Evidence Evaluation Report for Narrative Review in support of the NHMRC Recreational Water Quality Guidelines: Microbial Risks

Report Prepared for NHMRC

By Ecos Environmental Consulting Pty Ltd

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Executive Summary

Background

The National Health and Medical Research Council (NHMRC) commissioned Ecos Environmental Consulting Pty Ltd (Ecos) to conduct this Evidence Evaluation Report as part of a narrative review on microbial risks in recreational waters that will be used to update the *Guidelines for Managing Risks in Recreational Water* (NHMRC, 2008) (the Guidelines).

Microbial risks in recreational waters may arise from a range of point and non-point (diffuse) sources in the catchment area of the particular recreational water body. For the update to the Australian recreational water guidelines, NHMRC considers it important to determine if there are any site-specific issues that could lead to higher exposures or types of microbial pathogens that may be problematic in most recreational water use situations.

Methods

The review process followed a research protocol methodology developed specifically for this narrative review (O'Connor, 2020). The protocol involved a systematic search of several international databases of primary scientific research literature (Scopus, Web of Science, PubMed, Google Scholar) using search strings constructed from an extensive key word list. In addition to primary research literature, a search of grey literature, including existing recreational water quality guidelines and/or reports, was undertaken.

The search strings were constructed to identify literature citations relevant to a primary research question and two secondary research questions supplied by NHMRC's Recreational Water Quality Advisory Committee (the Committee). The primary research question was:

> *How can we monitor, assess and predict risks from diffuse and point source microbial contamination in recreational waters?*

The secondary questions were:

(i). *What are the indicators/surrogates of this/these risk/s?*
(ii). *What are the current practices to minimise or manage this/these risk/s?*

To assist in appropriate assessment of the literature, the search results were classified into two broad categories; (i) primary studies that were largely peer-reviewed journal articles and (ii) grey literature which were mainly regulatory guidelines or technical guidance publications produced by federal and state agencies in support of regulatory compliance goals.

The publication date-range for inclusion was from 2003 to October 30th, 2020.

The methodological quality of grey literature was assessed using administrative and technical criteria via an assessment tool which was developed by NHMRC based on common domains for assessing guidelines and systematic reviews such as the Appraisal of Guidelines, Research and Evaluation (AGREE) tool. For primary studies, a critical appraisal tool was used to undertake a quality assessment. The tool was based on approaches described by the Critical Appraisal Skills Program (CASP, Oxford CVTH, 2020) with a risk-of-bias rating similar to that used in the US Office of Health Assessment and Translation (OHAT) Risk-of-Bias Tool (OHAT, 2015).

A process based on the OHAT approach to using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system was used to assess the certainty of the body of evidence from primary studies and permit upgrading or downgrading of the body of evidence, as appropriate (OHAT, 2019). For existing guidelines, the assessment methodology for the body of evidence drew on the GRADE approach as well as the outcomes of relevance to the primary and secondary research questions.

Results

Searches for grey literature identified 38 documents. Each document was evaluated for its relevance to the primary and secondary research questions and if determined to not be relevant was screened out. This process identified 16 grey literature documents relevant to the primary and secondary research questions. Following quality assessments, 10 documents were considered ineligible on quality grounds, leaving six grey literature documents for evaluation and synthesis.

For primary studies, search results by database yielded 7381 citations initially and after removal of duplicates the numbers was 2563. Further screening using relevancy and logical exclusion criteria (e.g. limiting studies to comparable Organisation for Economic Co-operation and Development [OECD] countries) to control the volume of literature to review, identified 42 studies for final detailed assessment. These studies were assessed for eligibility on the basis of quality, including risk of bias, which resulted in 13 studies remaining for evaluation and synthesis.

For the six grey literature documents and 13 primary studies eligible for evaluation, data for evaluation was extracted from each document into standard forms developed for the purpose. The information contained in the quality assessment and data extraction forms was used in this Evidence Evaluation Report.

Conclusions

Primary research question

In response to the primary research question evidence was sought from quality grey literature and primary studies that addressed monitoring, assessment, and prediction of risks from microbial diffuse and point sources in recreational waters. Given the broad nature of the research question, there was substantial heterogeneity in the literature which made it challenging to distil the review findings. Similarly, there was some overlap with the secondary research questions, particularly with respect to the use of indicators and/or surrogates of microbial risk. Some broad themes, consistent with the primary research question, were present in the selected literature:

- The use of quantitative microbial risk assessment (QMRA) to evaluate alternative pollution events and management scenarios.
- Systematic review findings broadly supportive of current paradigms of sources of microbial risks to recreational waters, types of situations or events increasing risk of gastrointestinal illness (GI), and classificatory approaches to management of GI risk.

- Limited but emerging recognition of the greater susceptibility of children to health effects from exposure to pathogens in recreational waters.
- Retention of existing faecal indicator bacteria (FIB) *E. coli* and enterococci for monitoring and assessing the extent of faecal contamination of recreational waters and thus GI risk.
- Availability of quantitative polymerase chain reaction (qPCR) enterococci criteria values to trigger implementation of beach management actions and enterococci calibrated directly to observed health risk in epidemiological studies.
- Further research opportunities for microbial source tracking (MST), QMRA, antimicrobial-resistant bacteria (AMRB) and standardisation of methods.
- A positive relationship in recreational waters between the relative contribution of human faecal matter among pollution sources and greater health impacts on users compared to animal sources.
- Positive association between increased exposure rates in marine waters and higher levels of FIB and in GI and respiratory illness (RI) in swimmers including surfers.
- Recognition that certain beach geomorphology attributes and certain beach management practices greatly influence the probability of compliance with regulatory standards.
- Positive relationship between rivers flows and increased concentrations of FIB, biomarkers and pathogens in estuarine recreational waters.

GRADE assessment

The GRADE assessment of the overall quality of the primary studies body of evidence was undertaken for the outcome identified for the primary research question. The overall certainty rating was "moderate" for two studies (a systematic review and cohort study), leading to a final certainty rating of "*moderately confident in the reported associations*". The overall certainty rating was "low" for 11 qualitative studies and this led to a final certainty rating of "*limited confidence in the reported associations*". This result stems from the high degree of heterogeneity in study focus of the 13 studies that made it through the screening and quality assessment stages of the literature review. The broad nature of the primary research question resulted in a wide range of study types being eligible for inclusion and consequently it was not possible to apply gradings for some the categories in the GRADE assessment (e.g. magnitude of effect). None of the factors that could influence a change in the grading of certainty of the body of evidence were identified.

Secondary research questions

Evidence for the secondary research questions was limited to existing grey literature as outlined in the research protocol, with different suites of documents relevant depending on the research question.

In relation to secondary research question 1, from the review of grey literature documents commentary was provided on the use of genetic markers in MST where it was noted that although the concept behind MST is conceptually clear, quantification of sources relies on a number of assumptions which often are not fully met or are untested and that opportunities remain for further refinement of qPCR methodologies underpinning MST. It was concluded that such issues need to be clarified and some level of standardisation of methods is required to assist in the incorporation of MST methods within regulatory frameworks.

For secondary research question 2, evidence discussed in support retention of the existing NHMRC management framework which involves microbial-based categorisation of the water using a combination of sanitary inspection, microbial water-quality assessment, and prevention of exposure at times of increased risk. Furthermore information, supporting improvements and augmentation of details for the existing guidelines is described. Of particular relevance here is the recent NSW Department of Planning, Industry and Environment *Protocol for Assessment and Management of Microbial Risks in Recreational Waters.*

Concluding comments

An evaluation of evidence contained in six grey literature documents and 13 primary research studies provided satisfactory evidence to support detailed responses to the primary and secondary research documents. The findings from each study are described in the text and summarised in tables. This is followed by thematic summaries based on the major themes identified relevant to each of the three research questions (1 primary and 2 secondary).

Finally a section responding to a series of additional questions posed by the Committee is included which draws on the evidence identified for the review.

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Glossary

AGREE	Appraisal of Guidelines for Research and Evaluation
AHMC	Australian Health Ministers Conference
AMR/AMRB	Antimicrobial resistance/ Anti-microbial resistant bacteria
BAV	Beach action value
BMDL	Benchmark dose level
BWD	Bathing Water Directive
CASP	Critical Appraisal Skills Programme
CI	Confidence interval
DEFRA	Department for Environment, Food and Rural Affairs
DNA	Deoxyribonucleic acid
DWO	Dry weather overflow
EC	European Commission
EPHC	Environment Protection and Heritage Council
EPA	Environmental Protection Agency
EU	European Union
FIB	Faecal indicator bacteria such as *E. coli* or enterococci
GI	Gastrointestinal illness
GRADE	Grading of Recommendations Assessment, Development and Evaluation

HAdV	Human Adenovirus
HTS	High-throughput sequencing
ISO	International Organization for Standardization
LLOQ	lower limit of quantification
MAC	Microbial water assessment categories
MST	Microbial source tracking
NHMRC	National Health and Medical Research Council
NIWA	National Institute of Water and Atmospheric Research
NoV	Norovirus
NRMMC	National Resource Management Ministerial Council
NSW DPIE	New South Wales Department of Planning, Industry and Environment
OECD	Organisation for Economic Co-operation and Development
OHAT	Office of Health Assessment and Translation
OR	Odds ratio
PCR/qPCR	Polymerase Chain Reaction / Quantitative polymerase chain reaction
$p_{(ill)}$	Probability of illness (a metric commonly used in QMRA)
PLOQ	Process limit of quantification
PrCR	Primary contact recreation
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QMRA	Quantitative microbial risk assessment
RBT	Risk-based threshold
RI	Respiratory illness
RNA	Ribonucleic acid
RSD	Relative standard deviations
RWQAC	Recreational Water Quality Advisory Committee (the Committee)
RWQC	Recreational Water Quality Criteria
SeCR	Secondary contact recreation
SourceTracker	A statistical tool for assessment of faecal contamination of coastal waters
SEPP	State Environment Protection Policy
STP	Sewage treatment plant
US EPA	United States Environmental Protection Agency
USGS	United States Geological Survey
WHO	World Health Organization
WWO	Wet weather overflows

1 Introduction

The National Health and Medical Research Council (NHMRC) commissioned Ecos Environmental Consulting Pty Ltd (Ecos) to conduct narrative reviews on two of four research topics that will be used to update the *Guidelines for Managing Risks in Recreational Water* (NHMRC, 2008) (the Guidelines).

The two research topics to be addressed by Ecos are Microbial Risks and Chemical Hazards. The other two topics (Cyanobacteria and Algae and Free-living Organisms) will be addressed elsewhere. This document addresses Microbial Risks.

Microbial risks in recreational waters may arise from a range of point and non-point (diffuse) sources in the catchment area of the particular recreational water body. There may also be site-specific issues that could lead to higher exposures or types of microbial pathogens that may be problematic in recreational water use situations. For the update to the Australian recreational water guidelines, NHMRC considers it important to determine the current status of the evidence for any potential human health risks resulting from exposure to microbial risks in recreational water, including any site-specific issues. The review will provide NHMRC with an independent body of evidence to assure that the revision of the Guidelines is based on the most up-to-date and relevant scientific literature.

Ecos developed a research protocol to guide the review of the evidence (O'Connor, 2020). The research protocol sets out the methods to be used for the review including the research questions, population groups, health outcomes of interest, and a structured search and evaluation strategy. This Evidence Evaluation Report summarises the methodology used to find and select the studies and the findings of the literature search and evaluation process. It synthesises the results of key studies identified in the evaluation process into evidence statements and assesses this body of evidence taking into account its strengths or limitations.

A draft of this Evidence Evaluation Report was considered by the Committee who provided advice on its revision.

1.1. Purpose and objectives of review

The purpose of the microbial risks review is to inform the update to information provided in Chapter 5 of the 2008 NHMRC Guidelines (NHMRC, 2008) and any relevant sections throughout the rest of the document with respect to the microbial risks associated with the recreational use of water. This review, undertaken using a systematic approach, aims to provide NHMRC with an independent body of evidence to assure that the revision of the Guidelines is based on the most up-to-date and relevant scientific literature.

2 Methodology

2.1. Review Period

Publications published between January 1st, 2003, and 30th October 2020 were considered for the review. Although the current NHMRC Guidelines were published in 2008, extending the date range back earlier was done to assist in locating any documents that may have been overlooked, have become recognised as being of greater importance since that time or missed the cut-off period during the preparation of the guidelines.

2.2. Definitions

In this Evidence Evaluation Report, “microbial risk” refers to risk associated with the contamination of the water by frank human pathogens, mostly of faecal origin, and excludes risk associated with free-living microorganisms such as saprozoic bacteria and protozoa which are generally considered as opportunistic pathogens (these are to be covered in a separate review).

Definitions of types, uses and users of recreational water are given in Appendix 1 of the companion Technical Report to this study (O’Connor, 2022).

2.3. Research Questions

The research questions that form the basis of this review were developed by the Committee. There is one primary question and two secondary questions.

2.3.1. Primary question

The primary question is: *How can we monitor, assess and predict risks from diffuse and point source microbial contamination in recreational waters?*

To answer the primary question the Committee provided the following guidance points:

- Provide examples of what is done in Australian and international jurisdictions and their reasoning.
- Determine what is done in other settings and how this relates to the Australian context.
- Determine how specific target populations such as children, immunocompromised or the elderly are impacted.
- Determine the main factors impacting risk and its prediction (environmental, microbial, etc.).
- Identify gaps and opportunities to design a risk assessment framework that would provide an estimation of the risk truly reflective of adverse health outcomes in various settings relevant to the Australian context.

2.3.2. Secondary questions

The secondary questions are:

(i) *What are the indicators/surrogates of this/these risk/s?*

Committee guidance points:

- Review the new technologies available to assess and monitor risks and determine how they could be practically applied to Australian recreational waters
- Describe the relationship between the indicator and surrogates with adverse health outcomes. Include how this relationship been demonstrated in settings relevant to Australia.

