for coastal waters, and differences in how water quality sample results are interpreted.

Section 1.3 describes the features of inland and coastal waters with the potential to result in differences in the association of indicator levels with health effects. A thorough exploration of the differences in criteria for inland and coastal waters entails review of epidemiology, hydrodynamics, fecal pollution sources, use patterns, and performance of indicator measurement techniques. The reports and research articles reviewed herein were selected according to their coverage of all those subject areas. The specific factors pertinent to extending coastal water criteria to inland waters reviewed in each study are presented in Table 1.

Table 1. Key areas that the reviewed reports and publications used to compare and contrast Great Lakes and inland waters

Report/publication	Inland vs. coastal water factors addressed
<i>Final Report on the Experts Scientific Workshop on Critical Research and Science Needs for the Development of Recreational Water Quality Criteria for Inland Waters</i> (WERF 2009)	Epidemiology studies of inland and coastal sites; differences related to hydrology and resuspension; fecal source differences; differences related to performance of detection techniques; monitoring strategies for inland and coastal sites; data gaps and research prioritization
<i>Meeting Report: Knowledge Gaps in Developing Microbial Criteria for Inland Recreational Waters</i> (Dorevitch et al. 2010)	Epidemiology studies of inland and coastal sites; differences related to hydrology and resuspension; fecal source differences; data gaps and research prioritization
<i>Literature Review of Assessment of the Applicability of Existing Epidemiology Data to Inland Waters</i> (USEPA 2010a)	Epidemiology and watershed-scale studies of inland and coastal sites; differences related to hydrology and resuspension; fate and transport characteristics of indicators in coastal and inland settings
<i>Sampling and Consideration of Variability (Temporal and Spatial) for Monitoring of Recreational Waters</i> (USEPA 2010b)	The hydrology of inland and coastal sites; variability in indicator density at inland and coastal sites; sampling schemes and their association with inland and coastal sites
<i>Comparison of Different Methodologies for the enumeration of Fecal Indicator Organisms</i> (USEPA 2010c)	Differences in performance of enumeration techniques; identification of site features impacting comparison of qPCR and culture counts of fecal indicator organisms
<i>Quantification of Pathogens and Sources of Microbial Indicators for QMRA in Recreational Waters</i> (WERF 2010a)	Performance of enumeration techniques for different water matrices, indicators and settings; use of water quality data in a quantitative microbial risk assessment (QMRA) framework for assessing health risks (in the absence of epidemiology studies)
<i>Comparative Evaluation of Molecular and Culture Methods for Fecal Indicator Bacteria for use in Inland Recreational Waters</i> (WERF 2010b)	Performance of qPCR enumeration of fecal indicators for waters from different settings and for different laboratories; evaluation of the uncertainty of qPCR in enumeration of fecal indicator bacteria
<i>Single Lab Validation Study of Enterococcus qPCR and Bacteroidales qPCR</i> (USEPA 2010d)	Method performance of qPCR in a variety of fresh and marine water settings
"Covariation and Photoinactivation of Traditional and Novel Indicator Organisms and Human Viruses at a Sewage-Impacted Marine Beach" (Boehm et al. 2009)	Impact of photoinactivation on method performance for fecal indicator bacteria (FIB) monitoring
"Persistence of Nucleic Acid Markers of Health-Relevant Organisms in Seawater Microcosms" (Walters et al. 2009)	Impact of seawater quality and environmental factors on FIB DNA persistence in marine settings
"Discrimination of Viable and Dead Fecal Bacteroidales Bacteria by Quantitative PCR with Propidium Monoazide" (Bae and Wuertz 2009)	Comparison of method performance and significance of FIB viability for qPCR and culture-based assays in marine settings
"Linking Non-Culturable (qPCR) and Culturable Enterococci Densities with Hydrometeorological Conditions" (Byappanahalli et al. 2010)	Impact of hydrometeorological factors on method performance for FIB monitoring

Report/publication	Inland vs. coastal water factors addressed
"A Cross Comparison of QPCR to Agar-Based or Defined Substrate Methods for Determination of Escherichia coli and Enterococci in Municipal Water Quality Monitoring Programs" (Lavender and Kinzelman 2009)	Comparison of method performance for qPCR and culture-based assays in surface waters, municipal stormwater and wastewater

1.3. General descriptions of inland flowing, inland impounded, and Great Lakes coastal settings

Inland waters are waterbodies that are not coastal recreational waters as defined by the Clean Water Act (CWA). They are typically freshwater but can include some saltwater (estuarine) waterbodies (e.g., streams with tidal influences). They include flowing (rivers and streams) and impounded (lakes and reservoirs) waterbodies, but not the Great Lakes, which are defined as coastal waters under the Beaches Environmental Assessment and Coastal Health (BEACH) Act of 2000.

This section provides qualitative comparisons of coastal and inland waters. It is intended as background material and is included to help readers understand and interpret findings of the studies reviewed. Two types of comparisons are provided. In Section 1.3.1, the physical, hydrological, and biological differences in coastal and inland sites are described. Such differences influence indicator fate and transport and the association of indicators with specific fecal pollution sources. In Section 1.3.2, the findings of epidemiology studies of inland waters are reviewed. Epidemiology studies provide the best indication of the risks associated with recreation in inland and coastal waters and association of inland sites with specific fecal pollution sources and pathogens.

1.3.1. Qualitative comparison of indicator processes in inland and coastal waters

With the understanding that inland and coastal waters are diverse, generalizations about these waters are provided in this section as an introduction to the findings of the studies reviewed for this report. Intrinsic physical differences between inland and coastal settings include the following:

- the ways in which the sites are loaded with fecal pollution;
- the mechanisms by which indicators are advected into and out of sites;
- hydrograph and indicator organism density responses to rain events;
- the locations and mechanisms important in resuspension of sediment indicators;
- the dilution of fecal pollution loads; and
- average insolation (average incident solar radiation per water surface area).

Those differences are illustrated in Figure 1 and Figure 2 and are described below. Other important differences between recreational sites include the predominant fecal pollution source(s) and the frequency of use for recreation. Those differences are not intrinsic to inland and coastal waters, and no data sources providing means for quantitative assessment of their importance were reviewed for this report.

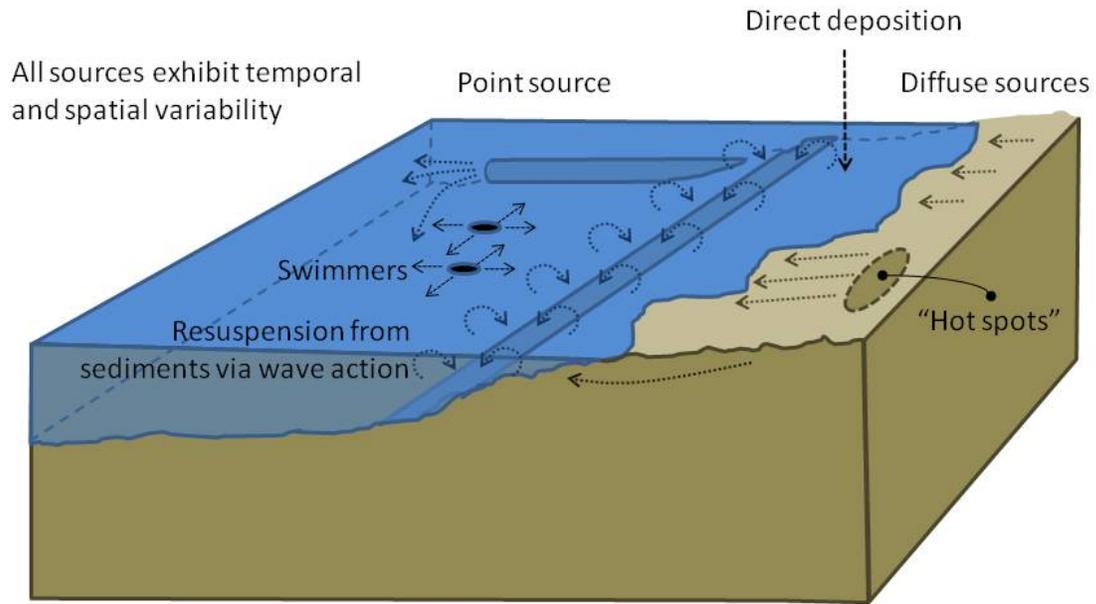


Figure 1. Illustration of fecal indicator organism sources for coastal waters

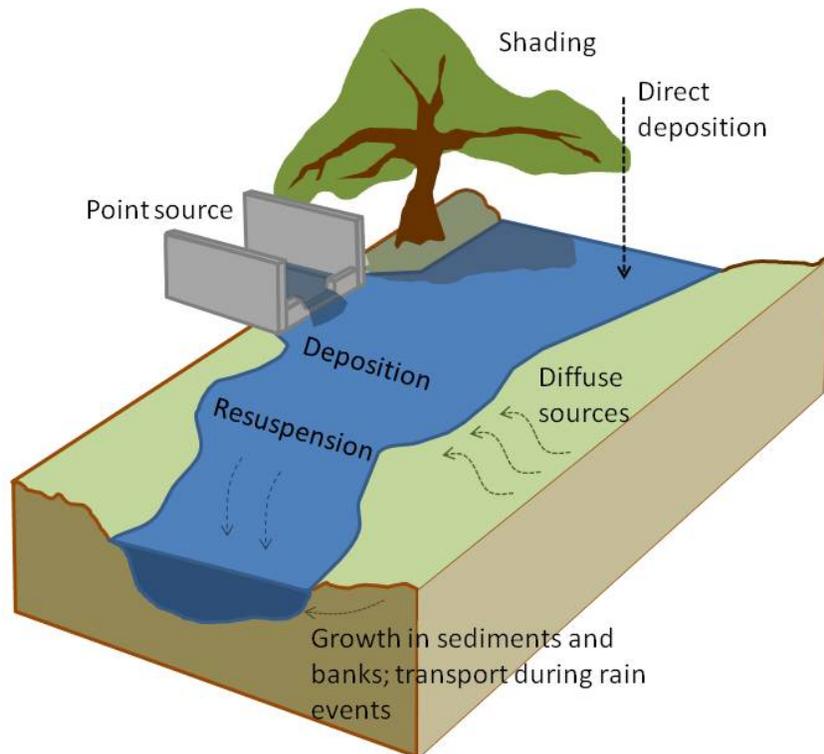


Figure 2. Illustration of fecal indicator organism sources for inland waters

Inland and coastal waters receive fecal indicator bacteria (FIB) from point sources, diffuse sources including nonpoint sources, direct deposition (e.g., gulls or cattle in a stream), and resuspension of FIB in sediments or overbanks. It is likely that the overall predominant sources in inland waters differ from those in coastal waters. However, for a specific stream compared to a specific coastal water, the sources might be the same or very similar. Human fecal pollution and nonhuman fecal pollution are associated with different types of pathogens. Those pathogens, in turn, pose different hazards, and the exposure necessary to observe a given response (e.g., adverse health effect) in the exposed population also differs.

FIB loading and hydrodynamics in POTW-impacted inland and coastal waters are generally similar. POTW discharges to both inland and coastal sites are relatively steady and have FIB densities that are variable but not dependent on whether a site is coastal or inland. Loading from non-POTW sources might differ significantly for inland and coastal sites. Non-POTW fecal pollution is loaded to receiving waters primarily during and immediately following rain events. Because non-POTW sources are usually in closer proximity to inland waters than to coastal waters and because the volumes of inland waters receiving fecal pollution are lower than those of coastal waters, fecal pollution in inland waters is expected to be generally less dilute than that in coastal waters. Because dose-response functions for individual pathogens are not linear, differences in dilution in coastal and inland waters might result in differences in incidence of illness typical of the two sites. Differences in incidence of illness are particularly important for pathogens such as *E. coli* O157:H7, which can result in very serious illness.

Inland and coastal waters differ in typical soils and sediments, the ratio of the sediment area to the water volume, and the mechanisms most responsible for resuspension of sediment-associated indicator organisms. Together, those factors can cause differences in the abundance of resuspended indicator organisms in the two settings. Because resuspended indicator organisms are not associated with a specific fecal pollution source, they are not good indicators of fecal pollution sources and might confound interpretation of microbial water quality from indicator density measurements. None of the studies reviewed for this report sought to associate inland or coastal waters with specific soil and sediment types or to compare and contrast the extent to which growth is likely in either setting, although one report (WERF 2009) asserts that growth is more likely in the soils and sediments of inland waters and at the water-sediment interface. In inland waters, resuspension is by means of turbulent shear at the stream bottom, while in coastal waters, resuspension is primarily due to wave action. The net resuspended indicator load at a given site is a function of both the abundance of sediment-associated indicators and the processes by which free or particle-associated organisms are drawn from the sediments. Although a quantitative comparison of resuspension in inland and coastal waters is not possible, it is likely that resuspension is different in inland and coastal waters because of the different sediments typical of the sites and the very different mechanisms that cause resuspension in the two settings. No information in the documents reviewed indicates whether the differences would affect the relationship between indicator density and adverse health outcomes in exposed persons.

1.3.2. Brief review of epidemiology studies conducted for inland waters

At present, epidemiology studies provide the best means for comparing recreation-associated risks in inland and coastal sites. However, due to the complex and variable processes associated with the transport of fecal pollution and indicators to recreational sites, only a few studies have demonstrated a correlation between indicators and health risks via modeling. Recently published

QMRA studies indicate the potential for connecting indicator densities and health effects via modeling (Schoen and Ashbolt 2010; Soller et al. 2010a, 2010b).

As context for the studies reviewed in this document, a brief review of epidemiology studies conducted at inland sites is presented below. These studies were conducted for different purposes and with different methodologies, and these differences hamper direct comparison of their results. In general, the studies have resulted in two types of data. First, they produce a measure of the increase in odds (or likelihood) of some health endpoint (usually gastrointestinal [GI] illness) for swimmers as compared to non-swimmers. Such an odds ratio does not relate to the water quality or indicator level. Second, some of the studies produce an association of the incidence of health endpoints with the level (density) of indicators to which swimmers were exposed. In some cases, no statistically significant association is observed. That lack of association could relate to a lack of association between the indicator and a specific fecal pollution source or could relate to the epidemiology study design. In a limited number of cases, studies have produced health effects curves predicting the incidence of illness as a function of indicator density.

To date, eight sets of U.S. and international epidemiological studies have been conducted to evaluate the association of swimming in inland surface waters with the incidence of GI illness (Stevenson 1953; Dufour 1984; Seyfried et al. 1985a, 1985b; Ferley et al. 1989; Calderon 1991; Weidenmann et al. 2006; European Commission [EC] 2009a, 2009b [referred to as Epibathe studies]; Marion et al. 2010). They are grouped below as follows:

- investigation of (presumably) POTW-impacted inland sites (Dufour 1984; Weidenmann et al. 2006; EC 2009a, 2009b; Marion et al. 2010);
- investigation of untreated domestic sewage affected inland flowing waters (Ferley et al. 1989)
- investigation of unspecified inland waters¹ with unspecified fecal sources (Seyfried et al., 1985a, 1985b)
- investigation of flowing and impounded inland waters (Dufour 1984; Stevenson et al. 1953; Weidenmann et al. 2006; EC 2009a, 2009b; Marion et al. 2010);
- investigation of avian and wildlife affected sites (Calderon 1991);
- use of randomized control trial study design (Weidenmann et al. 2006; EC 2009a, 2009b);
- use of prospective cohort study design (Calderon 1991; Dufour 1984; Marion et al. 2010; Stevenson et al. 1953; Seyfried et al. 1985a, 1985b); and
- use of retrospective cohort study design (Ferley et al. 1989).

In all of those epidemiology studies, statistically significant differences in the incidence of GI illness (and in some cases other health endpoints such as respiratory illness) were observed between swimmers and non-swimmers. In studies of POTW-impacted waters, three studies identified an association of the increased incidence of GI illness with indicator density (Dufour

¹ Seyfried et al. 1985b describes the locations of the study as being conducted “at various Ontario lakes.” It unclear whether or not this may include Lake Ontario, which would be considered a Great Lake and not an inland water for the purposes of applying recreational criteria.

1984; Seyfried et al. 1985b; Marion et al. 2010), and one study related indicator density to GI illness risk via estimation of a no adverse effect level of indicator organism density (Wiedenmann et al. 2006). No such relationships have been proposed for livestock and wildlife-affected inland waters, although two studies (Seyfried et al. 1985a, 1985b; Wiedenmann et al. 2006) pooled illness rates and indicator densities without regard to fecal pollution source. Calderon (1991) found no association between indicator level and incidence of GI illness in recreational waters with exclusively nonhuman impacts for the indicators *E. coli*, *Enterococcus*, fecal coliforms, and *Staphylococcus*—despite higher incidence of illness among swimmers than non-swimmers.

The Epibathe (EC 2009a, 2009b) study of four beaches on inland waters in Hungary is difficult to compare with the other epidemiology studies because the fecal pollution sources for the study sites are not characterized and they employed a randomized control trial (RCT) study design. At present, results from PC and RCT study designs cannot be used interchangeably, and no techniques currently exist for converting their statistical outputs to allow meaningful quantitative comparisons across study designs.

Based on these studies, *E. coli* (as measured by the culture-based method) seems a better index of GI illness in swimmers using fresh recreational waters than *Enterococcus* (as measured by the culture-based method), which appears to be the best predictor of such symptoms in marine waters (see also reviews by Pruss 1998; Wade et al. 2003; Zmirou et al. 2003).

2. Documents review

This section is comprised of a summary of findings (Tables 2 through 7) and a detailed review of the documents (Section 2.2). Five out of eight reports (#1 to 4 and 6) are discussed individually hereafter (Sections 2.2.1 to 2.2.5). The remaining reports (#5, 7, and 8), as well as the five published research articles (#9 to 13), are discussed together in Section 2.3 to compile aspects of methodology performance with respect to applicability of criteria to inland waters.

2.1. Summary of findings

Table 2. Study purpose, methodology, findings and limitations for the *Final Report on the Experts Scientific Workshop on Critical Research and Science Needs for the Development of Recreational Water Quality Criteria for Inland Waters (WERF 2009)*

Study purpose(s)	<ol style="list-style-type: none"> 1. Determine if or how marine coastal and Great Lakes recreational water research can be extrapolated to apply to inland waters 2. Identify additional research that could aid in the development of water quality criteria applicable to inland waters in both the near and the longer term
Methodology	<p>Findings are drawn from results of an experts workshop. Five major topic areas—indicators and pathogens, health effects, water matrix, sources, and implementation realities—were evaluated by separate teams of experts. Teams were asked to assess the state of the science in their topic area and use that information to assess the applicability of water quality criteria developed using Great Lakes epidemiology studies for inland waters. Experts also identified and prioritized data gaps, short-term, and long-term research needs.</p>
Major findings	<p><i>Indicators and pathogens group findings</i></p> <ul style="list-style-type: none"> • The group found that insufficient evidence exists for direct extrapolation of criteria based on Great Lakes studies for use for inland waters. The group speculated that swimmers in POTW-impacted Great Lakes and inland waters likely face similar risks but that the presence of non-fecal indicator sources at sites could result in differences in the meaning of indicator levels at inland and coastal sites. • Inland waters are generally more diverse, shallower (greater bacterial redistribution), and better suited to tree growth (creating additional shading and protection from sunlight inactivation) than coastal waters. • Soils and physical conditions in inland waters appear more conducive to extra-enteric indicator growth than those of coastal waters. <p><i>Health effects group findings</i></p> <ul style="list-style-type: none"> • The group recognized the imperative that new or revised criteria be developed and the low likelihood that additional epidemiology studies will be conducted in time for use in developing new or revised criteria. Under those circumstances, the group generally supported the position that AWQC derived from Great Lakes studies would likely be protective of public health at inland waters. • Epidemiology studies indicate that fecal source is more important than water type (marine or fresh) or setting (inland vs. coastal) in determining health effects related to swimming.

	<p>Water matrix group findings</p> <ul style="list-style-type: none"> • Inland water conditions that appear to favor indicator occurrence, growth and resuspension from sediments were thought to be the following: (1) higher ratio of sediment-water interface area to water volume for inland waters, (2) finer sediment sizes typical in inland waters, (3) greater presence of wetting and drying areas (per volume of water) at inland sites, and (4) relatively high velocities and higher potential for resuspension at inland water sediment-water interfaces. • Models appear to be the best avenue for exploring the impact of setting on indicator and pathogen occurrence. Models that should be developed for this purpose are regression models, mechanistic (watershed) models, and QMRA. <p>Sources group findings</p> <ul style="list-style-type: none"> • Even within a particular fecal pollution source (human treated, human nonpoint, livestock, companion animal, livestock) the relationship between indicator level and health effects for different fecal pollutions sources differs with the level of treatment of the waste, the proximity of the waste to the receiving water, the prevalence and abundance of pathogens in the fecal pollution, and the persistence of pathogens in the fecal pollution source relative to the persistence of indicators. • Inland waters are believed to be in closer proximity to sources and more influenced by on-site wastewater treatment facilities than coastal waters. <p>Implementation realities group findings</p> <ul style="list-style-type: none"> • Approaches that have been used or could be used to introduce flexibility into implementation of new or revised water quality criteria are the following: <ul style="list-style-type: none"> ○ different criteria for beaches with different use patterns; ○ discounting water quality measurements taken after rain events in concert with implementing risk management strategies for protecting human health; ○ using multiple metrics (sample analysis approaches and statistical measures) for assessing water quality; and ○ using temporary or permanent site-specific criteria. <p>Priority short- and longer-term research recommendations</p> <ul style="list-style-type: none"> • Short-term <ul style="list-style-type: none"> ○ Identify and quantify human pathogens in animal feces ○ Examine relationships between qPCR and culture-based FIB ○ Optimize and anchor QMRA models to observed health effects data obtained from epidemiologic studies ○ Develop QMRA tools for implementation of new AWQC • Longer-term <ul style="list-style-type: none"> ○ Characterize fate and transport of animal pathogens in relation to indicators <p>Conduct epidemiology studies in inland waters</p>
<p>Limitations</p>	<p>In many instances, findings were based on the best estimates or hypotheses of the experts and could not be assessed against actual data because they were asked to use their judgment in the absence of specific data. The major limitation/data gap identified in the WERF report was the scarcity of epidemiology studies of inland waters.</p>

Table 3. Study purpose, methodology, findings and limitations for Meeting Report: Knowledge Gaps in Developing Microbial Criteria for Inland Recreational Waters (Dorevitch et al. 2010)