(ii) *What are the current practices to minimise or manage this/these risk/s?*

Committee guidance points:

- Provide examples of how mitigation strategies have been developed based on scientific evidence.
- Provide examples/case studies of how this is achieved/implemented in settings relevant to the Australian context.

To keep the workload manageable within the available resources the Committee agreed that the secondary research questions could be addressed through a review of existing guidance or reviews rather than though a review of primary studies (see Section 2.5.1 for definitions of these categories).

2.3.3. Additional commentary and guidance from Committee

Whilst the above questions were the focus of the narrative review, the Committee noted that the 2008 NHMRC Guidelines were centred around marine waters and a risk model based on a study conducted in an oceanic setting in the United Kingdom (UK) with a point source of pollution of human origin. Such an approach excludes consideration of freshwaters as well as zoonotic pathogens and their sources with the exception of some pathogens or indicator organisms that infect both humans and other animals. Therefore, the current review considered the risks to recreational water quality from all sources of pathogens in marine, freshwater and estuarine environments.

Since the publication of the 2008 NHMRC Guidelines, the field of risk assessment (in particular QMRA) has become well established and new technologies to monitor indicators and pathogens have been developed. Therefore, in preparing our responses to the main research questions listed above, the Committee suggested that the narrative review should consider a number of additional questions based on the scientific evidence produced since 2003 (Table 2-1). These questions were addressed based on evaluation of the body of evidence.

Table 2-1. Additional questions and tasks required to answer questions for supporting main research questions for this narrative review.

Additional Questions	Tasks required to answer questions
(i) What are drawbacks of the interpretation of risks provided by the previous guidelines when applied to the Australian context? (ii) What happens when pollution is from non-point sources or when pollution is mainly associated with sources other than human? (iii) Can a new framework be developed to take into account these variations and truly reflect potential health outcomes in different settings (including in freshwaters)? (iv) Can the previous values be retained as default values in absence of a risk assessment process? (v) Can source tracking be a part this framework in identifying sources of contamination?	• A brief review of the current science relating faecal indicator bacteria to pathogen presence and public health risk to identify potential gaps in existing guidance. For example, can we use the same indicator(s) for fresh and marine waters? Are they relevant for all seasons and all regions of Australia? • Review of the potential alternatives or secondary indicators as reported in the literature and/or used in international guidance, regulation and practices (for example, *Clostridium perfringens*, bacteroides, 16s microbial community fingerprinting, bacteriophages, direct pathogen monitoring, non-microbial indicators, etc.) • A quick review of new technologies and methods for quantifying indicators, tracking sources and assessing risk. This should include sample analysis times and any issues associated with analytical variability. • Guidance on single-sample water quality triggers for short-term water quality assessment. • Review of QMRA approach to recreational water assessment to inform a methodology for inclusion in the Guidelines. • Practical implementation and consideration for a tiered approach to risk assessment. • State of knowledge for recreational waters in relation to climate change, emerging pathogens and antimicrobial resistance (AMR).

2.4. Search Strategy and Selection of Evidence

2.4.1. Overview

The specific steps involved in finding and selecting the evidence for review were:

- Preparation of a list of keywords (search terms) (Appendix 2) for approval by the Committee;
- Classification of the list into logical categories related to population, location, study type, property or attribute, method, health outcome, carrier (type of water), exposure pathway (includes split into sub-categories) and source. The classification was performed to assist in composing search strings;
- Development of search strings based on the keywords and preparing combinations of strings to create search strategies;
- Using the composed search strategies to search key life-science literature databases (Scopus, Web of Science, PubMed) and the general technical literature database, Google Scholar;
- In addition to the above literature, a search of grey literature, including existing recreational water quality guidelines and/or reports, was undertaken based on a list provided by the Committee (Technical Report Appendix 3: O’Connor, 2022) plus a search of websites of key international environmental and public health agencies such as the World Health Organization (WHO) and the United States Environmental Protection Agency (US EPA).;

- Lists of retrieved publications were exported to Microsoft Excel spreadsheets and sorted and filtered based on relevancy and quality, including risk of bias, using methods described in detail in subsequent sections below; and
- Shortlisted literature was imported from Excel into a bibliographic software package (Zotero) for management of associated PDF documents and for reporting on the results of the literature search.
- The shortlisted literature was subject to further screening during the quality assessment steps.

2.4.2. Inclusion and exclusion criteria

When conducting the literature search exercise, the criteria for literature inclusion or exclusion were:

- English language studies only were included;
- Human health outcomes only were included
- Publication date-range for inclusion was from 2003 onwards. As noted earlier the date range for review was extended back to 2003 which is 5 years prior to the publication of the 2008 NHMRC Guidelines. Extending the date range back earlier was done to assist in locating any documents that may have been overlooked or have become recognised as being of greater importance since that time or missed the cut-off period during the preparation of the guidelines;
- Peer reviewed publications only were considered, except for certain grey literature reports and guidelines from reputable international and national agencies (e.g., WHO, US EPA, State and Commonwealth Departments of Health, State EPAs, etc.). It is likely that such documents have undergone a peer-review process, however the process is not always clearly documented;
- Relevancy –studies that were not relevant to the Guideline or review scope were excluded, e.g., studies on dental hygiene.
- The study location was a developed country (excluding external territories) that was listed as a member of the OECD on the OECD website: (http:// http://www.oecd.org/about/members-and-partners/).

However, since initial search results yielded a very large selection of potentially relevant studies, the Committee approved some changes to the research protocol in the relation to the primary research question (Table 2-2) to reduce the large volume of literature required for review.

Table 2-2. Changes to the microbial risks research protocol to reduce the review workload.

Initial research protocol	Changes	NHMRC specifications
Method: Review relevant primary studies to answer the primary research question. *Literature search period*: Include all relevant primary studies (Australian and international) from 2003	The method was modified to allow for reviews to be used instead of primary studies over certain time periods. Literature search periods were amended accordingly to reflect the time coverage of the reviews. This reduced the number of primary studies to be assessed.	The selected reviews were appraised using the appropriate screening criteria outlined in this section (2.4). This includes assessing relevance for the Australian context and scope of our guidelines and the quality of the review process itself. The reviews were cross-checked against the results of the literature search to ensure that they cover the period 2003-2017. Any relevant primary

Initial research protocol	Changes	NHMRC specifications
onwards that meet inclusion criteria.	*International data:* Use three reviews (King, Exley, et al., 2014; US EPA, 2017; WHO, 2018) to cover the period up to 1 Jan 2017. Use primary studies from the literature search from 1 Jan 2017 to 30 Nov 2020. *Australian data:* Include all primary studies/reports found from 1 Jan 2003 to 30 Nov 2020	studies/reports from this period not listed in the reviews was also screened for eligibility. The findings from the reviews are included in a Summary of Findings table in the Evidence Evaluation Report, alongside the findings from the review of primary studies.

Apart from the exclusions listed above, all study other types were included (e.g., local and international surveys; peer-reviewed publications or government reports or guidelines for indicators). The resulting list of studies was subject to further screening and filtering based on more refined criteria for quality as described in Section 2.4.2 below.

2.5. Evidence Collection

2.5.1. Classification of the evidence

Literature types

To assist in appropriate assessment of the literature, the search results were classified into two broad categories;

(i) primary studies that were peer-reviewed journal articles and

(ii) grey literature which refers to literature produced by organisations other than conventional academic journal publishers and includes reviews, regulatory guidelines or technical guidance publications produced by federal and state agencies in support of regulatory compliance goals.

2.5.1.1. Grey literature

Grey literature documents are generally structured as larger documents seeking to integrate a wide range of topics in support of national regulatory goals (e.g. US EPA documents), more general international guidance (e.g. WHO documents) or strategic reviews for particular agencies in order to support subsequent regulations or guidance. In comparison, primary studies are mainly focused on the results of original research undertaken in response to narrowly focused study objectives. For the purposes of this evidence evaluation report and corresponding technical report, grey literature, although it may be constructed around reviews of the literature, should not be confused with the term “systematic review” used in the primary studies classification below as systematic reviews generally have a much narrower focus and are published in peer-reviewed journals. Grey literature is usually overseen by expert committees convened by national or international authorities and have many contributing authors, whereas systematic reviews may frequently be carried out by individual researchers or small groups of researchers.

2.5.1.2. Primary studies

The quality of each study to be included was assessed using an appraisal tool based on the CASP (Oxford CTVH, 2020)[1] quality assessment protocols for observational studies with an additional risk of bias rating similar to the OHAT Risk-of-Bias tool (OHAT, 2015). To assist in the selection of appropriate CASP tools, the studies were categorised according to a selected list of CASP study type definitions based on the adopted research protocol (O'Connor, 2020). These study categories were used to guide a critical appraisal of study quality and selection for the review.

1. Systematic review
2. Qualitative research
3. Case control study
4. Cohort study
5. Diagnostic test study
6. Randomised controlled trial
7. Cross-sectional study (mix of case-control and cohort)
8. Quantitative research

See the Technical Report (O'Connor, 2022) for definitions.

2.5.2. Quality assessment (by types)

2.5.2.1. Grey literature

The methodological quality of grey literature was assessed using administrative and technical criteria via a draft assessment tool developed by NHMRC. The criteria listed in the tool were based on common domains that have been evaluated in several existing tools for assessing guidelines and systematic reviews (e.g. AGREE tool: Brouwers, Kerkvliet, et al., 2016; AGREE Next Steps Consortium, 2017). A form for capturing the data is described in the Technical Report (O'Connor, 2022). Based on the responses in the form, a decision was made on whether a grey literature document should be included or excluded from the review on quality grounds.

In addition to this formal quality assessment approach, the close inspection of the full text document at this stage in some cases indicated that the evidence contained in the document did not satisfactorily contribute to answering the primary and/or secondary research questions. Where that was the case, the document was classed as "Excluded from further review" and the reason for the exclusion noted in the table.

2.5.2.2. Primary studies

Critical appraisal of evidence

As noted above, the CASP study categories were used to guide a critical appraisal of study quality and selection for the review

The CASP protocol considers three broad issues in appraising a study:

(i) Are the results of the study valid?

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[1] For further information on each CASP checklist see https://casp-uk.net/casp-tools-checklists/

(ii) What are the results?
(iii) Will the results help locally?

Depending on the type of study, 10 to 13 questions were posed within the three categories above that are designed to assist the reviewer to consider the issues systematically.

Primary data studies selected for review were assessed for internal validity, which is also known as "risk of bias", with bias classification according to the OHAT risk of bias assessment tool (OHAT, 2015). The tool provides a colour-coded visual scheme to summarise risk of bias assessments and this scheme was applied based on the response to the CASP questions (Table 2-3).

Table 2-3. OHAT risk of bias scheme categories (OHAT, 2015)

Symbol	Description
++	***Definitely Low*** *risk of bias*: There is direct evidence of low risk of bias practices. May include specific examples of relevant low risk of bias practices.
+	***Probably Low*** *risk of bias*: There is indirect evidence of low risk of bias practices **OR** it is deemed that deviations from low risk of bias practices for these criteria during the study would not appreciably bias results, including consideration of direction and magnitude of bias.
-	***Probably High*** *risk of bias*: There is indirect evidence of high risk of bias practices If there is insufficient information provided about relevant risk of bias practices, "not reported" or "NR" may be used instead of the minus symbol "-".
--	***Definitely High*** *risk of bias*: There is direct evidence of high risk of bias practices. May include specific examples of relevant high risk of bias practices.

OHAT (2015) provides rigorous protocols that can be applied to case control studies, cohort studies, diagnostic test studies, randomised controlled trials, and cross-sectional studies. For these study types it was originally planned to apply the OHAT Risk-of-Bias Tool (OHAT, 2015) and to develop similar risk of bias assessment criteria for the remaining categories of systematic reviews, qualitative and quantitative studies. However, it was determined that the OHAT tool was not suitable for many of the shortlisted studies remaining after the screening process. Consequently, the CASP critical appraisal tools were used to conduct quality assessments for all study types because they were simpler and more efficient to use systematically across different study types. While not completely overlapping, the CASP tools apply similar questions relating to some of the key OHAT risk of bias domains for evaluating the internal validity of a primary study and the quality of its research findings.

The OHAT tool provided a colour-coded visual scheme to summarise the risk of bias assessments and was applied based on the response to the CASP question. The combination of the two critical appraisal tools allowed for a reasonable assessment of study quality within the resources available for the review.

Once a determination of risk of bias for each domain was made, a visual summary of the risk of bias ratings for the included studies was prepared and used to determine overall risk of bias across the body of evidence.

2.6. Data Extraction

Data were extracted from individual studies using standardised data extraction forms designed for each class of literature. Samples of the data extraction forms are presented in the Technical Report (O'Connor, 2022).

2.7. Process for Assessing the Body of Evidence

2.7.1. Overview

The evidence collected and appraised for each research question was grouped by study type and outcome where possible and summarised in an Evidence Summary table that assigned the level of certainty (or confidence) in that body of evidence. Due to the different nature and quality of evidence between grey literature and primary studies, different approaches were required to review and evaluate the body of evidence for each class of literature. The assessment methodology for each literature class is described in the following sections.

2.7.2. Assessment of the body of evidence – primary studies

A process described by Ryan and Hill (2016) based on the OHAT approach to using the GRADE system (developed by Guyatt, Oxman, et al., 2011) was used to assess the certainty of the body of evidence from primary studies. Evidence streams for each research question were tabulated together by outcome, where possible. The domains used to assess certainty in the GRADE framework were applied to the body of evidence, after which an overall certainty rating was then assigned to each evidence stream. The domains are:

- Overall risk of bias across studies;
- Unexplained inconsistency;
- Imprecision;
- Indirectness; and
- Publication bias.