Study purpose(s)	Summarize the <i>WERF Inland Waters Workshop Results</i> (WERF 2009) in a peer-reviewed, widely distributed publication
Methodology	Principal investigators distilled the experts workshop report into a peer-reviewed publication and added contextual materials
Major findings	<ul style="list-style-type: none"> • The physical and biological processes that are most likely to cause FIB to relate to different risks in inland and coastal waters are the following: <ul style="list-style-type: none"> ○ differences in source types, suites of potential pathogens in the source materials, and severity of the diseases associated with the pathogens typical of inland and coastal waters; ○ closer proximity of inland waters to fecal pollution sources and lower dilution of fecal pollution in inland waters than in coastal waters; ○ differences in the potential for FIB growth in sediments typical of inland and coastal waters; and ○ differences in resuspension rates of sediment-associated indicators and pathogens. • Application of coastal water-based criteria to inland waters is expected to result in sporadic, mild illnesses at rates no higher and possibly lower than those experienced in coastal waters. However, coastal and inland waters might pose very different risks of severe illness. • Near-term research activities that will improve the understanding of indicator performance in inland waters are the following: <ul style="list-style-type: none"> ○ characterizing the spatiotemporal variability of indicator in inland waters by assessing the literature, collecting field data, and/or developing mechanistic models of indicator and pathogen fate and transport; ○ developing and standardizing a sanitary survey tool to use for inland waters; ○ anchoring QMRA using data and relationships from epidemiological studies; ○ developing a database describing the setting-specific relationships between molecular- and culture-based determinations of indicator densities; and ○ evaluating the viability of regression and mechanistic models for predicting indicator density and water quality for inland waters.
Limitations	This study is subject to the same limitations as WERF (2009)

Table 4. Study purpose, methodology, findings and limitations for Literature Review of Assessment of the Applicability of Existing Epidemiology Data to Inland Waters (USEPA 2010a)

Study purpose(s)	To assess and compare the performance of FIB in inland and coastal settings
Methodology	<p>A comprehensive literature survey was conducted to assemble studies reporting the following:</p> <ul style="list-style-type: none"> • epidemiology studies of inland waters; • occurrence of indicators in inland and coastal waters; • persistence of indicators in inland and coastal waters; and • co-occurrence of indicators and pathogens in inland and coastal waters. <p>Reports were reviewed, synthesized, and used in a qualitative comparison of inland and coastal sites</p>
Major findings	<ul style="list-style-type: none"> • The data and relationships developed for Great Lakes waters studies, which are affected primarily by POTW effluents, can be applied to inland waters that are also affected primarily by POTW effluents. • For inland waters that are affected predominantly by sources other than POTW effluent, the available science is not sufficient to support the extension of the relationships developed in the Great Lakes because of potentially important differences in fecal sources and hydrodynamics. • The reported occurrence ranges of FIB for coastal and inland waters are not substantially different; indicator densities are widely variable in both settings. • Reported decay rates for inland and coastal freshwaters are not substantially different. In all cases, predation, insolation, and the presence of sediments are the most important determinants of indicator organism persistence. • Growth of <i>E. coli</i> has been reported in sediments of both inland and coastal waters; insufficient data were obtained to assess the growth potential for <i>Enterococcus</i> in inland and coastal waters. • As anticipated, no consistent correlations between indicator and pathogen densities were observed, irrespective of setting.
Limitations	<p>The study was based on a literature survey and significant data gaps exist, including the following:</p> <ul style="list-style-type: none"> • epidemiology studies of agriculture-affected coastal and inland waters; • quantitative assessments of the relative importance of resuspension in inland and coastal settings; • quantitative assessment of the prevalence of different fecal pollution source types in inland and coastal waters; and • large-scale studies of indicator densities as determined by molecular methods and other alternatives to culture-based membrane filtration.

Table 5. Study purpose, methodology, major findings and limitations for *Sampling and Consideration of Variability (Temporal and Spatial) for Monitoring of Recreational Waters (USEPA 2010b)*

Study purpose(s)	<ul style="list-style-type: none"> • Describe and quantify temporal and spatial variability of FIB density at inland and coastal sites and the implications of variability for the design of sampling plans
Methodology	<ul style="list-style-type: none"> • A comprehensive literature survey was conducted to assemble physical and biological processes at all relevant temporal and spatial scales, and to assess FIB variability for coastal and inland waters.
Major findings	<ul style="list-style-type: none"> • Temporal variability: discrete events (e.g., precipitation events, CSOs) produce the greatest impact <ul style="list-style-type: none"> ○ Other factors affecting temporal variability include the following: diurnal, tidal, seasonal, and short-time-scale variability • Spatial variability: sample depth and along-stream sampling have the greatest impact for coastal and inland sites, respectively. Other factors affecting spatial variability are the following: <ul style="list-style-type: none"> ○ For coastal sites: site features, along shore variations, depth below surface at which samples are collected ○ For inland sites: depth below surface at which samples are collected, and cross-stream variations • Sanitary surveys and pilot monitoring are important components in the development of beach monitoring plans. These activities establish the likely fecal pollution sources and allow estimation of spatial variability in indicator density. • Monitoring considerations <ul style="list-style-type: none"> ○ Pilot monitoring studies and sanitary surveys are the best tools available for collecting data required to develop effective site-specific monitoring plans. ○ Where: area allowing most efficient characterization ○ When: morning samples most conservative; sampling frequency is site-specific, and providing best correlation between qPCR and culture-based results ○ How: approaches for choice of location and number of samples based on site-specific constraints and historical data
Limitations	<ul style="list-style-type: none"> • The study was based on a literature survey and limited to the studies reviewed. • Most of the studies reviewed reported results from studies of limited duration or spatial extent. Extrapolating data from those studies to the diverse set of recreational waters might not be warranted. • Few data are available to allow characterization of variability in qPCR FIB estimates. • No studies assembled as part of the literature survey proposed protocols for discounting or otherwise accounting for elevated indicator organism density counts during rain events; flexibility in accounting for rain events was a stated concern among stakeholders

Table 6. Purpose, methodology, major findings, and limitations of draft final report. *Quantification of Pathogens and Sources of Microbial Indicators for QMRA in Recreational Waters (WERF 2010a)*

Purpose(s)	<ul style="list-style-type: none"> • Identify and address data gaps pertaining to loadings and concentrations of waterborne pathogens and indicators in discharges to recreational waters that are affected by fecal pollution • Compile, analyze and synthesize the data in QMRA models and waterborne risk management frameworks
Methodology	<ul style="list-style-type: none"> • Compilation of existing pathogen data and collection of a new, comprehensive suite of bacteria, protozoa, and virus measurements for a variety of discharges-of-concern to recreational waters across the U.S. These data can serve as inputs for QMRA models. • Collection of additional pathogen and indicator data through field studies and surveys of water and wastewater professionals. • Evaluation of microbial source tracking tools, such as the source identifier bacteria <i>Bacteroidales</i>, for quantitative source apportionment and as a component of QMRA • Critical review and analysis of QMRA as a risk analysis tool, determination of the potential risks associated with measured discharges-of-concern, and consideration of the role of QMRA for implementation of new recreational water quality criteria.
Major findings	<ul style="list-style-type: none"> • Data gaps pertaining to waterborne pathogens and indicators in fecally-impacted discharges to recreational waters were identified and filled by targeted monitoring campaigns in three geographic regions. • QMRA analyses revealed norovirus as the most dominant health risk followed by rotavirus, regardless of setting (inland v. coastal). • Norovirus and <i>Enterococcus</i> both had significant correlations with a number of pathogens in discharges. • Using qPCR data on the fecal source identifier <i>Bacteroidales</i> a new model can predict the true amount of human fecal contamination in a water sample by relating a human-associated genetic marker to a universal assay for fecal sources. The model output can then be used to implement and evaluate management options intended to restore microbial water quality.
Limitations	<ul style="list-style-type: none"> • Inhibition and, for select pathogens, poor or widely varying recoveries must be identified and overcome • Weather conditions prevented some monitoring events • Resource limitations did not allow for flow-weighted composite sampling (instead, grab sampling was used), but for each sample the timing of the sampling with respect to the hydrograph was estimated. • The inability of molecular-based methods to distinguish between dead and viable cells is highlighted as a major limitation for risk assessment.

Table 7. Purpose, methodology, major findings, and limitations of Methodology Performance and Relevance to Applicability of Criteria to Inland Waters (compilation of findings from multiple reports)

<p>Purpose(s)</p>	<ul style="list-style-type: none"> • Describe important factors that influence enumeration method performance • Describe relevant differences in method performance between culture-based and qPCR assays with respect to POTWs, solar inactivation, persistence and other environmental factors • Review findings of studies comparing the relationship between qPCR and culture indicator densities for inland and coastal sites.
<p>Methodology</p>	<ul style="list-style-type: none"> • Review of relevant findings reports #5, 7, and 8, and peer-reviewed publications #9 through 13 (Section 1.2.1)
<p>Major findings</p>	<ul style="list-style-type: none"> • Accounting for bacterial viability and DNA persistence is an important consideration when comparing culture-based methods with qPCR assays. This leads to the following: <ul style="list-style-type: none"> ○ qPCR cell equivalent counts are consistently greater than culture-based CFU inventories due to the comprehensive detection of all DNA by qPCR (commonly, the culturable fraction of cells is relatively marginal with respect to the total count), prompting the design of live-only qPCR assays, such as PMA-qPCR; ○ accounting for different persistence in qPCR and culture targets when evaluating site water quality; and ○ coastal site morning samples analyzed by qPCR and culture-based assays exhibit better correlations than samples collected in the afternoon (less photoinactivation). • qPCR and culture-based results are well correlated at high densities but the correlation is lost at low density due to high uncertainty of the qPCR assay in this range and high variability in relationship between culture and qPCR density at low cell densities. One study reviewed indicated better correlation between qPCR and culture counts in Lake Michigan waters than in other waters including inland lakes and rivers. • Quantitative polymerase chain reaction (qPCR) and cultural enumerations of indicator organisms tend to be better correlated for fresh fecal material and very poorly correlated for aged fecal pollution, indicators that have been subjected to sunlight, and chlorinated waters. • qPCR <i>Enterococcus</i> and qPCR <i>Bacteroidales</i> methods are suitable for multi-lab validation. • Culture-based bacterial densities are strongly reduced (2-5 orders of magnitude) through wastewater treatment processes, especially by secondary treatment and disinfection; qPCR inventories experience smaller reductions or remain unchanged. This is probably due to the strong impact of disinfection on culturable cells. • qPCR results are not greatly affected by insolation while culture-based methods (e.g., membrane filtration [MF]) experience strong reduction by photoinactivation during sunlight exposure. If coastal recreational waters receive greater fluxes per volume of solar radiation (due to higher surface area-to-volume ratio compared with streams), afternoon differences in qPCR and culture counts for coastal waters are expected to be greater than for inland waters. • Inhibition under specific conditions (e.g., high salinity) can potentially affect the qPCR assay and lead to lower counts. This inhibition differs among inland and coastal sites and is not an intrinsic feature of either site type.

<p>Limitations</p>	<ul style="list-style-type: none"> • Data and experience with qPCR are limited; no single epidemiological study reviewed for this report has produced dose-response curves for both molecular- and culture-based methods. • Inhibition of the qPCR reaction likely differs among environmental waters and, at present, is not thoroughly characterized. • qPCR assays vary from one study to another with respect to bacterial targets and primer sets used, highlighting the need for harmonization/standardization for comparison of molecular-based results. • Finite number and variety of sites sampled.
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2.2. Detailed reviews

2.2.1. *Final Report on the Experts Scientific Workshop on Critical Research and Science Needs for the Development of Recreational Water Quality Criteria for Inland Waters (WERF 2009)*

In February 2009, EPA and WERF conducted the Inland Waters Expert Workshop with the following objectives:

1. Determine if or how marine coastal and Great Lakes recreational water research can be extrapolated to apply to inland waters.
2. Identify additional near- and long-term research that could aid in the development of water quality criteria applicable to inland waters.

The workshop was organized around the following five thematic areas:

- indicators and pathogens: biology, ecology, and methods;
- health risks: epidemiology and risk assessment;
- water matrix: hydrology, chemistry, geology, and modeling;
- sources: human vs. nonhuman and point vs. nonpoint; and
- implementation realities.

Workshop participants were assigned to one of the five topic areas, and each topic area group produced a separate section for inclusion in the WERF report summarizing the workshop. Findings in each topic area are described below.

Indicators and pathogens

The indicators and pathogens group explored differences in water quality at inland and coastal sites and based their recommendations on those differences and the differences noted in epidemiology studies. The water quality differences noted by the group are summarized below.

- Coastal and inland water indicator dynamics are different. Coastal indicator dynamics are more stable and less variable than those of inland waters. That is because inland waters are more diverse than coastal waters and have smaller volumes and less dilution potential than coastal waters.
- The banks of inland waters are more favorable for tree growth, which could result in greater shading of inland waters than coastal waters. Such shading is significant because

sunlight inactivation is a significant factor in the extra-enteric persistence of indicator organisms.

- Inland waters are generally more shallow than coastal waters. The group relates the difference to greater particle settling in inland waters than coastal waters. [Note that the authors of this summary report question that assertion. Flows in inland waters are characterized by higher velocities than those observed in coastal waters. Those higher velocities can keep particles and particle-associated bacteria suspended. When tributaries enter coastal waters, influent plumes disperse, velocities decrease, and particles tend to settle.]
- Inland waters are believed to be associated with soils and physical conditions that are more conducive to FIB growth than the soils and physical conditions associated with coastal waters. [Note that although the authors of this report find that assertion to be reasonable, it is important to note that growth in coastal sediments and sands has been documented in many studies, and no studies definitively support a greater propensity for FIB growth in the inland environment than the coastal environment.]

After reviewing water quality and the dynamics of indicators in inland and coastal settings, the group directly addressed the use of water quality criteria based on Great Lakes epidemiology studies for inland waters. That group's assessment is that insufficient evidence exists for direct extrapolation of criteria from Great Lakes studies for use in inland waters. The group speculated that swimmers in POTW-impacted Great Lakes and inland waters likely face similar risks but that the presence of non-fecal indicator sources at sites could result in differences in the meaning of indicator levels at inland and coastal sites.

Health risks

The group contrasted the results of epidemiological investigations conducted for the following types (setting) of water:

- fresh and marine waters,
- flowing and non-flowing waters, and
- waters with different fecal pollution sources.

Comparisons of epidemiology studies for fresh and marine sites showed that indicator organism levels do not relate to the same levels at either fresh and marine sites when culture-based methods are used for measuring indicator organism density. The comparison also identified fecal pollution source as more important than water type (marine or fresh) as a determinant of whether the indicator level is associated with observed adverse health effects.

The group was unable to contrast studies for flowing and non-flowing waters because limitations in study designs or differences between studies were too great to allow meaningful comparisons.

Comparisons of epidemiology studies conducted for waters with different fecal pollution sources were also hampered by differences in study designs and a relatively small number of studies. In general, the group members hypothesized that differences in fecal pollution source are likely more important than those in water quality and other features that differentiate inland and coastal sites.

Weighing the findings from the epidemiology studies described above, the group developed the following two positions:

- Position 1—applying the results from the Great Lakes studies to inland flowing waters is not supported by the scientific literature because directly comparable studies have not been conducted. It is unknown whether such application is underprotective of public health.
- Position 2—applying the results from the Great Lakes studies to inland flowing waters is a reasonable step based on supporting information in the literature and approaches taken worldwide and is unlikely to result in the underprotection of public health.

The group recognized the need for new criteria to be developed and the low likelihood that additional epidemiology studies will be conducted in time for use in developing new or revised criteria. Under such circumstances, the group generally supported position 2, stating, “It is the opinion of the Health Risks Group that water quality criteria derived from Great Lakes studies would likely be protective of public health at inland waters.”

Water matrix

The group noted the importance of fecal pollution source, water physical and chemical properties, microbial ecology, and hydraulics in the association of indicator organisms with health effects. Considering those factors, the group focused on the potential for settling, regrowth, and resuspension of indicators in inland waters, and the use of predictive models for evaluating differences between inland and coastal sites.

Inland water conditions that appear to favor indicator occurrence, growth, and resuspension from sediments were thought to be (1) higher ratio of sediment-water interface area to water volume for inland waters, (2) finer sediment sizes typical in inland waters, (3) greater presence of wetting and drying areas (per volume of water), and (4) relatively high velocities and higher potential for resuspension at sediment-water interfaces. The group acknowledged that additional research is required before the effects of those features on the loads of non-fecal indicators is known.

The group discussed the use of predictive models for evaluating differences between coastal and inland waters. Three types of models can be used for systematic evaluation of differences between inland and coastal sites:

- regression models,
- mechanistic models, and
- QMRA models.

Each of those models is associated with limitations. Regression models have, to date, been formulated on the basis of relatively limited sets of water quality and physical condition data. That limits the applicability of the models for sites outside those used for developing the model. Debate remains regarding the most appropriate independent variables, and it is possible that those variables differ between sites, further reducing the generality of regression models. Mechanistic models can be developed at many scales and including/excluding many processes. To allow comparison of inland and coastal waters, mechanistic models should include the following:

- three-dimensional models of indicator transport for coastal sites, inclusive of wind-driven flows;
- accurate modeling of dilution, turbidity, and shading;
- association of inland sites with finer particle sizes and greater association of microorganisms with particles; and
- higher organic content and potential for regrowth for inland sites.

Although the Water Matrix Group did not explicitly note it, the development of such models would require large amounts of data, additional data collection, and the simulation of multiple scenarios or development of techniques that would allow the generalization of results of individual simulations.

The group noted that QMRA modeling would have to overcome great uncertainty in parameters of the risk models. To lend credibility to QMRA modeling activities, the group strongly recommended studies in which QMRA models are *anchored* to epidemiology study results. Once anchored, QMRA would provide an avenue for the investigation of phenomena for which other modeling activities are less suited, including exploration of relative risks during events and of worst-case scenarios.

Sources

The group noted that even within a particular fecal pollution source (i.e., human treated, human nonpoint, livestock, companion animals, and livestock) the relationship between indicator level and health effects for different fecal pollution sources differs with level of treatment of the waste—including the proximity of the waste to the receiving water, the prevalence and abundance of pathogens in the fecal pollution, and the persistence of pathogens in the fecal pollution source relative to the persistence of indicator organisms. The prevalence and abundance of both FIB and pathogens varies widely between fecal pollution sources and within each fecal pollution source.

The Sources Group stated that because the prevalence of on-site wastewater treatment (septic) systems, which they considered to be nonpoint sources of pollution, is much greater for inland waters than for coastal waters, these human fecal pollution sources are more associated with inland waters than coastal waters. Note that septic systems differ widely in their design and operation and likely result in fecal pollution loads that vary widely. Additionally, runoff from urbanized areas with high proportions of impervious surface area differs from agricultural or rural areas, although the explicit implications of the differences between rural and urban runoff were not stated.

The group related differences between inland and coastal water settings to differences in proximity of fecal pollution sources to receiving waters, differences in pathogen and indicator density and prevalence in source materials, differences in loading during rain events, and differences in land use. The group hypothesized that coastal waters are generally more associated with urbanized land use, higher impervious surface areas, and point and diffuse human pollution sources than inland waters. However, the group acknowledged that inland waters comprise a diverse group and that the association of coastal and inland waters with specific sources of fecal pollution and characteristics are hypotheses.

The group concluded by stating that knowledge gaps—particularly for the abundance, prevalence, and pathogenicity of pathogens in livestock and wildlife wastes, are so profound and the inherent variability in nonpoint source-affected systems is so great that it could not assess whether water quality criteria based on Great Lakes/coastal studies could be extrapolated to inland waters.

Implementation realities

The group focused on flexibilities that are present in non-U.S. beach programs and might be considered for implementation with new or revised AWQC. Specific needs for flexibility should recognize the following factors/elements:

- water quality and risk vary dramatically with rain events;
- different fecal pollution sources pose different hazards; and
- the proportion of non-fecal, resuspended organisms varies with site type.

The current AWQC (USEPA 1986) implementation provides flexibility via use of different monitoring schemes for assessing water at beaches with different levels of usage, and through provision of an *off-ramp* by which site-specific water quality criteria could be established on the basis of sanitary surveys and epidemiology studies. Additionally, states can designate specific classes of waterbodies or specific circumstances for different, scientifically defensible water quality standards. Such a designation could be made for waters known to be affected primarily by animal sources or for temporary changes in microbial water quality criteria following rain events.

Flexibility is provided in World Health Organization (2003) standards by using sanitary survey findings in selecting water quality criteria appropriate for a specific site. Recreational sites without human fecal pollution sources and low bather density are considered to have good water quality at indicator densities higher than those for sites with known human fecal impacts or high bather densities.

European Union bathing water quality standards (EP/CEU 2006) provide flexibility through discounting of samples collected during short-term pollution events. Within this framework, up to 15 percent of the total samples at a site could be disregarded for classification purposes because of short-term pollution during the last assessment period. The Implementation Realities Group notes that inland waters are most influenced by wet weather events because they are more closely associated with urbanized areas. Note that this assessment is somewhat at odds with that of the Sources Group.

The Implementation Realities Group noted that beach sampling and water quality assessment are conducted in the context of other regulatory programs, including NPDES permitting, 303(d)/TMDL use attainment assessments, and BEACH Act monitoring. Monitoring needs and realities differ for these programs and techniques and methods for assessing water quality also differ. Flexibility in the overall regulatory context might be provided with the issuance of water quality criteria on the basis of multiple methods for counting indicator organisms, or through the provision of multiple statistical approaches for developing monitoring plans and assessing results of microbial water quality sampling.

Research priorities

After assessing the state of the science and knowledge gaps in each topic area, the expert groups developed prioritized lists of suggested research activities intended to provide improved information for development or extension of criteria for inland waters. The priority short- and longer-term research activities proposed by the workshop participants is presented in Table 8, and a comprehensive list is presented in Table 9.