Each evidence stream was assigned an initial certainty rating based on the form shown in Table 2-4, which is based on guidance from the OHAT Handbook (OHAT, 2019). For example, evidence from randomised controlled trials could initially be graded as high certainty and evidence from qualitative studies could be initially graded as low certainty.

Table 2-4. Summary of findings – body of evidence form (adapted from OHAT Handbook (OHAT 2019) and transposed to fit page).

Item	Classification	Description	Research question	Outcome 1.* Study Type **		Outcome 2. etc. Study Type	
				1	2	1	2
Body of evidence	*Evidence stream or study type*	*(# studies) initial certainty rating*	Research question: e.g. *How can we monitor, assess and predict risks from diffuse and point source microbial contamination in recreational waters?*				
Risk of bias	*Serious, not serious, unknown*	Describe trends, key questions, issues					
Unexplained inconsistency	*Serious, not serious, not applicable*	Describe results in terms of consistency, explain apparent inconsistency (if it can be explained)					
Indirectness	*Serious or not serious*	Discuss use of upstream indicators or populations with less relevance, any time-related exposure considerations (see OHAT Risk-of-Bias tool)					
Imprecision	*Serious, not serious, unknown*	Discuss ability to distinguish treatment from control, describe confidence intervals (if available)					
Publication bias	*Detected, undetected, unknown*	Discuss factors that might indicate publication bias (e.g., funding, lag)					
Magnitude of effect	*Large, not large, unknown*	Describe magnitude of response					
Dose Response	*Yes, no, unknown*	Outline evidence for or against dose response					
Residual confounding	*Yes, no, unknown*	Address whether there is evidence that confounding would bias toward null					
Consistency across species/model	*Yes, no, not applicable (NA)*	Describe cross-species, model, or population consistency					
Other reason to increase confidence?	*Yes or no*	Describe any other factors that increase confidence in the results					
Final certainty rating (GRADE assessment)	***High, moderate, low or very low***	List reasons for downgrading or upgrading					

*e.g. PECO listed hazard measured in recreational waters, ** e.g. Qualitative studies, Systematic review etc.

2.7.2.1. Outcome definition and prioritisation

Definitions of outcomes and the outcome measures to be included in the review were developed based on:

(i) general guidance supplied by NHMRC and the Committee,
(ii) the research questions, and;
(iii) the nature of the available literature.

The outcomes to be included in this review are presented in Table 2-5. Note that to keep the workload manageable within the available resources the Committee agreed that the secondary research questions could be addressed via a review of existing guidance or reviews (in the grey literature) rather than though a review of primary studies.

Table 2-5. Outcomes from the review to be included in the evidence evaluation

Outcome	Definition of outcome	Examples of health impacts under this outcome	Outcome measures	Rationale for selecting this outcome
Primary research question: *How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?*				
Method described for monitoring, assessing and predict risks from diffuse and point source microbial contamination in recreational waters	Study describes methods for assessing and predicting microbial risks in recreational waters from diffuse and point sources.	Rates of gastro-intestinal illness linked to exposure, or concentrations of pathogen or indicator measured above regional or national exposure criteria for protection of human health. Health impact recorded (GI illness), or inferred due to criteria exceedance for pathogens or indicators.	Measured or predicted rates of gastrointestinal illness, or concentration of pathogens or indicators in recreational waters in relation to criteria for human health protection.	Descriptions of methods for assessing and predicting microbial risks in recreational waters from diffuse and point sources match the stated requirements of the primary research question.

2.7.2.2. GRADE assessment

Under the GRADE system, the overall quality of the evidence for an outcome was categorised as High, Moderate, Low or Very Low, reflecting the degree of confidence in the effect estimate (Table 2-6).

Table 2-6. GRADE ratings and their interpretation from Ryan and Hill (2016)

Symbol	Quality	Interpretation
⊕⊕⊕⊕	**High**	We are very confident that the true effect lies close to that of the estimate of the effect.
⊕⊕⊕O	**Moderate**	We are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
⊕⊕OO	**Low**	Our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
⊕OOO	**Very Low**	We have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

To assist in the development of the GRADE assessment, an evaluation summary matrix for each primary study was constructed (Table 2-7). The summary matrices assisted in developing a response to each of the GRADE assessment categories.

Table 2-7. Evaluation summary matrix of individual primary studies

Item	Description
Study, Design, Quality	Study ID, Type of study, Quality assessment
Population	Population studied (e.g. adult, children, etc.)
Exposures	Exposure pathway, identity of microbial hazards studied
Location type	Type of location
Outcome	Metrics constructed for evaluation
Analysis	Nature of the statistical analysis conducted on the data
Results	The value of field measurements or metrics used to evaluate the effect studied
Effect estimate	The magnitude of the measured values or metrics
Significance	The level of significance of any test of a statistical hypothesis (i.e. p-value)

2.7.2.3. Upgrading or downgrading certainty of evidence

The certainty of the evidence was downgraded or upgraded from the initial rating if any of the conditions in Figure 2-1 (elaborated in Table 2-8) were met. If none were met, the initial certainty rating was kept. These domains are explained in more detail in the OHAT Handbook (OHAT, 2019). Conflicts of interest and funding sources were also considered as a reason to downgrade if there were serious concerns that these had influenced the findings from the body of evidence.

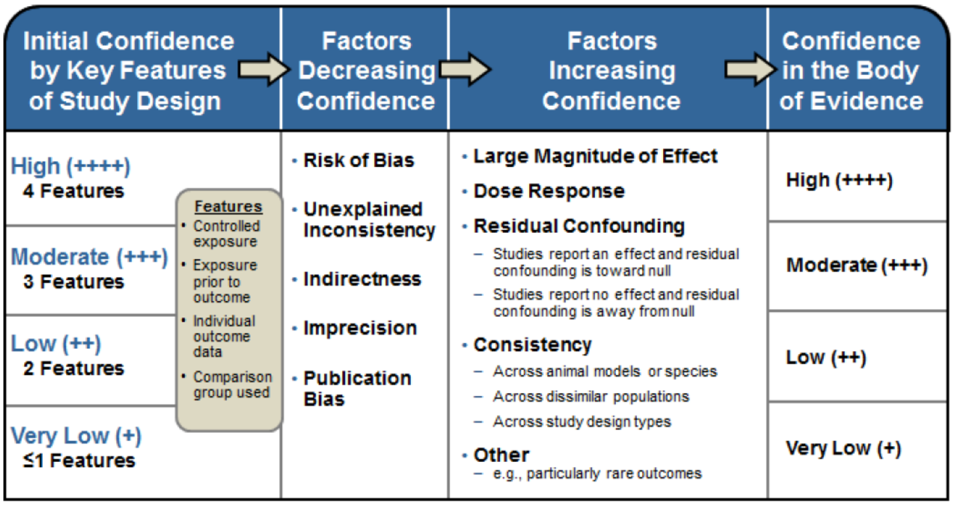

Figure 2-1. OHAT method for assessing confidence in the body of evidence (OHAT, 2019)

Table 2-8. OHAT reasons for down grading or upgrading certainty of evidence (OHAT, 2019)

Reasons to Downgrade	Reasons to Upgrade
• **Risk of bias** - Serious or very serious concerns about study quality across the body of evidence (reliability) (see Appendix 9 of Technical Report: O'Connor, 2022) • **Unexplained inconsistency** - Important inconsistency of results across the included studies that can't be explained by study design • **Indirectness** - Some or major uncertainty about directness (relevance to the research question that is being answered) • **Imprecision** - Imprecise or sparse data • **Publication bias** - High probability of reporting bias (selective reporting of results across the body of evidence that might skew results)	• **Consistency** - Strong or very strong evidence of association based on consistent evidence from two or more observational studies, with no plausible confounders • **Magnitude of effect** - Very strong evidence of association based on direct evidence with no major threats to validity • **Dose-response** - Evidence of a dose-response gradient • **Residual confounding** - All plausible confounders would have reduced the effect • **Other reasons** – any topic-specific reasons as determined by experts in the field

The results of the certainty assessment process were tabulated in a similar manner to that described for the OHAT risk of bias assessment tool (OHAT, 2019). Where a conclusion was unable to be made by the reviewer around any of the domains this was recorded as 'not applicable' or 'unknown'.

2.7.3. Assessment of the body of evidence for grey literature

Grey literature documents have been largely developed with the goal of providing guidance for management of water quality for differing environmental requirements and generally contain no primary data but are usually informed by an evidence review. If the GRADE criteria (summarised in Table 2-4) were to be applied to the grey literature, the results would often be weak or null responses, and since no effect estimates are reported, no determination of a final certainty rating can be made. The grey literature documents do contain authoritative information that collectively represents the current state of knowledge and practice on microbial risks from recreational water quality, and therefore is worthy of inclusion in this Evidence Evaluation Report.

Consequently, the assessment methodology for the body of evidence from grey literature drew on the GRADE approach as well as the outcomes of relevance to the primary and secondary research questions described in Table 2-4. The criteria for assessing the body of evidence for grey literature in Table 2-9 were largely derived from the guidance and commentary supplied by the Committee (Section 2.3.1) to assist in developing responses for the research questions.

Table 2-9. Criteria for assessing the body of evidence for grey literature

Item	Description (responses)
Grey literature	Document identity
Contribution to primary research question outcome?	Does the document contain any information useful for responding to the primary research question? (Yes or No)
Contribution to secondary research questions outcomes?	Does the document contain any information useful for responding to the secondary research questions? (If Yes list which question, No)
Outcomes relevant to Australian conditions	Does the document contain descriptions of methods or approaches relevant to Australian conditions (If Yes, list information, No)
Addresses target populations	Does the document address specific target populations such as children, immunocompromised or the elderly and describe how they may be impacted? (If Yes, list information, No).
Identifies main factors impacting risk and its prediction	Does the document clearly identify the main factors impacting risk and its prediction? (If Yes, list information, No)
Reviewer’s comments	Reviewer’s comments on key attributes of the document justifying its inclusion in the review.
Overall assessment	Reviewer’s concise assessment of the overall contribution made by the document in responding to the primary and secondary questions.

3 Literature search results

3.1. Grey literature

Searches for grey literature[2] identified 38 documents. Twelve documents were screened out on the basis that they were non-Australian studies and preceded the agreed cut-off date for inclusion, whilst each of the remaining documents was evaluated for its relevance to the primary and secondary research questions and if not relevant was screened out.

This process identified 16 documents relevant to the primary and secondary research questions (i.e., microbial risks in recreational water). Each of the 16 shortlisted documents was further assessed for eligibility by a full-text quality assessment process described in Section 2.5.2.1 which resulted in 10 documents being screened out on quality grounds. The final list of 6 grey literature documents retained by meeting the eligibility criteria are shown in Table 5-1, Section 5.1.

The screening process is summarised graphically in Figure 3-1.

3.2. Primary studies

Search results by database yielded 7381 citations initially and after removal of duplicates within each database (i.e. Scopus, Web of Science, PubMed) this number was reduced to 3629 citations. After combining the lists, further removal of citations that were duplicated between the databases brought the number of citations down to 2563. Text recognition script in the programming language, R, was used to identify duplicates at this stage, although some duplicate records remained due to minor character differences that the script was not able to detect. These duplicates were removed in later screening using Excel tools.

The combined list of citations from the literature search, after removal of duplicates was subjected to an iterative screening process via a number of steps. The first steps involved screening titles for relevancy and exclusion of non-OECD citations. At this stage, an additional 29 citations were added from lists supplied by the Committee. These steps left 349 citations for screening via; (i) removal of non-Australian studies that were cited in the key reviews; (ii) removal of non-Australian studies addressing a topic explicitly addressed in the key reviews, or (iii) assessment of abstracts gave other reasons for exclusion (e.g. obsolete methods, out of scope etc. see section 3.2.1 of the Technical Report (O'Connor, 2022) for details).

After screening, 42 full text articles were assessed for eligibility (see Section 1.1) of which 29 were excluded on the basis of quality. A description of the stepwise screening process is summarised graphically in Figure 3-2.

[2] See Technical Report (O'Connor 2022), Section 2, for method.

PRISMA Flow Diagram – Grey Literature

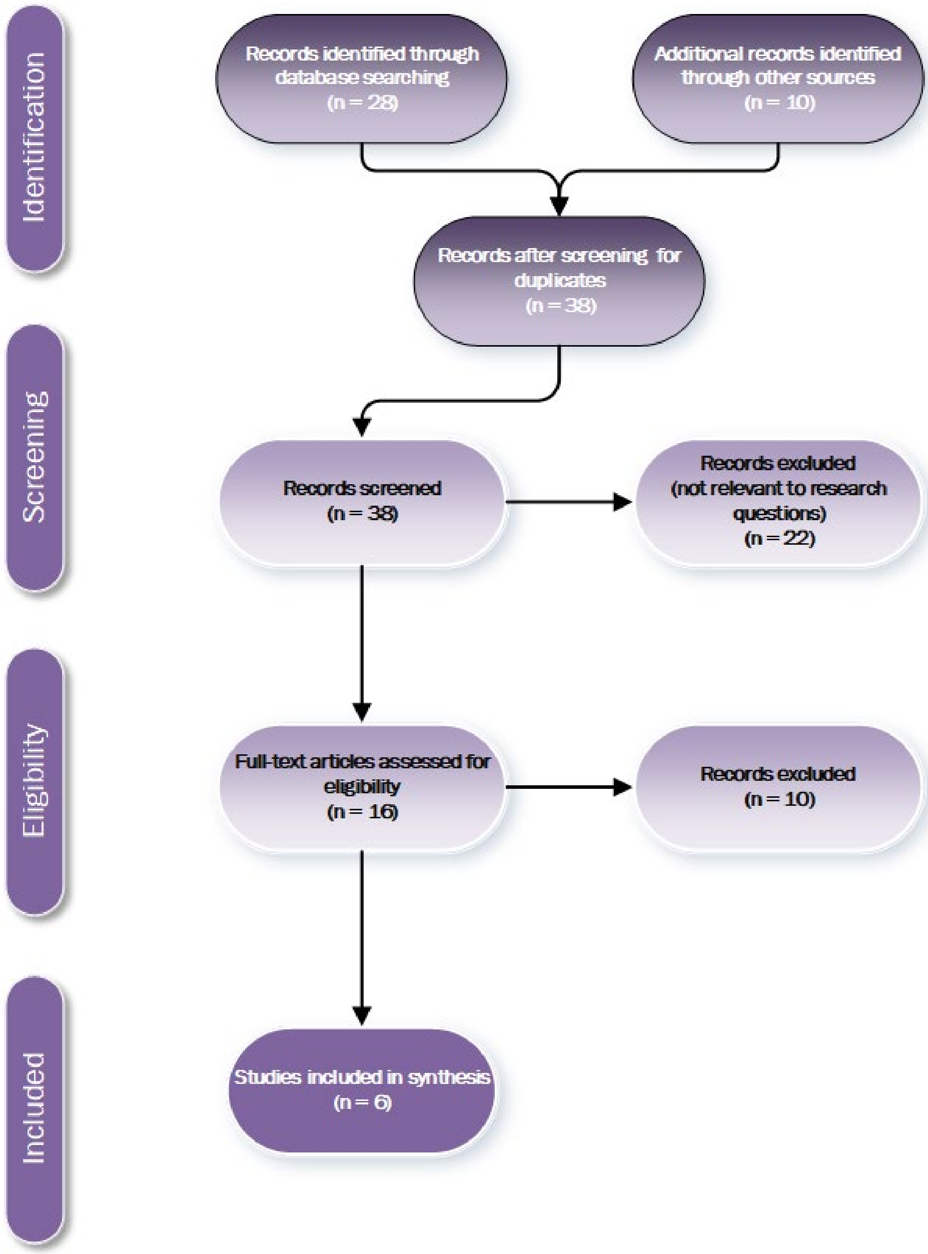

Figure 3-1. PRISMA summary of the citation review process for grey literature

PRISMA Flow Diagram – Primary Studies

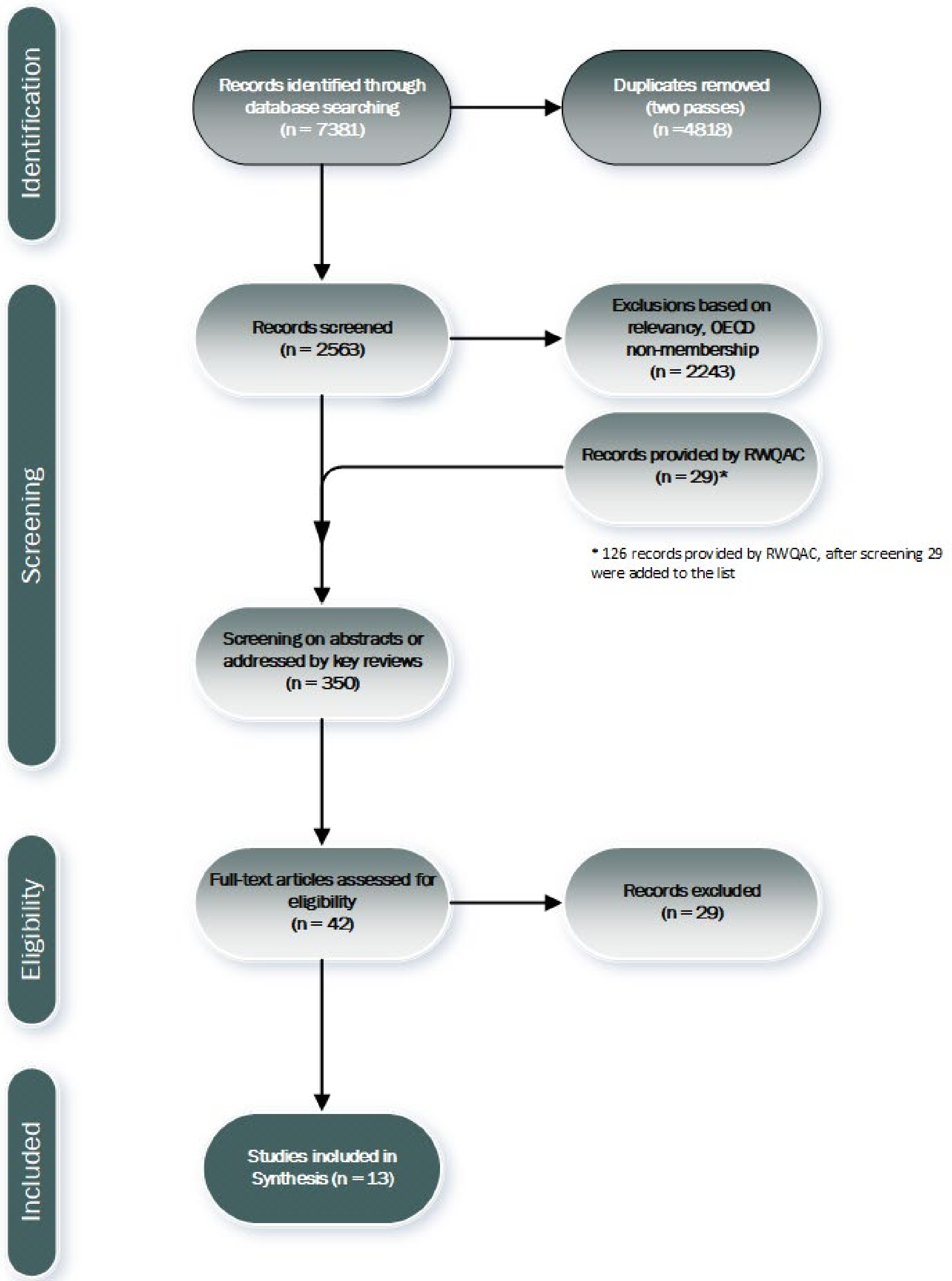

Figure 3-2. PRISMA summary of the citation review process for primary studies

4 Quality of evidence

4.1. Grey literature

4.1.1. Quality of included grey literature

Out of 16 grey literature documents identified from the literature search or suggested by the Committee, six were found to be relevant to answering the research questions and were included in the final synthesis. They were also found suitable for potential adoption/adaption based on their administrative and technical processes (Table 4.1). Full details on the completed quality assessment forms for the six grey literature documents considered eligible for inclusion in the final synthesis can be found in Section 5 of the Technical Report (O'Connor, 2022).

When measured for compliance with the administrative and technical assessment criteria outlined in the assessment tool, the overall quality of the body of literature was moderate to low with respect to methodological quality. Such a finding indicates standards and methods for the development of grey literature produced as guidance in the area of recreational water quality have not kept up with standards in the broader public health technical domain. In addition, several documents summarised existing information from a few leading publications and did not contain new material. Others had a different focus and, whilst being of good quality, lacked sufficient relevant and new material to be included, e.g. *Australian Guidelines for Water Recycling* (NRMMC, EPHC, et al., 2006). The six documents considered for the final synthesis were among the best resourced and comprehensive, e.g., US EPA (2017); WHO (2018) or were well constructed and written documents with a high degree of relevance to the research questions, e.g., NSW DPIE (2020).

Table 4-1. Form for administrative and technical criteria for assessing existing guidance or reviews. Criteria have been colour-coded to assess minimum requirements as follows: 'Must have', 'Should have' or 'May have'. Y, N, n/a, NR =Yes, No, or Not Applicable, Not Reported. Individual assessments are available in the Technical Report (O'Connor 2022).

Administrative and Technical Criteria	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16
Overall guidance/advice development process																
Are the key stages of the organisation's advice development processes compatible with Australian processes?	Y	Y	Y	Y	Y	N	N	Y	Y	Y	Y	N	Y	Y	N	Y
Are the administrative processes documented and publicly available?	N	N	N	N	N	N	N	Partly	N	Y	N	N	Y	N	N	N
Was the work overseen by an expert advisory committee? Are potential conflicts of interest of committee members declared, managed and/or reported?	N	Y N	N	N	N	N	N	Y N	Y	Y	N	Y	Can't tell	Can't tell	N	Y N
Are funding sources declared?	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Was there public consultation on this work? If so, provide details.	N	Can't tell	N	N	N	N	N	Y	Can't tell	Can't tell	N	N	Y	Can't tell	N	Y
Is the advice peer reviewed? If so, is the peer review outcome documented and/or published?	N	Y N	n/a	N	N	N	N	Y N	Can't tell	Can't tell	N	N	Can't tell	Y N	N	Can't tell
Was the guidance/advice developed or updated recently? Provide details.	N	N	Y	N	Y	N	N	N	N	N	N	N	N	Y	N	Y
Evidence review parameters																
Are decisions about scope, definitions and evidence review parameters documented and publicly available?	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Is there a preference for data from studies that follow agreed international protocols or meet appropriate industry standards?	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Does the organisation use or undertake systematic literature review methods to identify and select data underpinning the advice? Are the methods used documented clearly?	N	N	N	Y	N	N	N	N	N	N	N	N	Y	Y	N	N
If proprietary/confidential studies or data are considered by the agency, are these appropriately described/recorded?	n/a	n/a	n/a	N	N	N	N	n/a	n/a	n/a	N	N	n/a	n/a	N	N
Are inclusion/exclusion criteria used to select or exclude certain studies from the review? If so, is justification provided?	N	N	N	Y	N	N	N	N	N	n/a	N	N	Y	Y	N	N
Does the organisation use or adopt review findings or risk assessments from other organisations? What process was used to critically assess these external findings?	Y	N	N	N	Y	N	N	Y	Y	Y	N	N	N	N	N	N
Can grey literature such as government reports and policy documents be included?	Y	Y	Y	N	Y	N	Y	Y	Y	Y	N	N	Y	Y	N	Y
Is there documentation and justification on the selection of a toxicological endpoint for use as point of departure for health-based guideline derivation?	N	Y	N	N	N	N	N	N	N	Y	N	N	n/a	n/a	N	N
Evidence search																
Are databases and other sources of evidence specified?	N	N	N	Y	N	Y	N	N	N	n/a	N	N	Y	Y	N	N
Does the literature search cover at least more than one scientific database as well as additional sources (which may include government reports and grey literature)?	N	N	N	Y	N	N	N	N	N	n/a	N	N	Y	Y	N	N

	Administrative and Technical Criteria	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16
	Is it specified what date range the literature search covers? Is there a justification?	N	N	N	Y	N	N	N	N	N	N	N	N	Y	Y	N	N
	Are search terms and/or search strings specified?	N	N	N	Y	N	N	N	N	N	N	N	N	Y	Y	N	N
	Are there any other exclusion criteria for literature (e.g. publication language, publication dates)? If so, what are they and are they appropriate?	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N
	Critical appraisal methods and tools																
	Is risk of bias of individual studies taken into consideration to assess internal validity? If so, what tools are used? If not, was any method used to assess study quality?	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N
	Does the organisation use a systematic or some other methodological approach to synthesise the evidence (i.e. to assess and summarise the information provided in the studies)? If so, provide details.	N	N	N	Y	N	N	N	N	N	N	N	N	Y	N	N	N
	Does the organisation assess the overall certainty of the evidence and reach recommendations? If so, provide details.	N	N	N	Y	N	N	N	N	N	N	N	N	Y	Y	N	N
	Derivation of health-based guideline values*																
	Is there justification for the choice of uncertainty and safety factors?	n/a	n/a	n/a	n/a	n/a	n/a	n/a	N	N	Y	n/a	n/a	n/a	N	n/a	n/a
	Are the parameter value assumptions documented and explained?	n/a	n/a	n/a	n/a	n/a	n/a	n/a	N	N	Y	n/a	n/a	n/a	Y	n/a	n/a
	Are the mathematical workings/algorithms clearly documented and explained?	n/a	Y	n/a	n/a	n/a	n/a	n/a	N	N	Y	n/a	n/a	n/a	Y	n/a	n/a
	Does the organisation take into consideration non-health related matters to account for feasibility of implementing the guideline values (e.g. measurement attainability)?	Y	N	n/a	n/a	n/a	n/a	n/a	N	N	N	n/a	n/a	n/a	N	n/a	n/a
	Is there documentation directing use of mechanistic, mode of action, or key events in adverse outcome pathways in deriving health-based guideline values?	n/a	Y	n/a	n/a	n/a	n/a	n/a	N	N	N	n/a	n/a	n/a	Can't tell	n/a	n/a
	If expert judgement is required, is the process documented and published?	n/a	Y	n/a	n/a	n/a	n/a	n/a	N	N	N	n/a	n/a	n/a	N	n/a	n/a
	Is dose response modelling (e.g. BMDL) routinely used?	n/a	Y	Y	N	n/a	n/a	n/a	N	N	N	n/a	n/a	n/a	Can't tell	n/a	n/a
	Has the organisation's policy for dealing with substances for which a non-threshold mode of action may be applicable in humans been articulated and recorded?	n/a	Y	n/a	n/a	n/a	n/a	n/a	N	N	Y	n/a	n/a	n/a	Can't tell	n/a	n/a
	If applicable: For carcinogens, what is the level of cancer risk used by the organisation to set the health-based guideline value?	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	N	n/a	n/a	n/a	n/a	Can't tell	n/a	n/a
	Comments*																
	Useful for answering primary research question?	N	N	Y	Y	N	N	N	Y	N	N	Y	N	Y	N	N	Y
	Useful for answering secondary research questions?	N	N	Y	Y	N	N	N	N	N	N	Partly	N	Y	N	N	Y
	Include in review	**N**	**N**	**Y**	**Y**	**N**	**N**	**N**	**Y**	**N**	**N**	**Y**	**N**	**Y**	**N**	**N**	**Y**

* These questions primarily relate to the derivation of health-based guideline values for chemicals and may not be applicable for this research topic.