Table 8. Priority research activities from the 2009 Inland Waters Workshop (WERF 2009)

Time frame	Research
Short-term	Identify and quantify human pathogens in animal feces
	Examine relationships between qPCR and culture-based FIB
	Optimize and anchor QMRA models to observed health effects data obtained from epidemiology studies and develop QMRA tools for implementation of new AWQC
Longer-term	Characterize fate and transport of animal pathogens in relation to indicators
	Conduct epidemiology studies in inland waters

Table 9. Comprehensive list of proposed research activities from the 2009 Inland Waters Workshop (WERF 2009)

Group	Short-term research priorities	Longer-term research priorities
Indicators and Pathogens	<ul style="list-style-type: none"> Examine relationships between qPCR- and culture-based FIB and develop a database of results to date of other reliable potential new monitoring methods 	<ul style="list-style-type: none"> Conduct studies on watershed assessment information to be used as input for site-specific water quality criteria Investigate the potential for speciation of enterococci to identify fecal-specific strains (preferably human) from environmental strains, and then apply results to future epidemiology studies Conduct epidemiology studies that take into account urban runoff and nonpoint sources of fecal contamination. Include the use of culture-based and molecular-based analytical methods. Include sensitive populations (particularly children). Conduct epidemiology studies incorporating the measurement of pathogens of interest along with indicators to determine the correlations of these organisms and to better understand their associations with diseases at downstream recreational locations

Group	Short-term research priorities	Longer-term research priorities
Health Effects	<ul style="list-style-type: none"> • Optimize and anchor QMRA models to epidemiology studies • Incorporate source characterization methods into ongoing epidemiology studies • Optimize and standardize qPCR methods and enhance their interpretation • Conduct meta-analysis of epidemiology studies by source 	<ul style="list-style-type: none"> • Conduct further epidemiology research to identify how much uncertainty exists in using the results of epidemiology studies conducted in Great Lakes and coastal settings to establish criteria for inland flowing waters
Water Matrix	<ul style="list-style-type: none"> • Apply model-driven nowcasting or forecasting (or both) in current epidemiologic studies to determine a relationship of model variables to health outcome • Further develop and test regression models in inland waters (at rivers with different morphologies from those in Ohio, Georgia, and Kansas) • Focus on prior epidemiology studies to build models after the fact and compare the results from such retrospective models to the observed human health effects • Include an in-depth evaluation of target microbe/pathogen sources and environmental conditions in epidemiology studies to evaluate the sources and parameters needed for coupling health outcomes with model output • Evaluate reverse QMRA for its applicability in developing site-specific criteria • Apply data mining activities to identify data gaps that limit application of models and identify longer-term research goals 	<ul style="list-style-type: none"> • Use modeling approaches to characterize source impacts as an integral part of the design of all recreational water epidemiology studies • Further develop and test regression models in all types of inland waters to clarify their applicability, limits, and future research needs • Focus on developing the fundamental understanding and knowledge needed for establishing mechanistic models

Group	Short-term research priorities	Longer-term research priorities
Sources	<ul style="list-style-type: none"> • Identify and quantify human pathogens in animal feces • Identify and quantify human pathogens in various types of on-site systems, especially during wet weather and including disinfection efficacy of systems and their rate of failure 	<ul style="list-style-type: none"> • Identify fate and transport of important human pathogens identified in animal feces • In future and ongoing epidemiology studies, include agricultural, nonpoint source runoff, measure human pathogens identified in animal feces • In characterization of land-use and sanitary surveys, define consistent template and quantify important inputs • In developing water quality safety plans and developing best management practices (BMPs), determine recreational component of water safety plans involving EPA, utilities, scientists, and community collaboration • In developing water quality safety plans and developing BMPs, undertake research to quantify effectiveness of various BMPs and their applicability to various watershed scenarios

Group	Short-term research priorities	Longer-term research priorities
Implementation Realities	<ul style="list-style-type: none"> • Perform analyses of all existing inland water epidemiological data (domestic and international), including multiple sub-analyses (e.g., flowing versus quiescent, source type, relationships between indicators, relationships of indicators to human health risk) • Develop more science to understand regrowth or resuspension of enterococci in sediment of inland flowing waters • Research the effects of prolonged holding times on microbiological analytical results • Develop early communication on aspects or options of implementation guidance and national level expectations in terms of adoption for inland waters • Use longer averaging periods for assessment purposes to deemphasize short-term excursions • Consider modification or use suspensions during defined high-flow conditions • Consider developing site-specific criteria based on QMRA or other methods • Designate a new designated use (or uses) on the basis of physical waterbody characteristics or the types of activities that a waterbody is used for (shallow water use or secondary contact use) • Develop a guidance document to facilitate criteria adoption by states 	<ul style="list-style-type: none"> • Develop data to quantify risk in waters affected by nonhuman sources • Develop a flowing water sample design on the basis of stream characteristics • Develop a translation between current criteria and any new criteria • Standardize methods including an evaluation of method robustness and a certification program for regulated laboratories • Conduct studies evaluating the impact of sediment-borne resuspension events especially during high-flow periods • Conduct studies to determine appropriate indicators for each CWA purpose and a method to bridge current assessment criteria and methodologies to new ones • Develop data on pathogens/infectivity and exposure information for input to site-specific QMRA models • Use QMRA for estimating health risks and developing appropriate and detailed exposure pathways for inland waters

2.2.2. Meeting Report: Knowledge and Gaps in Developing Microbial Criteria for Inland Recreational Waters (Dorevitch et al. 2010)

As described in Section 2.2.1, in February 2009, WERF with support from EPA planned and conducted a workshop to explore similarities and differences between inland and coastal waters. That workshop resulted in a peer-reviewed publication (Dorevitch et al. 2010) describing the workshop findings and knowledge gaps related to the extension of criteria developed for coastal to inland waters. In the following section, the report findings are summarized. Differences between inland and coastal waters with the potential to necessitate different criteria are summarized first. Then research questions and a research agenda are presented. Note that the

study is a synthesis of the full report (WERF 2009) from the workshop (Section 2.2.1). Because the authors of the study interpreted and prioritized elements from the full study, the peer-reviewed version of the report summary is also reviewed in this report.

Differences between inland and coastal waters

Dorevitch et al. (2010) identifies the following three assumptions that must be made to extend AWQC developed for sewage-affected coastal sites to inland sites:

1. Similar densities of FIB reflect a similar risk in inland and coastal settings, presumably because they reflect a similar exposure to pathogens of similar infectivity and virulence.
2. Hydrogeochemical differences among inland lakes, rivers, and coastal waters have nondifferential effects on the transport and fate of indicators and pathogens.
3. The criteria derived from the studies conducted at sewage-affected coastal beaches protect against illness in inland settings, where the predominant source could be wildlife or agricultural animals.

Foremost, the report notes that the *fecal pollution source* is the primary site feature determining the risk of illness from recreational exposure. The authors assert that inland waters are predominantly in rural areas and have a greater likelihood than coastal waters of being affected by agricultural or wildlife fecal pollution sources. Further, pathogens present in livestock and wildlife fecal wastes differ in both abundance and human health effects from those present in sewage. Zoonotic agents can differ from pathogens of human origin either in their ability to initiate infection or in the hazard they pose. The authors of this summary report note that although the reviewed report notes the importance of source and etiology of illnesses arising from recreation, it does not quantify the extent to which coastal and inland waters are affected by different fecal pollution sources. Though many river miles are in rural areas, rivers are loaded by POTWs, combined sewer overflows (CSOs), septic systems, and other human sources. For some streams during dry periods, POTW effluent can constitute a significant portion of flow. Likewise, animal sources affect coastal sites. For example, coastal counties of the Great Lakes states support large populations of dairy cattle, while coastal counties on the Delaware and Chesapeake bays are locations of intensive chicken production (NASS 2010). Shorebirds and dogs are also considered important fecal pollution sources for coastal waters.

Dorevitch et al. (2010) identifies *indicator growth and mobilization in sediments* as a significant potential difference between inland and coastal water indicator performance. Specifically, the report implies that the inland water sediment and soil environment is more favorable to indicator organism growth than the coastal environment and that inland water hydraulics generates greater suspended indicator loads than coastal hydraulics. Inland water sediments are believed to generate larger FIB loads because the extra-enteric growth conditions for FIB are thought to be most favorable at the water-sediment boundary, and the ratio of the water-sediment boundary length to the stream cross-sectional area is higher than that for coastal sites. The study also asserts that indicator organisms harbored or growing in sediments are more likely to be resuspended into the water column due to more favorable hydraulics for resuspension in inland waters because boundary layers (indicative of the shear stress on bottom sediments) occupy a larger fraction of inland water volume than of coastal water volume. Together, those phenomena result in a larger proportion of indicators in inland waters arising from non-fecal sources than the proportion in coastal waters. Thus, for inland and coastal sites with the same fecal pollution source, an indicator density observed in inland water is likely to correspond to a different

presence of fecal pollution and a different risk than for the coastal water at which the same indicator density is observed. Such a decoupling of indicator organisms from fecal pollution sources represents a significant difference in indicator performance for inland and coastal waters.

Hydrogeological differences might influence indicator performance in inland and coastal waters. First, fecal pollution sources are often closer to receiving waters for inland water than for coastal waters. Given the smaller water volumes typical of inland waters, less dilution of the fecal pollution is expected for inland waters than for coastal waters. This finding does not pose a difficulty in use of indicators for inland and coastal waters; the higher indicator densities observed in inland waters are related to higher fecal pollution densities and higher public health risk. Increased risk is expected to be associated with proximity to the fecal pollution source. Decreased risk is expected to be associated with exposure to more dilute fecal pollution. Indicators shed by the bathers can be diluted less in inland waters than in coastal waters. All other factors being equal, higher indicator densities are associated with higher fecal pollution concentration and increased risk.

An additional difference in hydrogeology for inland and coastal waters with significance to indicator performance is the flow typical of the settings. Inland waters are subject to highly variable volumetric flow rates, bottom shear stresses, and turbulence. Those parameters are less variable for coastal waters. Advection dominates fecal pollution transport in flowing inland waters, whereas advection via longshore currents, other large-scale currents, or wind-driven water flows are the drivers behind most transport of fecal pollution into and out of coastal sites. This difference results in more extreme variation in indicator densities during storm events for inland waters than for coastal waters, as observed by Nevers et al. (2007) for coastal streams near Great Lakes beaches. Furthermore, as described above, turbulence can promote decoupling of indicator density from fecal pollution sources.

Critical research questions and a research agenda

The report proposes four areas encompassing the research that should be conducted to improve the understanding and estimation of health risks arising from recreation in inland waters. The four areas are presented below, along with suggested short- and long-term research activities for closing data gaps and improving understanding.

1. Microbial indicators as predictors of risk

The spatiotemporal variability and determinants of FIB need to be characterized for hydrologically diverse settings. Although not detailed in the report, these diverse settings include small rural streams, small urban streams, large flowing waters, and impounded waters. Short- and long-term research suggested include meta-analysis of epidemiological relationships developed for waters affected by different fecal pollution sources (short-term), and mechanistic fate and transport modeling of FIBs for the range of water types expected to be important as recreational waters.

2. Fecal pollution sources as predictors of pathogen exposure and health risk

In the topic of fecal pollution sources as predictors of pathogen exposure and health risk, short-term research activities suggested in the report are development of a sanitary survey tool for use in inland water epidemiology and QMRA studies, optimization and anchoring of QMRA by means of data and relationships from epidemiology studies, and field sampling of feces from farm animals and wildlife to quantify the occurrence and dynamics of pathogens of human

concern. In the long term, the report suggests that epidemiology studies should be conducted at inland sites with different dominant fecal pollution sources. Epidemiological studies should differentiate between pathogens posing different hazards, particularly those associated with very serious health outcomes (e.g., zoonotic *E. coli* O157:H7).

3. Molecular methods for water quality testing

Given the likelihood that molecular methods will be employed in new AWQC, molecular methods should be better understood and optimized. A database describing the relationships between molecular- and culture-based determinations of indicator densities should be developed on the basis of findings reported in the technical literature. Given the differences in transport and fecal pollution loading for inland and coastal waters, that database would likely include relationships for a wide variety of settings ranging from small inland waters to large inland waters to coastal sites. The persistence of molecular method targets should be established. This step is critical given that one performance criterion of indicator organisms is persistence similar to that of the pathogens they indicate. Quantitative PCR methods, particularly for source-specific markers, should be optimized, standardized, and applied in epidemiology studies.