** These questions relate to relevance (usefulness) of the guidance/guideline document for answering the primary or secondary research questions.

Study ID: G1: Deere et al. (2015), G2: EnHealth (2012), G3: EPA Victoria (2021), G4: King et al. (2014), G5: McBride et al. (2019), G6: McCarthy (2017), G7: Milne et al. (2017), G8: NHMRC (2008), G9: NRMMC et al. (2006), G10: NRMMC et al. (2008), G11: NSW DPIE (2020), G12: NZMoH (2018), G13: US EPA (2017), G14: US EPA (2019), G15: Visby and Weller-Wong (2020), G16: WHO (2018)

4.2. Primary studies

4.2.1. Quality of included studies

Forty-two primary studies remained after screening and were further assessed for eligibility through a risk of bias assessment. Classifying the 42 studies by type (see section 2.5.1.2) gave the following breakdown:

- Qualitative Research = 33
- Cohort Study = 4
- Systematic Review = 5

Eligibility assessment summaries for each study type are described below. The full quality assessment results including justifications of all assessment decisions are given in Sections 6 and 7.2 of the associated Technical Report (O'Connor, 2022)

4.2.1.1. Qualitative Research Studies

Of the 33 Qualitative Research studies, 22 were classified overall as having either a probable or definitely high risk of bias (Table 4-2). These studies were excluded from further consideration. Of the remaining 11 studies, one study (Schoen, Boehm, et al., 2020) was classified as having a definitely low risk of bias and the remaining 10 were found to have a probable low risk of bias. The study by Schoen, Boehm, et al. was a high-quality study by a research team with extensive experience in microbiological risk assessment and recreational water quality.

Table 4-2. Primary studies [Qualitative research] overall risk of bias (body of evidence) (protocol adapted from the CASP appraisal tool [Oxford CVTH, 2020] and the OHAT Risk-of-Bias ratings system [OHAT, 2015]OHAT, 2019). Study authors and titles are listed on the following page.

	Study ID																															
Q.	**J2**	**J3**	**J4**	**J5**	**J7**	**J9**	**J10**	**J11**	**J12**	**J13**	**J15**	**J16**	**J17**	**J18**	**J19**	**J20**	**J21**	**J23**	**J24**	**J25**	**J28**	**J30**	**J32**	**J33**	**J35**	**J36**	**J37**	**J38**	**J39**	**J40**	**J41**	**J42**
Section A1: Are the results valid?																																
1	++	++	+	++	+	+	+	+	--	+	+	--	+	+	-	+	-	+	+	+	++	+	--	+	+	+	+	+	+	+	+	+
2	+	+	+	++	+	+	+	+	--	--	+	+	+	++	+	+	+	+	+	-	+	+	--	+	++	++	+	+	+	+	+	+
Section A2: Is it worth continuing?																																
-	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	++	++	+	++	-	--	+	+	-	--	+	--	+	++	-	+	-	+	+	--	+	+	nil	--	++	+	+	+	--	+	+	+
4	+	+	+	n/a	n/a	+	+	n/a	+	n/a	+	+	+	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	nil	n/a	+	+	+	n/a	n/a	n/a	n/a	n/a
5	+	++	+	++	+	+	+	-	-	--	+	+	+	+	-	+	-	n/a	+	-	+	+	nil	+	++	+	+	+	+	+	+	+
Section B: What are the results?																																
8	+	+	+	++	+	+	+	+	-	--	+	+	--	+	--	+	+	+	-	+	-	+	nil	+	++	++	+	++	--	+	+	-
9	++	-	-	+	-	--	+	+	+	--	+	+	-	+	-	+	-	+	-	+	-	+	nil	-	++	++	+	+	-	-	+	-
Section C: Will the results help locally?																																
10	+	+	+	++	-	-	--	-	-	--	+	-	--	+	--	+	-	+	-	-	-	+	nil	-	++	+	-	-	-	-	-	-
Overall	+	+	+	+	-	-	-	-	-	--	+	--	--	+	--	+	-	+	-	-	-	+	--	-	++	+	-	-	-	-	-	-

Key to Table 4-2

++	***Definitely Low** risk of bias:*	+	***Probably Low** risk of bias*	-	***Probably High** risk of bias:*	--	***Definitely High** risk of bias:*

Questions:

1. Was there a clear statement of the aims of the research?
2. Is the methodology appropriate?
3. Was the research design appropriate to address the aims of the research?
4. Was the chosen hypothetical population or subpopulation appropriate for addressing the study research aims?
5. Was the data collected in a way that addressed the research issue?
8. Was the data analysis sufficiently rigorous?

9. Is there a clear statement of findings?
10. How valuable is the research? Is the research of satisfactory value?
Overall = Overall risk of bias rating
Not Shown (responses were all given as "n/a" as no epidemiological studies were included among the qualitative research group):

6. Has the relationship between researcher and participants been adequately considered?
7. Have ethical issues been taken into consideration?

Abbreviations: n/a = not applicable. nil = response not recorded as response to earlier questions indicated it was not worth continuing the assessment.

Study ID for Table 4-2

J2: Ahmed et al. (2018) Quantitative microbial risk assessment of microbial source tracking markers in recreational water contaminated with fresh untreated and secondary treated sewage
J3: Ahmed et al. (2019) Enhanced insights from human and animal host-associated molecular marker genes in a freshwater lake receiving wet weather overflows
J4: Ahmed et al. (2020) Sewage-associated marker genes illustrate the impact of wet weather overflows and dry weather leakage in urban estuarine waters of Sydney, Australia
J5: Ahmed et al. (2019) Comparative decay of sewage-associated marker genes in beach water and sediment in a subtropical region
J7: Aslan et al. (2018) The Impact of Tides on Microbial Water Quality at an Inland River Beach
J9: Boehm et al. (2019) Risk-based water quality thresholds for coliphages in surface waters: effect of temperature and contamination aging
J10: Brown et al. (2017) Estimating the probability of illness due to swimming in recreational water with a mixture of human- and gull-associated microbial source tracking markers
J11: Cazals et al. (2020) Near real-time notification of water quality impairments in recreational freshwaters using rapid online detection of β-D-glucuronidase activity as a surrogate for Escherichia coli monitoring
J12: Craig et al. (2003) Effectiveness of guideline faecal indicator organism values in estimation of exposure risk at recreational coastal sites
J13: Craig et al. (2004) Use of microcosms to determine persistence of Escherichia coli in recreational coastal water and sediment and validation with in situ measurements
J15: Gitter et al. (2020) Human health risks associated with recreational waters: Preliminary approach of integrating quantitative microbial risk assessment with microbial source tracking
J16: Goodwin et al. (2017) Consideration of Natural Sources in a Bacteria TMDL-Lines of Evidence, Including Beach Microbial Source Tracking
J17: Hart et al. (2020) Examining coastal dynamics and recreational water quality by quantifying multiple sewage specific markers in a North Carolina estuary
J18: Henry et al. (2016) Into the deep: Evaluation of SourceTracker for assessment of faecal contamination of coastal waters
J19: Hughes et al. (2017) Cross-Comparison of Human Wastewater-Associated Molecular Markers in Relation to Fecal Indicator Bacteria and Enteric Viruses in Recreational Beach Waters
J20: Kelly et al. (2018) Effect of beach management policies on recreational water quality
J21: Kinzelman et al. (2020) Utilization of multiple microbial tools to evaluate efficacy of restoration strategies to improve recreational water quality at a Lake Michigan Beach (Racine, WI)
J23: Lugg et al. (2012) Estimating 95th Percentiles from Microbial Sampling: A Novel Approach to Standardising their Application to Recreational Waters
J24: McKee et al. (2020) Microbial source tracking (MST) in Chattahoochee River National Recreation Area: Seasonal and precipitation trends in MST marker concentrations, and associations with *E. coli* levels, pathogenic marker presence, and land use
J25: Mulder et al. (2020) Tracing the animal sources of surface water contamination with *Campylobacter jejuni* and *Campylobacter coli*
J28: Oun et al. (2017) Microbial pollution characterization of water and sediment at two beaches in Saginaw Bay, Michigan
J30: Robins et al. (2019) Viral dispersal in the coastal zone: A method to quantify water quality risk
J32: Roser et al. (2006) Microbial exposure assessment of an urban recreational lake: A case study of the application of new risk-based guidelines
J33: Rosiles-González et al. (2019) Norovirus and human adenovirus occurrence and diversity in recreational water in a karst aquifer in the Yucatan Peninsula, Mexico
J35: Schoen et al. (2020) Contamination Scenario Matters when Using Viral and Bacterial Human-Associated Genetic Markers as Indicators of a Health Risk in Untreated Sewage-Impacted Recreational Waters
J36: Shrestha et al. (2019) Evaluation of rapid qPCR method for quantification of *E. coli* at non-point source impacted Lake Michigan beaches
J37: Shrestha et al. (2020) Fecal pollution source characterization at non-point source impacted beaches under dry and wet weather conditions
J38: Shrestha et al. (2019) *Campylobacter jejuni* strains associated with wild birds and those causing human disease in six high-use recreational waterways in New Zealand
J39: Steele et al. (2018) Quantification of pathogens and markers of fecal contamination during storm events along popular surfing beaches in San Diego, California
J40: Xue et al. (2018) Using Bacteroidales genetic markers to assess fecal pollution sources in coastal waters
J41: Xue et al. (2018) Assessment of fecal pollution in Lake Pontchartrain, Louisiana
J42: Zimmer-Faust et al. (2020) The challenges of microbial source tracking at urban beaches for Quantitative Microbial Risk Assessment (QMRA)

4.2.1.2. Cohort Studies

Eligibility assessment of the four cohort studies resulted in three studies being excluded from further consideration and one study (Arnold, Schiff, et al., 2017) being retained for final review (Table 4-3).

Table 4-3. Primary studies [Cohort studies] overall risk of bias (body of evidence) (protocol adapted from the CASP appraisal tool [Oxford CVTH,2020] and the OHAT Risk-of-Bias ratings system [OHAT, 2015].

		Study ID			
Q.	**Questions**	**J6**	**J26**	**J27**	**J29**
	Section A1: Are the results valid?				
1	Did the study address a clearly focused issue?	++	++	++	--
2	Was the cohort recruited in an acceptable way?	+	+	+	-
	Section A2: Is it worth continuing?	**Yes**	**Yes**	**Yes**	**No**
3	Was the exposure accurately measured to minimise bias?	+	-	-	+
4	Was the outcome accurately measured to minimise bias?	+	+	+	+
5a	Have the authors identified all important confounding factors?	+	-	-	+
5b	Have they taken account of the confounding factors in the design and/or analysis?	+	-	-	-
6a	Was the follow up of subjects complete enough?	+	+	+	n/a
6b	Was the follow up of subjects long enough?	+	+	+	n/a
	Section B: What are the results?				
7	What are the results of this study?	+	-	-	+
8	How precise are the results?	+	-	-	-
9	Do you believe the results?	+	--	--	+
	Section C: Will the results help locally?				
10	Can the results be applied to the local population?	+	--	--	+
11	Do the results of this study fit with other available evidence?	+	-	-	+
12	What are the implications of this study for practice?	+	--	--	-
-	**Overall risk of bias rating**	+	--	--	-

Key to Table 4-2

++	***Definitely Low** risk of bias:*	+	***Probably Low** risk of bias*	-	***Probably High** risk of bias:*	--	***Definitely High** risk of bias:*

J6: Arnold et al. (2017) Acute Illness Among Surfers After Exposure to Seawater in Dry- and Wet-Weather Conditions
J26: Napier et al. (2017) Exposure to human-associated fecal indicators and self-reported illness among swimmers at recreational beaches: A cohort study
J27: Napier et al. (2018) Exposure to Human-Associated Chemical Markers of Fecal Contamination and Self-Reported Illness among Swimmers at Recreational Beaches
J29: Polkowska et al. (2018) An outbreak of Norovirus infections associated with recreational lake water in Western Finland, 2014

4.2.1.1. Systematic reviews

Eligibility assessment of the five systematic reviews resulted in one study (Boehm, Graham, et al., 2018) being retained for final review (Table 4-3) and the remaining four studies being excluded from further consideration.

Table 4-4. Primary studies [Systematic reviews] overall risk of bias (body of evidence) (protocol adapted from the CASP appraisal tool [Oxford CVTH, 2020] and the OHAT Risk-of-Bias ratings system [OHAT, 2015]OHAT, 2019).

				Study ID		
Q.	**Paper for appraisal and reference:**	**J8**	**J14**	**J22**	**J31**	**J34**
	Section A1: Are the results valid?					
1	Did the review address a clearly focused question?	+	--	-	-	++
2	Did the authors look for the right type of papers?	+	+	-	--	+
	Section A2: Is it worth continuing?	**Yes**	**Yes**	**Yes**	**No**	**Yes**
3	Do you think all the important, relevant studies were included?	++	++	-	-	++
4	Did the review's authors do enough to assess quality of the included studies?	--	--	--	--	++
5	If the results of the review have been combined, was it reasonable to do so?	-	n/a	--	n/a	++
	Section B: What are the results?					
6	What are the overall results of the review?	+	-	-	n/a	+
7	How precise are the results?	--	n/a	--	n/a	+
	Section C: Will the results help locally?					
8	Can the results be applied to the local population?	-	--	--	-	+
9	Were all important outcomes considered?	-	-	-	-	+
10	Are the benefits worth the harms and costs?	--	--	--	--	+
-	**Overall risk of bias rating**	--	--	--	--	+

Key to Table 4-3

++	***Definitely Low** risk of bias*:	+	***Probably Low** risk of bias*	-	***Probably High** risk of bias*:	--	***Definitely High** risk of bias*:

J8: Boehm et al. (2018) Can We Swim Yet? Systematic Review, Meta-Analysis, and Risk Assessment of Aging Sewage in Surface Waters

J14: Federigi et al. (2019) The application of quantitative microbial risk assessment to natural recreational waters: A review

J22: Korajkic et al. (2018) Relationships between microbial indicators and pathogens in recreational water settings

J31: Rodrigues et al. (2017) Assessment of the microbiological quality of recreational waters: indicators and methods

J34: Russo et al. (2020) Evaluating health risks associated with exposure to ambient surface waters during recreational activities: A systematic review and meta-analysis

5 Full list of included studies

5.1. Grey literature

The six grey literature documents remaining after screening and quality assessment included one report on based on original research, three comprehensive reviews, and two guideline documents (Table 5-1). The research report was prepared by EPA Victoria and consisted of a QMRA based on monitoring of FIB, reference pathogens (virus, bacteria, and protozoa) and qPCR biomarkers for human gut bacteria (human Bacteroides) focused on recreational waters in Port Phillip Bay.

The two guideline documents were the 2008 NHMRC Guidelines and the NSW DPIE 2020 Protocol for Assessment and Management of Microbial Risks in Recreational Waters. Chapter 5 (Microbial Quality of Recreational Water) of the NHMRC 2008 Guidelines deals with topics of relevance to the primary research question. Although the purpose of this review is to support an update to the Guidelines, the material contained in the Chapter 5 could be considered to provide a baseline for reference with other guidance documents appraised in this review and to identify areas for improvement in the updated Guidelines. On that basis it was included for further review, noting that the 2008 NHMRC Guidelines will be rescinded when the updated Guidelines are published.

The 2020 NSW DPIE guideline provides detailed guidance for implementing Chapter 5 of the 2008 NHMRC Guidelines. Specifically, the guideline provides a clear and detailed practical methodology for monitoring, assessing, and predicting risks from diffuse and point source microbial contamination in recreational waters. Since it augments the information in the 2008 NHMRC Guidelines with some practical detail, it can be considered to be useful for informing the response to the primary research question, although it does not provide new scientific information.

The three reviews on recreational water quality were undertaken by the UK Department for Environment, Farming and Rural Affairs (DEFRA), the US EPA and WHO. The DEFRA document examined the relationship between water quality and gastrointestinal illness. The US EPA document consisted of a detailed review of US 2012 Recreational Water Quality Criteria, whilst the WHO study derived recommendations based on scientific, analytical, and epidemiological developments relevant to the parameters for bathing water quality in the European Union 2006 Bathing Water Directive.

Table 5-1. List of grey literature included after screening and quality assessment

ID	Title	Type of Study
G3: EPA Victoria (2021)	EPA Victoria, 2021. Quantitative Microbial Risk Assessment (QMRA) for assessing risks to recreational users in Port Phillip Bay. EPA Publication 2007, June 2021. Environment Protection Authority, Victoria.	Report
G4: King et al. (2014)	King, S., Exley, J., Winpenny, E., Alves, L., Henham, M.-L., Larkin, J., 2014. The health risks of bathing in recreational waters. A rapid evidence assessment of water quality and gastrointestinal illness. Final report WT1530. A report of research carried out by RAND Europe, on behalf of the UK Department for Environment, Farming and Rural Affairs (DEFRA). United Kingdom.	Review
G8: NHMRC (2008)	NHMRC, 2008. Guidelines for managing risks in recreational water. National Health and Medical Research Council (Australia).	Guidelines

ID	Title	Type of Study
G11: NSW DPIE (2020)	NSW DPIE, 2020. Protocol for Assessment and Management of Microbial Risks in Recreational Waters. NSW Department of Planning, Industry & Environment, [online]	Guidelines
G13: US EPA (2017)	US EPA, 2017. 2017 Five-Year Review of the 2012 Recreational Water Quality Criteria (No. Office of Water 823-R-18-001). US Environmental Protection Agency, Office of Water, Office of Science and Technology, Washington, DC, United States.	Review
G16: WHO (2018)	WHO, 2018. WHO recommendations on scientific, analytical and epidemiological developments relevant to the parameters for bathing water quality in the Bathing Water Directive (2006/7/EC): Recommendations. World Health Organization.	Review

5.2. Primary studies

The 13 primary studies included consisted of 11 qualitative research studies, one cohort and one review (Table 5-2). Studies classified as qualitative using the CASP classification criteria for quality and risk of bias include observational studies that measure or predict concentrations of pathogens or microbial indicators in recreational water environments and on the basis of those concentrations infer the extent of exposure against health guidelines. In other contexts, such studies are commonly considered quantitative risk assessments. For the CASP approach used here, a classification as qualitative was considered appropriate, since no health impacts were observed and measured. Many of the qualitative studies incorporated QMRA methodologies to predict concentrations of pathogens or microbial indicators under a range of scenarios in order to identify the conditions that give rise to high risk of gastrointestinal illness amongst recreational water users.

The cohort study by Arnold, Schiff, et al., (2017) was a large study that addressed acute illness among surfers after exposure to seawater in dry- and wet-weather conditions and followed the health of 654 surfers in San Diego, California over two seasons and involved over 10,000 surf sessions.

The review study by Russo, Eftim, et al., (2020) evaluated health risks associated with exposure to ambient surface waters during recreational activities. The study was a high-quality literature review and notable for its consistency with best practice for systematic reviews.

Table 5-2. List of primary studies included after consideration of risk of bias

Study ID	Citation	CASP classification
J2: Ahmed, Hamilton, et al., (2018)	Ahmed, W., Hamilton, K. A., Lobos, A., Hughes, B., Staley, C., Sadowsky, M. J., and Harwood, V. J. (2018) Quantitative microbial risk assessment of microbial source tracking markers in recreational water contaminated with fresh untreated and secondary treated sewage. Environment International, 117, 243–249. [online] https://www.scopus.com/inward/record.uri?eid=2-s2.0-85047077932&doi=10.1016%2fj.envint.2018.05.012&partnerID=40&md5=c97413bc0249240d20d0a19774172eeb.	Qualitative research
J3: Ahmed, Payyappat, et al., (2019)	Ahmed, W., Payyappat, S., Cassidy, M., and Besley, C. (2019) Enhanced insights from human and animal host-associated molecular marker genes in a freshwater lake receiving wet weather overflows. Scientific Reports, 9(1), 12503. [online] http://www.ncbi.nlm.nih.gov/pubmed/31467317.	Qualitative research

Study ID	Citation	CASP classification
J4: Ahmed, Payyappat, et al., (2020)	Ahmed, W., Payyappat, S., Cassidy, M., Harrison, N., and Besley, C. (2020) Sewage-associated marker genes illustrate the impact of wet weather overflows and dry weather leakage in urban estuarine waters of Sydney, Australia. The Science of the Total Environment, 705, 135390. [online] http://www.ncbi.nlm.nih.gov/pubmed/31838427.	Qualitative research
J5: Ahmed, Zhang, et al., (2019)	Ahmed, W., Zhang, Q., Kozak, S., Beale, D., Gyawali, P., Sadowsky, M. J., and Simpson, S. (2019) Comparative decay of sewage-associated marker genes in beach water and sediment in a subtropical region. Water Research, 149, 511–521. [online] http://www.ncbi.nlm.nih.gov/pubmed/30500686.	Qualitative research
J6: Arnold, Schiff, et al., (2017)	Arnold, B. F., Schiff, K. C., Ercumen, A., Benjamin-Chung, J., Steele, J. A., Griffith, J. F., Steinberg, S. J., Smith, P., McGee, C. D., Wilson, R., Nelsen, C., Weisberg, S. B., and Colford, J. M., Jr. (2017) Acute Illness Among Surfers After Exposure to Seawater in Dry- and Wet-Weather Conditions. American Journal of Epidemiology, 186(7), 866–875. [online] https://doi.org/10.1093/aje/kwx019 (Accessed March 10, 2021).	Cohort study
J15: Gitter, Mena, et al., (2020)	Gitter, A., Mena, K. D., Wagner, K. L., Boellstorff, D. E., Borel, K. E., Gregory, L. F., Gentry, T. J., and Karthikeyan, R. (2020) Human health risks associated with recreational waters: Preliminary approach of integrating quantitative microbial risk assessment with microbial source tracking. Water (Switzerland), 12(2). [online] https://www.scopus.com/inward/record.uri?eid=2-s2.0-85081546265&doi=10.3390%2fw12020327&partnerID=40&md5=f48942230d1dbe0b5426d168ad1f982d.	Qualitative research
J18: Henry, Schang, et al., (2016)	Henry, R., Schang, C., Coutts, S., Kolotelo, P., Prosser, T., Crosbie, N., Grant, T., Cottam, D., O'Brien, P., Deletic, A., and McCarthy, D. (2016) Into the deep: Evaluation of SourceTracker for assessment of faecal contamination of coastal waters. Water Research, 93, 242–253. [online] https://www.scopus.com/inward/record.uri?eid=2-s2.0-84958973030&doi=10.1016%2fj.watres.2016.02.029&partnerID=40&md5=857b1be61a85ed86ed706842b9f6584f.	Qualitative research
J20: Kelly, Feng, et al., (2018)	Kelly, E. A., Feng, Z., Gidley, M. L., Sinigalliano, C. D., Kumar, N., Donahue, A. G., Reniers, A. J. H. M., and Solo-Gabriele, H. M. (2018) Effect of beach management policies on recreational water quality. Journal of Environmental Management, 212, 266–277. [online] https://repository.tudelft.nl/islandora/object/uuid%3A4cae77ec-45fd-4029-b8e5-5c3e68015b34/datastream/OBJ/download.	Qualitative research
J23: Lugg, Cook, et al., (2012)	Lugg, R. S. W., Cook, A., and Devine, B. (2012) "Estimating 95th Percentiles from Microbial Sampling: A Novel Approach to Standardising their Application to Recreational Waters" in D. Kay and C. Fricker (eds.), The Significance of Faecal Indicators in Water: A Global Perspective. Cambridge, RSC Publishing, 62–71. [online] http://ebook.rsc.org/?DOI=10.1039/9781849735421-00062 (Accessed December 17, 2014).	Qualitative research
J30:Robins, Farkas, et al., (2019)	Robins, P. E., Farkas, K., Cooper, D., Malham, S. K., and Jones, D. L. (2019) Viral dispersal in the coastal zone: A method to quantify water quality risk. Environment International, 126, 430–442. [online] https://www.scopus.com/inward/record.uri?eid=2-s2.0-85062216772&doi=10.1016%2fj.envint.2019.02.042&partnerID=40&md5=72fcd9a789a4b4dd475a16a0e31978dc.	Qualitative research
J34: Russo, Eftim, et al., (2020)	Russo, G. S., Eftim, S. E., Goldstone, A. E., Dufour, A. P., Nappier, S. P., and Wade, T. J. (2020) Evaluating health risks associated with exposure to ambient surface waters during recreational activities: A systematic review and meta-analysis. Water Research, 176. [online] https://www.scopus.com/inward/record.uri?eid=2-s2.0-85082875333&doi=10.1016%2fj.watres.2020.115729&partnerID=40&md5=b8d28f8b8576aa1d50ad124e4649f95f.	Systematic review
J35: Schoen, Boehm, et al., (2020)	Schoen, M. E., Boehm, A. B., Soller, J., and Shanks, O. C. (2020) Contamination scenario matters when using viral and bacterial human-associated genetic markers as indicators of health risk in untreated sewage-impacted recreational	Qualitative research

Study ID	Citation	CASP classification
	waters. Environmental Science & Technology. [online] http://www.ncbi.nlm.nih.gov/pubmed/32969642.	
J36: Shrestha and Dorevitch, (2019)	Shrestha, A. and Dorevitch, S. (2019) Evaluation of rapid qPCR method for quantification of *E. coli* at non-point source impacted Lake Michigan beaches. Water Research, 156, 395–403. [online] https://www.scopus.com/inward/record.uri?eid=2-s2.0-85063941252&doi=10.1016%2fj.watres.2019.03.034&partnerID=40&md5=8d0a3a8e1bd9c65175f11c60ca03b025.	Qualitative research

6 Significance of microbial risks to human health in recreational waters

6.1. Review of existing grey literature

6.1.1. Primary research question

How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?

As noted earlier, the grey literature available for review following quality and risk of bias assessments consisted of one original report, two guideline documents and three systematic reviews (Table 5-1). While the six documents address different aspects of microbial risks to recreational waters, each document is comprehensive and there is significant overlap in issues covered. Each document also contained material relevant to the primary research question.

G3: EPA Victoria (2021). *Quantitative Microbial Risk Assessment (QMRA) for assessing risks to recreational users in Port Phillip Bay*[3]

The report by EPA Victoria (2021) *Quantitative Microbial Risk Assessment (QMRA) for assessing risks to recreational users in Port Phillip Bay* describes original research conducted by Monash University on behalf of EPA Victoria to better understand the health risks associated with water-based recreation in Port Phillip Bay, a large 1,930 km^2 shallow estuarine embayment around which greater metropolitan Melbourne is located. EPA Victoria commissioned QMRAs at three popular beaches in the bay, combined with microbial source tracking at each location over the summer season 2017-2018. With respect to the primary research question relevant study objectives were:

(i). to compare the probability of illness from water-based recreational activities at the three beach locations within the Bay, as calculated using a QMRA approach, to the risk portrayed by the Victorian State Environment Protection Policy [SEPP (Waters)] and the 2008 NHMRC Guidelines;

(ii). to provide an example of how a QMRA could be conducted and provide parameters and key assumptions for future application.