4. Other approaches for predicting inland water recreation health risks

The other approaches suggested by Dorevitch and colleagues for predicting risks include predictive modeling (such as Nowcast forecasting) and QMRA. Regression models have been demonstrated to be more predictive of whether an AWQC will be exceeded than simple use of a prior day's indicator density for coastal sites. Flow models can be used to improve regression models, though real-time multidimensional modeling of coastal sites has not been demonstrated to date. Modeling research activities suggested include evaluation of the viability of regression models and mechanistic models for risk management and as supplements to FIB monitoring for inland waters. There are several indications that regression and mechanistic models are feasible for use in inland water risk management. Monitoring and forecasting systems are in use for managing risks to drinking water systems with surface sources. Numerous well-tested contaminant transport models are available for mechanistic modeling of FIB or pathogens in streams; given the hydraulics and geometry of streams, these models are much less complex than flow models of coastal sites and have the potential for use in real time. Challenges to use of regression and mechanistic models include the following:

- sporadic nature of fecal indicator and pathogen loads to streams;
- knowledge gaps regarding FIB and pathogen growth in sediments and resuspension;
- incomplete knowledge of the persistence of the microorganisms in diverse inland water settings; and
- need for sensors and protocols for collecting real-time data for use in predictive models.

The QMRA framework is flexible and can be used to relate indicator and pathogen fate and transport to risk. When used in tandem with epidemiological analyses, QMRA can add context to epidemiology study findings. In the absence of epidemiology studies (as is currently the case for inland waters), QMRA can be used to develop estimates of human health risk that can be compared to those observed for coastal waters. To be used in either of those modes, QMRA must have credibility with the scientific community and must provide sufficient data to allow realistic risk estimates specific to the geography, biology, and hydrology of inland waters. The report advocates *anchoring* QMRA (retrospective studies) as a short-term research need. Such an

exercise would necessitate generating improved models and can be used to advance the credibility of QMRA with the scientific and policy communities.

Report conclusions

Dorevitch et al. (2010) conclude that the distinction between inland and coastal waters is less important than differences in the fundamental processes and variables of the systems. The processes and variables include the scale of the waterbody, the fecal pollution source(s), indicator and pathogen dynamics in sediments, and other factors related to the transport and fate of indicators and pathogens. Of particular importance is a clear understanding of the proportion of indicators that is directly related to a specific fecal pollution source and the proportion not related to fecal pollution sources and thus indicative of a different and likely lower risk.

The authors reason that applying criteria derived on the basis of epidemiology studies of coastal sites to inland waters should result in sporadic mild illnesses at rates no higher and possibly lower than those experienced in coastal waters. However, coastal and inland waters might pose very different risks of severe diseases such as hemolytic uremic syndrome arising from exposure to *E. coli* O157:H7 and other pathogenic *E. coli* strains. The origin of those different risks is the difference in the dilution capacity of typical inland waters and coastal waters, as well as the difference in pathogenic organisms in sources typical of coastal and inland waters.

2.2.3. Literature Review of Assessment of the Applicability of Existing Epidemiology Data to Inland Waters (USEPA 2010a)

Before the 2009 WERF workshop whose findings are described above (Dorevitch et al. 2010; WERF 2009), background material was assembled describing and comparing the occurrence, fate and transport of indicator organisms in inland and coastal waters. After the workshop, the draft report was revised to expand upon sections pertaining to epidemiological data and health effects. The document (USEPA 2010a) underwent a second round of revisions in August 2010. That version of the report is reviewed below.

The purpose of the report is to assess and compare the performance of indicators in inland and coastal waters. The authors compare the performance of indicators in coastal and inland waters on the basis of the following features:

- demonstrated correlation with health risk;
- similar or greater survival time than the target pathogen;
- similar or greater transport than the target pathogen;
- presence in greater numbers than the pathogen; and
- specificity to a fecal source or an identifiable source of origin.

Indicators demonstrate all those features for both inland and coastal waters.

The report presents data and analysis from the peer-reviewed literature grouped into three sections (1) epidemiology and modeling studies of the health effects associated with recreation in surface waters; (2) the occurrence and variability of indicators in coastal and inland settings; and (3) the persistence and growth of indicators and the association of indicators with pathogens. Absent from the report are discussion of differences in resuspension for typical inland and coastal waters, discussion of differences in typical fecal pollution sources for different settings,

discussion of suites of pathogens characteristic of different fecal pollution sources, and evaluation of the role of dilution in indicator performance.

Epidemiology and modeling studies

To date, epidemiology studies have demonstrated correlations of health risk with indicator level only for waters primarily affected by POTW discharge. For freshwater sites, studies have demonstrated association of health risk with indicator level for *E. coli* enumerated by cultural methods (Dufour 1984; EC 2009a, 2009b; Marion et al. 2010) and for *Enterococcus* enumerated by qPCR (Wade et al. 2006, 2008). Among those studies, the only two that are potentially directly comparable are the Dufour and Marion studies, because both used the prospective cohort (PC) study design and the same illness definition. Direct comparison of the results of those studies should be done cautiously, because the health effects relations in the Dufour study are based on seasonal averages of indicator densities whereas in the Marion study they are based on individual days' samples taken at an inland reservoir. In general, the results of both epidemiology studies indicate that health effects observed in the two studies are comparable, despite the studies being conducted more than two decades apart (the GI definition was the same in both studies).

Other epidemiology studies will require analyses and conversion of data prior to comparison. For example, both health effects and water quality data from randomized control trial (RCT) epidemiology studies could require conversion for comparison with PC epidemiological health effects data.

Modeling provides an alternative to epidemiology studies for assessing health risks associated with recreational use of surface waters. As noted above, epidemiology studies provide the firmest basis on which to develop criteria. However, in the absence of epidemiology studies, modeling studies could provide a means for evaluating health risks. Modeling studies described in the USEPA (2010a) include QMRA and watershed modeling approaches.

Recently published QMRA studies (Schoen and Ashbolt 2010; Soller et al. 2010a,b) have linked indicator level to the occurrence of pathogens for non-POTW sources—including cattle manure, swine manure, chicken manure, and gull droppings. Those studies rely on pathogen and indicator occurrence and abundance as reported in the literature for estimating the pathogen suite and resulting illnesses rates associated with ingestion of runoff from livestock operations or from beaches contaminated with gull droppings. Those models are stochastic and account for the variability in the occurrence and abundance of pathogens and indicators in fecal sources. The QMRA models indicate that, at a given indicator level, recreation in waters affected by cattle runoff poses a similar risk to recreation in waters affected by human fecal pollution sources, whereas recreation in swine-, chicken-, and gull-affected waters poses a significantly lower health risk. The results will be refined in future studies to account more accurately for fate and transport processes and manure treatment. The finding that different fecal pollution sources have different risks at the same indicator level does not consider inland and coastal sites to be substantially different. Rather, it is presumed that the source, not the setting, is the critical feature of a site.

Watershed modeling is a related but alternative modeling technique that can be used as an alternative to epidemiology studies for assessing health risks association with recreation in surface waters. Like QMRA models, watershed models rely on data collected from external sources as input for models that predict net pathogen loads watersheds discharge to receiving

waters. The watershed models can be used in developing sampling schemes, in assessing differences of risk in different portions of a watershed, or in comparing the contribution of different fecal pollution sources to the pathogen loads to receiving waters. Because the models are site-specific, they might have a greater role in implementing new or revised criteria than in developing the criteria.

FIB occurrence in inland and coastal waters

Nearly all available data on the occurrence of FIB are presented as ranges, not as statistical distributions. Accordingly, more meaningful comparison of ranges of indicator densities with metrics such as geometric means, measures of spread and of skew in distribution of occurrence, could not be used. Moreover, studies were limited to relatively few waterbodies and each study had different designs and objectives, so how representative their data are with respect to the overall distribution of FIB among inland and coastal waters is uncertain. Thus, comparisons of the occurrence of indicators in coastal and inland waters were considered *screening level* and were made on the basis of reported ranges.

The report presented indicator occurrence density ranges for *E. coli*, enterococci, fecal coliforms, total coliforms, and fecal streptococci. To allow comparison across setting types, ranges for the following setting types were determined:

- coastal freshwaters;
- coastal marine waters;
- estuarine;
- inland flowing, main;
- inland flowing, small; and
- inland non-flowing.

Coastal marine sites exhibited the highest variation in both *E. coli* and *Enterococcus* densities, somewhat contrary to expectations. Coastal waters are subject to much greater dilution than inland waters and are farther from fecal pollution sources than typical inland waters. Given the limitations of the occurrence data, the report concludes that the occurrence of indicators in inland waters and in coastal waters is not substantially different. It is important to note that none of the studies used in developing the occurrence ranges attempted to ascertain the source of the indicator organisms.

Growth and persistence of FIB

Indicator survival curves typically exhibit *shoulder* behavior (the shoulder being the initial curve before the exponential portion, representing that damage has to accumulate to a certain level before cells begin to die), followed by first-order decay. The factors most important in determining the decay rate are presence of sediments, insolation, and the presence/absence of predators. Less significant factors are temperature and salinity. Nutrient availability and pH also influence persistence, but they do not vary sufficiently among waters to result in significant differences in persistence among sites.

Fecal indicator bacteria growth has been observed in coastal and inland settings and in both large and small streams. Fewer studies have reported growth of enterococci than of *E. coli* and fecal coliforms; however, it is not certain whether this is because enterococci growth is less prevalent than that of the other indicators or whether there are fewer studies on enterococci. The presence

of sediments is a primary factor in determining whether growth is observed. Because inland and coastal waters differ in the sediments present and the ratio of the sediment-water interface area to the water column volume, that difference could result in a different proportion of indicators in inland waters arising from sediments than the proportion in coastal waters. The proportion of indicators attributable to resuspension from sediments is determined by the density of indicators in the sediments and the resuspension of the indicators via turbulence arising either from shear stress at an inland water streambed or from wave action or tidal processes in coastal sites.

Reported *E. coli* inactivation rates do not differentiate indicator performance in Great Lakes waters from that in inland waters. In both water types, indicator decay rates depend on the same physical factors and are widely variable. Contrary to expectations, reported inactivation rates for marine and freshwaters overlapped. Studies have established decreased persistence of *E. coli* with salinity, so the overlapping ranges indicate that the combination of factors determining survival are such that their net effect is inactivation rates spanning the same range. Fewer studies were found providing *Enterococcus* inactivation rates. The studies reviewed in USEPA (2010a) indicate that the same factors govern the persistence of enterococci in inland and coastal waters and that persistence is comparable in the two settings.

Co-occurrence of FIB and pathogens

At the time that report was completed, only two epidemiology studies have yielded health effects relationships for culture-based indicator enumeration in inland waters and there are no available studies that have established health effects relationships for inland waters based on qPCR. Therefore, the health effects associated with inland waters and coastal waters must be deduced either from a direct association of pathogens and indicators or from an association of indicators and pathogens with fecal pollution sources. In USEPA (2010a), studies attempting to correlate pathogen and indicator occurrence were reviewed. The relevant studies included studies of bacterial, protozoan, and viral pathogens for inland and coastal waters. In short, co-occurrence and correlation between pathogens and *E. coli* and *Enterococcus* were not observed for any setting. Though not reported, it is clear that pathogen variability differs from that of indicators, even for a given fecal pollution source. Other processes, including the following, can cause pathogen-to-indicator ratios to vary:

- different removal rates of indicators and pathogens via settling in flowing and non-flowing settings;
- different removal rates of indicators and pathogens via ultraviolet (UV) inactivation (assuming the incident UV radiation is different in coastal and inland settings because of shading or other features); and
- different sources or loading rates associated with different settings.