The QMRA consisted of baseline scenario and a series of sensitivity scenarios, where the sensitivity of some of the assumptions and uncertainties involved in the baseline QMRA were explored. The baseline scenario was constructed by generating exposure volumes for 100 people randomly drawn from ingestion volume probability distribution functions derived from the literature and exposing these 100 people to 1000 different days of pathogen concentrations, randomly drawn from the monitoring datasets acquired from the three beaches used in the study. The resultant doses

[3] Reviewers comment: A draft version of this study dated 2019 was supplied to the reviewer by the Committee. However a later final and almost identical version of the same document was published on the Victorian EPA website in early 2021 after the late 2020 cutoff. Since it was essentially the same document and published as the review process was beginning, it was the version used for this review.

were used in dose-response models to calculate the probability of infection (or probability of illness for *Salmonella*). The probability of infection was then used to determine the probability of illness using the p(ill/inf) distributions. This was repeated for each chosen pathogen and the aggregate probabilities of illness were calculated and used to determine statistical distributions for ingestion exposure during both primary and secondary contact.

The baseline QMRA for primary contact recreation predicted a mean probability of illness (p(ill)) of 0.33% [95th percentile 1.07%] per exposure event, mostly due to norovirus 0.23% [0.82%] (which had a highly uncertain dose-response model). Adenoviruses contributed the next highest proportion of total risks at 0.07% [0.23%], whilst bacteria and protozoa had p(ill) at or below 0.01%.

The baseline QMRA suggested that the beaches were:

- in category 1 (swim safely) with a predicted probability of illness of ≤1% per primary contact recreational exposure about 94% of the time
- in category 2 (swim at your own risk) with a probability of illness between 1% and 10% only 6% of the time, and
- never in category 3 (do not swim).

For secondary contact recreation, the baseline QMRA predicted 48 illnesses from 100,000 events, which equates to a mean probability of illness of 0.05% (95th percentile 0.18%). As with the primary contact QMRA, most of this probability was derived from the norovirus dose-response model which appears conservative.

The QMRA Sensitivity analyses showed that adjustment of the *Cryptosporidium* and adenovirus dose-response relationships did not change the outcomes significantly or result in reduced probabilities of illnesses per contact event. Regardless of the dose-response model chosen, the 95th percentile probability of enteric illness due to a single primary contract recreational event rarely exceeded 2.02%.

The QMRA for primary and secondary contact recreational events using data collected from the three beaches showed that the probabilities of contracting an illness were very low compared to the rates of illness expected using the SEPP (Waters) and NHMRC interpretation (NHMRC 2008). Furthermore, the probabilities of contracting an illness were comparable to those found in the limited number of epidemiological studies with similar water body-types and pollution sources.

The study concluded that the current practice of using indicators testing could not accurately predict the densities of pathogens in Port Phillip Bay. *E. coli* and enterococci correlated with the calculated rates of gastrointestinal illnesses due to a primary contact event. However, meaningful bay-specific objectives could not be directly derived from this relationship.

G4: King et al. (2014). *The health risks of bathing in recreational waters. A rapid evidence assessment of water quality and gastrointestinal illness.*

To evaluate the epidemiological literature published between 2003 and 2014, King et al examined the relationship between recreational water use (i.e. exposure to marine water and freshwater recreational waters) and gastrointestinal illness (GI). The authors also sought to highlight any significant new research and/or evidence gaps which may help inform future bathing water quality guidelines.

Overall, 21 papers (from 16 studies), including two randomised controlled trials and 14 observational studies, met the inclusion criteria of the review. With respect to the primary research question, King et al. posed the question: What is the post 2003 evidence for the health risks of recreational bathing in general – and also to specific groups of bathers?

The authors found that based on studies included in the review, there is continuing evidence that bathing in recreational water poses some increased risk of GI to bathers compared with non-bathers. Specifically:

- There appears to be little or no significant difference between GI in bathers compared with non-bathers at marine beaches.
- In contrast, there appears to be a consistent and significantly higher risk of GI in bathers compared with non-bathers in freshwater sites in temperate climates (up to 3.2 times higher).
- There is some evidence to suggest that increased bather exposure (i.e. head immersion or swallowing water) results in a higher risk of GI, particularly for freshwater bathers.
- There is evidence to suggest that an increase in time spent in water is associated with an increase in GI.

Only two studies reported results separately by age group of bathers, and only one study investigated the risk of GI among other water users (e.g. in people canoeing, fishing, kayaking, motor boating, or rowing), so the data on these specific population groups remain limited. Findings were:

- There is very little evidence on how the risk of GI varies with age.
- There is a lack of recent studies which have evaluated the risk of GI in recreational water users other than bathers (e.g. in people canoeing, fishing, kayaking, motor boating, or rowing).

King et al., were highly critical of the quality of the literature reviewed. They reported difficulty in drawing firm conclusions from the evidence because of the heterogeneity of study protocols and methodological limitations, including self-selection and misclassification biases. The authors suggested that the various results presented by the study authors could be an artefact of the range of methods used.

G8: NHMRC (2008). *Guidelines for managing risks in recreational water*.

The 2008 NHMRC Guidelines provide a tool for states and local agencies for use in developing legislation and standards appropriate for local conditions and to encourage a nationally harmonised approach to managing recreational water quality. Section 5.1 of the guidelines implicitly recommends a classificatory approach to the management of recreational water quality. The approach involves microbial-based categorisation of the water using a combination of sanitary inspection and microbial water-quality assessment.

Further combining such categorisation with prevention of exposure at times of increased risk leads to the framework for assessing and managing recreational water quality recommended by the guidelines. This ultimately leads to the classification matrix shown in Table 5.13 of the guidelines which is based on four classes of microbial water quality (as 95th percentiles of enterococci/100 ml) by four sanitary inspection categories (rating susceptibility of the site to faecal influence).

The classification matrix for faecal pollution of recreational water environments:

- emphasises faecal contamination from humans, with lesser importance placed on faecal contamination from other sources.
- enables local management to respond to sporadic or limited areas of pollution and thereby upgrade a recreational water body's classification, provided that appropriate and effective actions are taken to control exposure.
- provides triggers for actions to reduce risk.
- provides incentives for taking action locally and reducing pollution.
- produces a generic statement of the level of risk, thereby supporting informed personal choice, and it helps to identify appropriate management and monitoring actions.

The structure of Chapter 5 of the 2008 NHMRC Guidelines is based on a microbial-based classificatory approach, combining sanitary inspection and microbial water-quality assessment, to create a risk matrix to guide management. Although the content of the chapter needs updating, the structure still describes an effective approach for management of recreational water quality.

G11: NSW DPIE (2020). *Protocol for Assessment and Management of Microbial Risks in Recreational Waters*.

The NSW DPIE Protocol for assessment and management of microbial risks in recreational waters provides guidance to support the implementation of Chapter 5 of the 2008 NHMRC Guidelines. The Protocol is closely aligned with and builds on the 2008 NHMRC Guidelines. The Protocol describes a framework for managing microbial risks in recreational water with the most innovative components being methods for:

- Selection of sites for assessment
- Sanitary inspections
- Microbial water quality monitoring
- Microbial assessment and beach classification
- Reporting

Key elements of the protocol that contribute to the primary research question are:

- The inclusion of an initial site prioritisation step which prioritises beaches to provide a basis for determining resource allocation. This step provides a mechanism for identifying high priority swimming locations that should attract more monitoring and reporting resources to ensure the greatest benefit is obtained. High priority beaches become the subjects of more detailed risk assessments, and programs for monitoring, reporting and microbial risk management.

- A detailed Sanitary Inspection process with 5 steps:
    1. Define the swimming area and catchment.
    2. Identify sources of faecal contamination and gather information on the frequency, duration, and intensity of impact.
    3. Assess likelihood for each identified source of faecal contamination.
    4. Determine the Sanitary Inspection Category for the site (overall likelihood).
    5. Hold a workshop or meeting with stakeholders to review pollution sources and likelihood assessment.
- A microbial water quality monitoring program with specific guidance for sampling design and documentation, quality control aspects including sampling procedures, lab methods and accreditation, data management and work health and safety.
- A microbial assessment and beach classification program which describes the methodology for determining beach suitability grades
- Reporting of: Annual Classifications, Weekly Star Ratings, Advisories following rainfall events, daily beach pollution forecasts, communication planning and methods of communication.
- Appendices with high quality templates for data collection and reporting for such items as: Sanitary inspection major attributes (e.g. pollutions sources), and water quality sample log sheets.

A further noteworthy innovative aspect of the Protocol is the inclusion of methods for assessing and scoring likelihoods of contamination from a wide variety of pollution sources including bather shedding, toilet facilities, wastewater treatment plant discharges (including bypass events), sewer chokes and leaks, onsite sewage treatment systems, wastewater reuse, stormwater, river discharges, lagoons, boat discharges and animals. Templates for scoring are also provided in the appendices.

Overall, the Protocol is clearly laid out, is of a high standard, and provides much more explicit guidance for the development of recreational water microbial risk assessment and risk management than the 2008 NHMRC Guidelines. For example there are 48 pages of guidance material and 26 pages of templates vs the NHMRCs 30 pages and 9 pages respectively.

G13: US EPA (2017). 2017 Five-Year Review of the 2012 Recreational Water Quality Criteria.

The US EPA "2017 Five-Year Review of the 2012 Recreational Water Quality Criteria" is a 5-year review of its 2012 Recreational Water Quality Criteria (RWQC), as required by amendments to the 1972 US Clean Water Act.

The review was based on 3 main review components:

1. A systematic review of available peer-reviewed literature published between 2010 and 2017 conducted for EPA by Dr Graham McBride of the NZ National Institute of Water and Atmospheric Research (NIWA).

2. A supplemental review by EPA of literature resulting from systematic searches and from other sources such as technical documents from US states and the United States Geological Survey (USGS).
3. Informal interviews with recreational water public health practitioners and members of the academic community with relevant expertise in beach monitoring.

The results of the above review components informed an extensive review section of the document entitled "Findings of the Review". This section provides a general discussion of the review findings. While the review was unable to draw any concise conclusions, several suggestions for further research are provided. Summaries of the findings are presented in the responses to the primary research questions below as well as to the secondary research questions in following sections.

The review contains authoritative information on a broad range of topics and research findings from particular sources. Due to the highly heterogeneous subject matter, an effective synthesis was unable to be conducted to draw concise conclusions.

The authors split the summary of review findings into two components; (i) Science Review and (ii) Implementation Review. Note that implementation review components only related to Secondary Research Question (ii). The key elements of each component relevant to the current review are presented alongside the relevant research questions below. With respect to the primary research question, key findings were:

- Health Studies: A growing body of evidence suggests that children can be disproportionately susceptible to health effects resulting from exposure to pathogens in recreational waters. There are opportunities for further resolution of epidemiological relationships, especially around children's health protection and wider application of *Enterococcus* spp. qPCR techniques for monitoring.
- Antimicrobial Resistance. Although of increasing interest, US EPA suggests more research is needed to better understand the role the environment plays in transferring antimicrobial resistant bacteria (AMRB) to primary contact recreators.

G16: WHO (2018). WHO recommendations on scientific, analytical and epidemiological developments relevant to the parameters for bathing water quality in the Bathing Water Directive (2006/7/EC).

The WHO 2018 bathing water review is based on a detailed and systematic review of the scientific literature and can be considered an authoritative report on the subject. Details of there review process and methods could be more clearly reported; however the report contains a useful, high-quality review of key themes relevant to microbial risk and recreational water use which can be considered compatible with Australian processes.

The document represents the advice of the WHO, as a series of recommendations, for consideration in a potential review of the current European Commission (EC) Bathing Water Directive (BWD) – Directive 2006/7/EC3. The document summarises, in a series of fact sheets, the recent scientific literature on the existing Bathing Water

Directive parameters (intestinal enterococci and *E. coli*). It also examines the feasibility of possible additional parameters (viral indicator(s) and harmful algal blooms) [not in scope of current narrative review] and considers wider/emerging issues (antimicrobial resistance, microplastics, other infectious agents) also as a series of fact sheets.

With respect to the primary research question, WHO recommendations were:

Monitoring

(i). Intestinal enterococci and *E. coli* should be retained.
(ii). The four levels within the current classification system (excellent, good, sufficient, and poor) should be retained.
(iii). The classification system for each category should be based on a 95^{th} percentile value and not a mixture of 95^{th} and 90^{th} percentile water quality standards.
(iv). The annual minimum number of samples for an EU bathing water site should be increased to 20.
(v). Data from bathing water sites (with at least 80 samples) should be tested for log10 normality. Where the data are shown to be log10 normally distributed, the calculation method in Annex II of the 2006 Bathing Water Directive should be used. Where the data do not exhibit log10 normality the Hazen calculation should be used. Where there are inadequate data available, it is suggested that the Hazen calculation is used.
(vi). The ISO method (9308-1) for *E. coli* analysis is no longer appropriate for the measurement of bathing water quality. Sampling and sample analysis should be conducted by laboratories accredited for the methods being used.

Good practice

(vii). Bathing water quality should be representative of the whole bathing area. This should be confirmed by occasional spatial/beach shoreline transect sampling.
(viii). Temporal variability in water quality should be addressed by sampling at different times to characterise the bathing day in the overall compliance data set, or by taking a precautionary approach and sampling when water quality is generally poorest.
(ix). Where predictive modelling is used to inform the public, the choice of model and methods of public information dissemination should be reported. The models should meet minimum requirements (including an explained variance of at least 50-60%) and the approach taken should be justifiable and auditable.
(x). In a number of cases (such as MST techniques and QMRA for use in bathing water profiling) it would be valuable to commission a detailed state of the art review, to provide standardised information and advice on their practical application to Member States.