2.2.4. Sampling and Consideration of Variability (Temporal and Spatial) for Monitoring of Recreational Waters (USEPA 2010b)

As noted in Section 1.1.2, stakeholders have expressed concerns over both science and the application of new or revised AWQC to inland waters. Concerns repeatedly expressed by stakeholders are (1) sampling and interpretation of results for inland waters at which there is the potential for recreation but recreation does not occur, and (2) concerns that samples taken during or shortly after rain events, when indicator densities are extremely high, will unduly influence water quality assessment. Data and interpretations germane to those concerns are presented in

Sampling and Consideration of Variability (Temporal and Spatial) for Monitoring of Recreational Waters (USEPA 2010b). That report describes the temporal and spatial variations in indicator densities for both inland and coastal waters and at all relevant spatial and temporal scales.

Indicator variability in inland and coastal waters

A full review of the findings regarding indicator spatial and temporal variability provided in USEPA (2010b) is outside the scope of this report. In brief, a review of the literature produced the following findings:

- Regardless of site type, the greatest variations in indicator density arise from rain events.
- Temporal variability, from greatest to least, is as follows: Event variability (rain events) > diurnal variability > tidal-time-scale variability (coastal sites only) > monthly/seasonal variability (considering only the recreational use season) > short-time-scale variability (for samples taken at knee depth and greater).
- For coastal sites, sources of spatial variability, from greatest to least, are as follows: variation with depth of sample collection > variation with site features such as point sources or features inhibiting mixing > along-shore variation > variation with depth below the water surface where sample is collected.
- For inland sites, sources of spatial variability, from greatest to least, are as follows: along-stream variation > variation with depth below the water surface where sample is collected > cross-stream variation (i.e., downstream of the mixing zone for point sources).

Developing monitoring plans

Monitoring plans chosen for specific sites should be designed on the basis of the variability in indicator density anticipated at that site. Thus, data should be collected before monitoring scheme development to quantify spatial and temporal variability. Two vehicles suggested for collecting those data are sanitary surveys and pilot monitoring activities. Perhaps most important, sanitary surveys include collection of data on the fecal pollution sources with the potential to affect a site and the route by which fecal pollution could be delivered to the site. For coastal waters, transport modeling can be complex, given variability in currents, wave-generated turbulence, and such. For inland waters, identification of sources can be complex because many inland water fecal pollution sources are diffuse.

None of the studies reviewed in USEPA (2010b) directly addressed the consideration of extremely high indicator densities during rain events. As noted in that report, extremely high indicator densities can arise in both inland and coastal waters during rain events, although given the lower dilution and proximity to sources of inland waters, the impact on inland waters is expected to be greater than that on coastal waters.

Inland sites can require different monitoring strategies because of their accessibility, length, and frequency of use. Ideally, the number of samples is chosen using a variation of power analysis, with the detectable difference related to the acceptable range in risk or a range of risks that is measurable within the overall population. Because of cost or logistical considerations, beach managers might not be able to sample with the density or frequency suggested by power analysis. Sampling locations should be selected based on the ability of a small number of samples to adequately describe water quality at the site and should target areas of beaches in

closer proximity to fecal pollution sources (portions of the beach with significantly different mixing should be sampled separately). In addition, collection of samples in the morning appears to offer the best balance between practicality and generation of data that are conservative estimators of human health effects. It also provides the best correlation between culture-based and qPCR results.

2.2.5. Quantification of Pathogens and Sources of Microbial Indicators for QMRA in Recreational Waters (WERF 2010a)

The overall objectives of this study are (1) to identify and address data gaps pertaining to loadings and concentrations of waterborne pathogens and indicators in discharges to recreational waters that are affected by fecal pollution; and (2) to compile, analyze and synthesize the data in QMRA models and waterborne risk management frameworks. These objectives are related to the comparison of inland and coastal waters because of the following:

1. Pathogen densities typical of specific fecal pollution sources are characterized. These data can be used to compare the relative risks posed by inland and coastal sites if it is found that inland and coastal sites are impacted predominantly by different fecal pollution sources.
2. The study develops a method by which the relative contributions of different fecal pollution sources can be determined for a particular site. Knowing the contribution of the sources will allow improved risk estimates for both inland and coastal sites and will provide information regarding the how the dynamics of indicators and pathogens differ for inland and coastal sites. At present, the general features of indicator and pathogen dynamics are known for inland and coastal sites, but quantitative data for relating different processes affecting risk are not available.
3. QMRA models for recreational exposure are becoming increasingly available. New methodologies and data for use in QMRA are provided in this study. Although the new data and methodologies will be useful if QMRA is used for comparing the relative risks of inland and coastal sites, because the QMRA model developed in this study does not consider the characteristics of the waters receiving fecal pollution, the results do not directly address differences in indicator performance for inland and coastal sites.

Elements of this study were: data collection on pathogen and indicator occurrence and abundance in diverse fecal pollution sources (literature survey and field study); development and characterization of the performance of microbial methods for all of the relevant pathogens and indicators; development of a quantitative microbial source tracking (MST) procedure for estimating the contributions of various sources to the density of indicators at a specific site; and QMRA and modeling to relate fecal pollution sources to health risks. In a generic sense, all of these activities are pertinent to comparison of indicator performance in inland and coastal sites. The activities with the most direct relevance to differences between these settings are the quantification of pathogens and indicator prevalence and abundance in specific fecal pollution sources, and the development of source-specific QMRA models. Each of these areas is reviewed below, with findings most closely related to differences in inland and coastal waters highlighted.

Data collection

Literature searches and field studies were used to quantify the prevalence and abundance of several waterborne pathogens (e.g., *Salmonella*, *Campylobacter*, *Cryptosporidium*, *Giardia*, adenoviruses, enteroviruses, noroviruses, and rotaviruses) and indicator organisms (e.g.,

Bacteroidales, *Enterococcus*, and *E. coli*) in treated and untreated sewage, urban runoff, runoff from undeveloped sites, livestock feces, wildlife feces, and companion animal feces.

Field studies involved sampling wastewater treatment plant, CSO, and stormwater effluents prior to mixing with receiving waters. Because the drainages contributing to the discharges are well-characterized, they might allow generalization of the findings to other drainages with similar characteristics. Sampling was conducted during (precipitation) event and non-event conditions. Both culture- and molecular-based methods were used to enumerate pathogens and indicators (to the extent that both methods are possible for a given microorganism). Novel or particularly relevant findings from the field studies include the following:

- *Cryptosporidium* density in runoff from forested land was much higher than that in runoff from lands with other uses;
- *Salmonella* occurred far more frequently in all fecal pollution sources than the other bacterial pathogens; and
- the dominant viruses differed by fecal pollution source, with enterovirus and norovirus most prevalent in runoff from residential and commercial/light industrial drainages, rotavirus more plentiful in discharges from agricultural operations, and different adenovirus types occurring with different prevalences among the fecal pollution sources.

The occurrence of some pathogens was found to be correlated with other pathogens. This finding might be significant in assessing risk related specific settings, because risks due to multiple pathogens are additive.

Spiking experiments were conducted such that filtration efficiencies, detection limits, and the effect of hold times could be established for the bacterial and protozoan organisms chosen for the study. From those experiments, the authors determined that using holding media stabilized bacterial populations without adversely effecting protozoa. The authors also noted that from raw data that, in some cases, recoveries above 100 percent were realized, and in many cases there were declines in recovery during the 72-hour hold times. The results of the spiking studies do not contribute directly to the comparison of inland and coastal waters. However, they do provide information with which pathogen abundance might be characterized in QMRA studies. Correctly characterizing pathogen densities is very important given the low densities at which pathogens normally occur and at which some pathogens can initiate infection and illness in humans.

QMRA

A QMRA model was developed for each fecal pollution source. The model incorporated data on the prevalence and abundance of all the priority pathogens in each fecal pollution source and used two exposure scenarios—direct exposure to effluent/runoff and exposure to diluted effluent/runoff. As noted above, it is difficult to relate the QMR results directly to differences between inland and coastal sites because there are no quantitative data describing and comparing the distribution of fecal pollution sources among coastal and inland waters.

In general, viruses were consistently found to be the risk drivers for all sources, with norovirus producing the dominant health risk, even for agricultural runoff. The authors do not comment on the degree to which animal noroviruses are host-adapted and their potential to be infectious to exposed persons. It was also found that dilution (as simulated in the QMRA) was not associated with significant risk reduction. This finding is significant, in that one significant difference between inland and coastal suggested by participants in the WERF experts workshop (Dorevitch

et al. 2010; WERF 2009) was the much higher dilution of fecal pollution in coastal waters as compared with inland waters.

The authors discuss methods by which QMRA might be used for generating new recreational water criteria or in implementation of those criteria. An important precursor to such uses is calibration of the models based on findings of epidemiology studies and development of models with specific components that reflect site characteristics ascertained through sanitary surveys or other data collection. Underlying the discussion of QMRA is the need to formulate the model for consistency with the pathogens and exposure scenarios for sites, whether they are inland or coastal.

Quantitative MST

Along with QMRA model development, the authors developed a quantitative MST methodology based on quantification of universal, human, cow, and dog *Bacteroidales*, and that was capable of determining the extent to which different fecal pollution sources (human, dog, and cow) contributed fecal indicators in a fecal pollution sample. Given the assumption that fecal pollution source is the most important determinant of risk for a given receiving water, this tool, along with sanitary surveys, has the potential to allow comparison of risks between sites (including inland and coastal sites). The performance of the quantitative MST method was found to be sensitive to the selection of the different host-specific indicators included in the methodology, and on the measurement error associated with each of the indicators. Two illustrations of model performance showed that the model performs well under some circumstances and might be improved such that it could also perform well when fecal pollution from species other than humans, dogs, or cows is present.

2.3. Methodology Performance and Relevance to Applicability of Criteria to Inland Waters (compilation of findings from multiple reports)

This section contains a review of the findings from reports #5, 7, and 8, and peer-reviewed publications 9 through 13 (Section 1.2.1) that are relevant to the difference in performance of indicator-method combinations in inland and coastal waters. The emphasis of this section is method performance and relative differences in qPCR and culture targets, rather than the association of indicators (as measured by different methods) with health effects. Some of the factors reviewed in this section are common to both inland and coastal waters. For example, chlorinated POTW effluent typically has much higher indicator counts via qPCR than culture methods, regardless of whether the plant is discharging to an inland water or a coastal water. Other features reviewed herein differentiate inland from coastal waters, such as the degree of qPCR inhibition exhibited in specific water types.

The section begins with a review of qPCR enumeration of indicator bacteria in environmental waters and the factors that may impact qPCR performance. Those factors, which include solar radiation, chlorination, die-off and predation of qPCR and culture targets, and inhibition, may differ among inland and coastal waters, though the extent to which those factors differ in inland and coastal settings is yet to be established. The section concludes with a review of several studies directly comparing culture and qPCR method performance in inland and coastal waters.

Factors influencing method performance

Generally, FIB densities derived from qPCR have been consistently reported higher by several orders of magnitude than those derived from culture-based assays in both coastal and inland water settings, and under a variety of conditions (e.g., throughout wastewater treatment, at POTW effluent, different times of day). This is mainly due to the comprehensive or unspecific nature of DNA amplification in the qPCR assay. All DNA present in a sample is amplified equally, regardless of the viability status of its host. This total DNA inventory is comprised of free and dead cell DNA (constituting ambient background DNA), viable but not culturable bacterial (VBNC) DNA, which is predominant with respect to the last culturable and viable fraction (corresponding to the cells enumerated by culture-based methods).

In report # 7 (Final report: Comparative Evaluation of Molecular and Culture Methods for Fecal Indicator Bacteria for use in Inland Recreational Waters (WERF 2010b)) the study authors view, based on the results of their study, is that qPCR methods, as currently optimized, cannot be applied universally across all inland water bodies. Differences among inland sites (and presumably among coastal sites as well) that may limit the performance of a particular qPCR assay are the fecal contamination source, unresolved inhibition, between-lab method variability (e.g., difference in extraction efficiency or development of calibration curves), and the relative contribution of DNA from viable v. non-viable cells. All of these factors vary among waterbodies and laboratories and are not intrinsic to inland waters per se. None-the-less, this finding implies that method performance can differ from site to site and should be considered when interpreting indicator levels for a given site or comparing indicator levels among sites.

In a study comparing qPCR and culture counts of Enterococci, Haugland et al. (2005) noted that geometric mean values of cell equivalents (qPCR counts) for samples taken on a given day were nearly always one order of magnitude and frequently two orders of magnitude greater than those for the MF method. Haugland and colleagues suggested that the much higher densities indicated by the qPCR assays are a result of the inability of the qPCR technique used to distinguish between DNA from live and dead cells (see the discussion on distinguishing live and dead cells, below). This finding is significant to the difference in inland and coastal waters in that the persistence of indicators and relative abundance of live culturable cells and qPCR targets may differ for inland and coastal sites.

A discrepancy in correlation between qPCR and culture-based results for morning and afternoon samples was consistently observed for samples taken at Great Lakes beaches during the NEARR epidemiology studies (USEPA 2010c; report 5 reviewed in this summary). Plots of qPCR counts of *Enterococcus* against culture counts of *Enterococcus* for one of the beaches for data collected at 8 AM and 3 PM are shown in Figure 3 and 4. Slopes of linear regression models of the log-transformed densities of the two data sets are significantly different, with the 8 AM samples exhibiting a slope much closer to 1 than the samples collected at 3 PM. These plots demonstrate the importance of sunlight inactivation on culture counts and the relative insensitivity of qPCR targets to solar radiation. In dark conditions culture and qPCR targets have shown different persistences, with persistence of naked DNA in seawater mesocosms on the order of three times longer than that of viable culturable enterococci (Walters et al., 2009). The insensitivity of qPCR targets to solar radiation and the much slower decay of qPCR targets underscore the necessity to account for ambient background DNA at recreational sites when using molecular-based monitoring techniques. Culture-based and qPCR results are well correlated at high FIB densities (viable and culturable fraction predominant). However, this correlation is lost at low

culture-based densities due to high variability of the qPCR in this range and predominance of VBNC over culturable bacteria and possibility due to the high uncertainty of qPCR assays when fewer than 100 cells (or qPCR targets) are present (WERF 2010b). These findings may be of significance in the comparison of inland and coastal waters if inland waters are generally more shaded than coastal waters, if beaches on inland waters tend to be impacted by “fresher” fecal pollution, or if the microbial ecology of inland and coastal sites are sufficiently different to impact the persistence of culturable cells and DNA from non-intact cells.

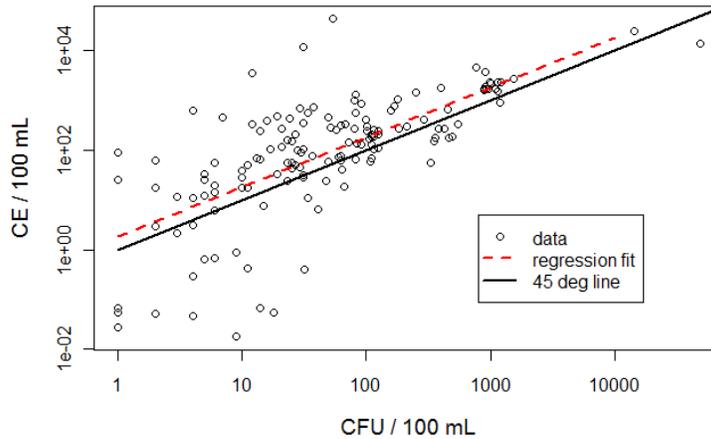


Figure 3. Paired qPCR and Culture *Enterococcus* Data: Huntington Beach: 8 AM Samples Only

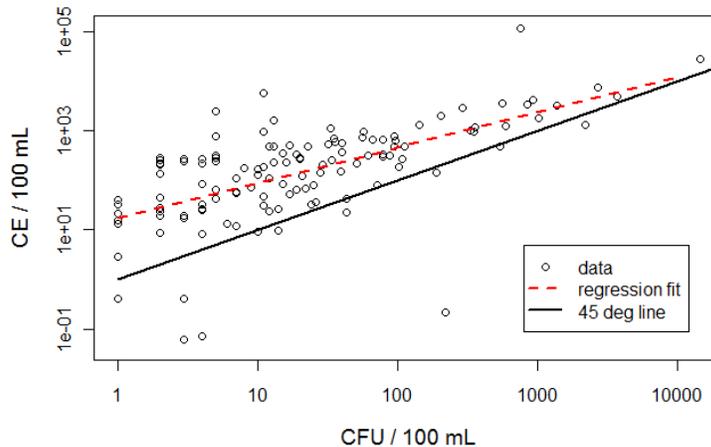


Figure 4. Paired qPCR and Culture *Enterococcus* Data: Huntington Beach: 3 PM Samples Only

Alternative molecular methods or refinements to qPCR may improve the ability of molecular methods to distinguish between live and dead cells. For example, Bae and Wuertz (2009) developed a modified qPCR propidium monoazide (PMA) to remove DNA from non-intact cells from the PCR reaction, resulting in better correlations with culture-based methods. For samples from the wastewater plant effluent, gene copies from qPCR with PMA were only 30 percent of those from qPCR without PMA. The difference between qPCR with and without PMA was greater than two orders of magnitude for samples of wastewater plant effluent. If optimized, these new techniques have the potential for making the analysis of coastal and inland waters more consistent.

POTW effluent

Strong reductions of cultivable FIB (2-5 orders of magnitude) have been observed throughout wastewater treatment, especially during secondary treatment and disinfection. Although this illustrates the ability of such treatments to inhibit cultivability, it does not necessarily demonstrate that the treatment trains have caused cell death. This is because qPCR counterpart inventories experience only small reductions or remain unchanged. Such dichotomy between method outputs is critical both from the standpoint of criteria design but also from the standpoint of public health, highlighting the potential for a large proportion of VBNC bacteria being released to recreational waters. In addition, because inland waters generally receive less-dilute chlorinated POTW effluent than coastal waters, the difference in qPCR and culture densities for inland waters impacted by POTW effluent could be greater than that for coastal waters.

Some water matrices may have a strong influence on the comparison of qPCR cell equivalents and MF CFUs. For example, PCR amplification efficiency may be lower in high turbidity waters than low turbidity waters. He and Jiang (2005) developed a qPCR assay for *Enterococcus* and evaluated the assay against MF for unchlorinated primary and secondary POTW effluent, chlorinated secondary effluent, and marine samples from multiple sites. For unchlorinated sewage, the difference between qPCR and culture enumerations of *Enterococcus* varies widely, with qPCR enumerations exceeding culture enumerations part of the time and below culture enumerations for other samples. Two plausible explanations for this finding, in addition to matrix-related effects, are that (1) qPCR results are uncertain and vary significantly between samples, or (2) qPCR and culture methods measure different features of bacteria and those features vary differently between samples. In the case of chlorinated secondary effluent, relatively high enumerations by qPCR potentially indicate the presence of dead cells or extra-cellular DNA. For samples from a marine environment (Table 4), qPCR results are consistently higher than those of culture methods.

Impact of Setting (Inland v. Coastal) on the Relative Abundance of qPCR and Culture Targets

Byappanahalli et al. (2010) found that the relative abundances of enterococci as measured by cultural methods and qPCR differed for samples taken at beach sites and for samples taken from a tributary discharging to Lake Michigan in the vicinity of the beaches. The authors found that the mean of the samples enumerated via cultural methods was not significantly different from that of the samples enumerated via cultural methods in the tributary. For the two beaches monitored in the study, the mean of the qPCR counts were 1.6 and 2.1 times the mean CFU counts and the mean cultural and qPCR counts were significantly different for both beaches. The authors discount inhibition in the lake water as the cause for significantly higher qPCR counts than culture counts because samples were analyzed after a 1:5 dilution employed to prevent inhibition. Further, the authors report that the qPCR *Enterococcus* counts in the tributary and the lake water were not significantly greater than those on the beach. These findings support slower removal of qPCR targets than culturable indicator bacteria as discharge from the tributary is advected to the beaches or other loading of qPCR targets to the beach sites from sources other than the tributary.

Telech et al. (2009) developed a series of regression models using several rapidly-measured environmental variables (e.g., rainfall, turbidity, number of bathers, wind speed and direction) for predicting qPCR and culturable (membrane filtration) *Enterococcus* counts at four Great Lakes beaches. Although the explanatory variables differed by beach for regression models

using either type of analytical detection method (i.e., qPCR or membrane filtration), the variables exhibiting the strongest relationship with Enterococcus densities in the models were consistent within the analytical detection method. The authors noted that meteorological, physical, water, and beach characteristics explained more variability in FIB densities measured by membrane filtration than by qPCR.

Lavender and Kinzelman (2009) developed an empirical model for correcting qPCR *Enterococcus* and *E. coli* densities (as CE) and improving their correlation with culture counts. Although empirical, their methodology relies upon a condition-specific correction and as such is related to the theoretical model described above. The study entailed collection of samples at several locations and analysis of the samples for *Enterococcus* and *E. coli* via membrane filtration and qPCR. Analysis of data found discordance between qPCR and culture data during rain events or when wave height was above a certain threshold. The authors attributed poor correlation under those conditions to increased densities of background DNA (not associated with viable culturable cells). To improve correlation between culture and qPCR densities, the authors proposed use of a correction factor for conditions associated with high background DNA densities. In the resulting model, if specific rainfall and wave heights are observed, the qPCR indicator density is reduced by a correction factor associated with those conditions and estimated from the data. The correction factors proposed by the authors were different for different sites. It is likely that correction factors and the conditions under which they are applied would be substantially different for inland and coastal waters.

Finally, report # 7 (Final report: Comparative Evaluation of Molecular and Culture Methods for Fecal Indicator Bacteria for use in Inland Recreational Waters (WERF 2010b)) reviewed for this study reports stronger associations of culture indicator density with qPCR indicator density of Great Lakes waters than for an effluent dominated river or an inland lake. This finding was based on logistic regression modeling performed on paired culture and qPCR analyses from Great Lakes waters, rivers, inland lakes, effluent dominated waters, and waters not dominated by POTW effluents. The authors of that study also noted that for the 3 qPCR methods used, low levels of qPCR targets were hard to analyze for cell numbers below 100 cells. This shortcoming of qPCR may influence the correlation between culture and qPCR cell densities if consistently low indicator densities are typical of an inland or a coastal site.

Summarizing, correlations between qPCR and culture counts differ among all sites, with distinct differences observed among inland and coastal sites when the two site types were compared directly. These differences may not be the result of intrinsic differences in inland and coastal sites but rather the result of site-specific differences in water quality or source characteristics for the sites studied.

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