The review material is clearly presented and targets issues of relevance to the European Bathing Water Directive, but overall does not report on the expert analysis of the constituent research papers in detail. This may be a deliberate to keep the document concise.

Table 6-1. Summary of grey literature review results for the Primary Research Question: How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?

ID	Summary
G3: EPA Victoria (2021)	Using a QMRA approach, the probability of illness p(ill) from water-based recreational activities at three beach locations within Port Phillip Bay, was compared to that which would be expected to occur when indicators were at the thresholds listed in the Victorian State Environment Protection Policy [SEPP (Waters)] and the 2008 NHMRC Guidelines. The QMRA for primary contact recreation (PrCR) predicted a mean probability of illness (p(ill)) of 0.33% [95th percentile 1.07%] per exposure event, mostly due to norovirus 0.23% [0.82%]. Adenoviruses contributed the next highest proportion of total risks at 0.07% [0.23%], whilst bacteria and protozoa had p(ill) at or below 0.01%. For secondary contact recreation (SeCR), the QMRA predicted a mean probability of illness of 0.05% [0.18%). As with the PrCR QMRA, most of this probability was derived from norovirus. Sensitivity analyses showed that the QMRA models were not sensitive to changes in the dose-response relationship. Regardless of the dose-response model chosen, the 95th percentile probability of enteric illness due to a single PrCR event rarely exceeded 2.02%. Using data collected from the three beaches, the QMRA showed that for both PrCR and SeCR the probabilities of contracting an illness were very low compared to that expected using at the thresholds listed in the SEPP (Waters) and the NHMRC guidance (2008). The study concluded that the current practice of using indicators testing could not accurately predict the densities of pathogens in Port Phillip Bay. Although *E. coli* and enterococci correlated with the calculated rates of gastrointestinal illnesses due to a PrCR event, meaningful bay-specific objectives could not be directly derived from this relationship.
G4: King et al. (2014)	To evaluate the epidemiological literature published between 2003 and 2014, King et al examined the relationship between recreational water use (i.e. exposure to marine water and freshwater recreational waters) and gastrointestinal illness (GI). The authors found that there is continuing evidence that bathing in recreational water poses some increased risk of GI to bathers compared with non-bathers. Specifically: • There appears to be little or no significant difference between GI in bathers compared with non-bathers at marine beaches. • In contrast, there appears to be a consistent and significantly higher risk of GI in bathers compared with non-bathers in freshwater sites in temperate climates (up to 3.2 times higher). • There is some evidence to suggest that increased bather exposure (i.e. head immersion or swallowing water) results in a higher risk of GI, particularly for freshwater bathers. • There is evidence to suggest that an increase in time spent in water is associated with an increase in GI. • There is very little evidence on how the risk of GI varies with age. • There is a lack of recent studies which have evaluated the risk of GI in recreational water users other than bathers (e.g. in people canoeing, fishing, kayaking, motor boating, or rowing). King et al., were highly critical of the quality of the literature reviewed and suggested that the various results presented by the study authors could be an artefact of the range of methods used.
G8: NHMRC (2008)	The 2008 NHMRC Guidelines provide guidance for state and local agencies towards a nationally harmonised approach to managing recreational water quality. The Guidelines implicitly recommend a classificatory approach to the management of recreational water quality. The approach involves microbial-based categorisation of the water using a combination of sanitary inspection and microbial water-quality assessment. Combining such categorisation with prevention of exposure at times of increased risk provides an effective framework for assessing and managing recreational water quality. The guidelines include a classification matrix which is based on four classes of microbial water quality (as 95th percentiles of enterococci/100 ml) by four sanitary inspection categories (rating susceptibility of the site to faecal influence).
G11: NSW DPIE (2020)	The NSW DPIE Protocol for assessment and management of microbial risks in recreational waters provides guidance to support the implementation of Chapter 5 of the 2008 NHMRC Guidelines. Key elements of the protocol that contribute to the primary research question are:

ID	Summary
	• The inclusion of an initial site prioritisation step which prioritises beaches to provide a basis for determining resource allocation. This step provides a mechanism for identifying high priority swimming locations that should attract more monitoring and reporting resources to ensure the greatest benefit is obtained. High priority beaches become the subjects of more detailed risk assessments, and programs for monitoring, reporting and microbial risk management. • A detailed Sanitary Inspection process with 5 steps: 1. Define the swimming area and catchment. 2. Identify sources of faecal contamination and gather information on the frequency, duration, and intensity of impact. 3. Assess likelihood for each identified source of faecal contamination. 4. Determine the Sanitary Inspection Category for the site (overall likelihood). 5. Hold a workshop or meeting with stakeholders to review pollution sources and likelihood assessment. • A microbial water quality monitoring program with specific guidance for sampling design and documentation, quality control aspects including sampling procedures, lab methods and accreditation, data management and work health and safety. • A microbial assessment and beach classification program which describes the methodology for determining beach suitability grades • Reporting of: Annual Classifications, Weekly Star Ratings, Advisories following rainfall events, daily beach pollution forecasts, communication planning and methods of communication. • Appendices with high quality templates for data collection and reporting for such items as: Sanitary inspection major attributes (e.g. pollutions sources), and water quality sample log sheets. The protocol also includes methods and templates for assessing and scoring likelihoods of contamination from a wide variety of pollution sources.
G13: US EPA (2017)	The US EPA "2017 Five-Year Review of the 2012 Recreational Water Quality Criteria" is a 5-year review of its 2012 Recreational Water Quality Criteria (RWQC), as required by amendments to the 1972 US Clean Water Act. With respect to the primary research question, key findings were: • Health Studies: A growing body of evidence suggests that children can be disproportionately susceptible to health effects resulting from exposure to pathogens in recreational waters. There are opportunities for further resolution of epidemiological relationships, especially around children's health protection and wider application of *Enterococcus* spp. qPCR techniques for monitoring. • Antimicrobial Resistance. Although of increasing interest, US EPA suggests more research is needed to better understand the role the environment plays in transferring antimicrobial resistant bacteria (AMRB) to primary contact recreators.
G16: WHO (2018)	With respect to the primary research question, the WHO 2018 bathing water review recommendations were: **Monitoring** (i). Intestinal enterococci and *E. coli* should be retained. (ii). The four levels within the current classification system (excellent, good, sufficient, and poor) should be retained. (iii). The classification system for each category should be based on a 95th percentile value and not a mixture of 95th and 90th percentile water quality standards. (iv). The annual minimum number of samples for an EU bathing water site should be increased to 20. (v). Data from bathing water sites (with at least 80 samples) should be tested for log10 normality. Where the data are shown to be log10 normally distributed, the calculation method in Annex II of the 2006 Bathing Water Directive should be used. Where the data do not exhibit log10 normality the Hazen calculation should be used. Where there are inadequate data available, it is suggested that the Hazen calculation is used. (vi). The ISO method (9308-1) for *E. coli* analysis is no longer appropriate for the measurement of bathing water quality. Sampling and sample analysis should be conducted by laboratories accredited for the methods being used. **Good practice**

ID	Summary
	(vii). Bathing water quality should be representative of the whole bathing area. This should be confirmed by occasional spatial/beach shoreline transect sampling. (viii). Temporal variability in water quality should be addressed by sampling at different times to characterise the bathing day in the overall compliance data set, or by taking a precautionary approach and sampling when water quality is generally poorest. (ix). Where predictive modelling is used to inform the public, the choice of model and methods of public information dissemination should be reported. The models should meet minimum requirements (including an explained variance of at least 50-60%) and the approach taken should be justifiable and auditable. (x). In a number of cases (such as MST techniques and QMRA for use in bathing water profiling) it would be valuable to commission a detailed state of the art review, to provide standardised information and advice on their practical application to Member States.

6.1.2. Secondary research question (1)

1. What are the indicators/surrogates of this/these risk/s (of microbial contamination from diffuse and point sources in recreational waters)?

G3: EPA Victoria (2021). *Quantitative Microbial Risk Assessment (QMRA) for assessing risks to recreational users in Port Phillip Bay*

EPA Victoria (2021) used microbial source tracking (MST) techniques to identify sources of microbial contamination and assess risks to recreational waters. The authors state (p.29) that on average, sewage and dog faeces were the highest contributors to faecal pollution at the beaches, although elsewhere (p.35) it is stated that the main contributions to faecal contamination were of avian and canine origins (which carry comparatively lower risks to human health). Data in Table 6 on p.29 suggests that human sewage, canine and avian sources are all significant although contributions vary with site. On average, 13% of the total faecal contamination originated from a human source, which is believed to drive the risk at each of the three beaches studied. The total proportion of human sewage in samples ranged from less-than-detection to 0.29%, with an average of 0.03% across all sites.

The study used the qPCR marker Bacteroides HF183/BacR287 as an indicator of human sewage and found that there was a statistically significant correlation between the concentrations of the qPCR marker and the proportion of the microbial communities in the beach samples that were like human sewage ($p=0.008$). Similarly there was a significant correlation between enterococci concentrations and the proportion of the microbial community within the beach samples that were like human sewage communities ($p=0.004$). There was also a statistically significant relationship between enterococci concentrations and the total proportion of faecal microbial communities ($p<0.001$), perhaps confirming that enterococci provide an estimate of the overall level of faecal contamination.

G4: King et al. (2014). ***The health risks of bathing in recreational waters. A rapid evidence assessment of water quality and gastrointestinal illness (GI).***

In relation to research question, King et al. (2014) sought evidence to support the different classification standards outlined in the European Bathing Directive.

The authors found that:

- There is little evidence for a significant dose-response between faecal indicator organisms and GI in marine water.
- There appears to be a significant dose-response between faecal indicator organisms and GI in fresh water.
- Very high levels of pollution due to heavy rainfall and urban run-off or sewage contamination are associated with increased GI.

G8: NHMRC (2008). ***Guidelines for managing risks in recreational water*****.**

The guidelines contained no evidence to support a response to this question.

G11: NSW DPIE (2020). ***Protocol for Assessment and Management of Microbial Risks in Recreational Waters*****.**

The Protocol adopts the 2008 NHMRC Guidelines recommended approach for the use of enterococci as the indicator organism for assessing risks from microbial contamination in recreational waters.

G13: US EPA (2017). ***2017 Five-Year Review of the 2012 Recreational Water Quality Criteria.***

Relevant findings under the Science Review component described by US EPA (2017) to the secondary research question (1) were:

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

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

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

A further conclusion drawing on the review undertaken for the US EPA by Dr Graham McBride (elaborated in the text in section IV, A, 4: Health Relationships and Alternative Indicators) noted that although observations show that development and use of alternative faecal indicators is a rich and evolving field, no strong case was made for changing the indicators currently. However, alternative method-indicator combinations might be supported in certain situations and warrant further study especially in specific settings, such as tropical waters.

G16: WHO (2018). ***WHO recommendations on scientific, analytical, and epidemiological developments relevant to the parameters for bathing water quality in the Bathing Water Directive (2006/7/EC)*****.**

With respect to the secondary research question (1), the WHO general recommendations were:

(i). Current evidence does not support the inclusion of a viral indicator (or viral pathogen) as a regulatory parameter within the BWD.

Under good practice and research, the WHO recommendations were:

(ii). Viruses have a valuable role to play in microbial source tracking investigations and also quantitative microbial risk assessment, and it is suggested that these tools should be considered more widely in bathing water profiling

(iii). Research needs to include the identification of suitable candidate viral organisms and the development of standard methods suitable for bathing water use.

Table 6-2. Summary of guideline review results for Secondary Research Question 1: What are the indicators/surrogates of this/these risk/s (of microbial contamination from diffuse and point sources in recreational waters)*?*

ID	Summary
G3: EPA Victoria (2021)	EPA Victoria (2021) used microbial source tracking (MST) techniques to identify human sewage, canine and avian sources of microbial contamination and assess risks to recreational waters. The study measured qPCR marker Bacteroides HF183/BacR287 as an indicator of human sewage and enterococci concentrations and found a significant correlation between proportion of the microbial communities were like human sewage microbial communities and the qPCR marker ($p=0.008$) and enterococci ($P<0.001$). Similarly a significant relationship was reported between enterococci concentrations and the total proportion of faecal microbial communities ($p<0.001$), perhaps confirming that enterococci provide an estimate of the overall level of faecal contamination.
G4: King et al. (2014)	King et al. (2014) found that: • There is little evidence for a significant dose-response between faecal indicator organisms and GI in marine water. • There appears to be a significant dose-response between faecal indicator organisms and GI in fresh water. • Very high levels of pollution due to heavy rainfall and urban run-off or sewage contamination are associated with increased GI.
G8: NHMRC (2008)	The guidelines contained no evidence to support a response to this question.
G11: NSW DPIE (2020)	The Protocol adopts the 2008 NHMRC Guidelines recommended approach for the use of enterococci as the indicator organism for assessing risks from microbial contamination in recreational waters.

ID	Summary
G13: US EPA (2017)	Under the Science Review component US EPA (2017) conclusions were: • *Coliphage as an indicator:* Because evidence strongly suggests most illnesses in recreational waters are due to enteric viruses, development and implementation of viral indicators, such as coliphage, may yield advances in public health protection. • *Indicators and Performance of qPCR Methods*: qPCR methods have contributed to improved beach monitoring programs where implemented, yet opportunities remain for further refinement of qPCR methodologies. *Enterococcus* spp. measured by qPCR is a better predictor of swimming-associated GI illness and more timely than current culturable bacterial indicators. Although observations show that development and use of alternative faecal indicators is a rich and evolving field, no strong case was made for changing the indicators currently.
G16: WHO (2018)	The findings from the WHO's general recommendations were: (i). Current evidence does not support the inclusion of a viral indicator (or viral pathogen) as a regulatory parameter within the European Bathing Water Directive. (ii). Viruses are important in MST investigations and QMRA and these tools should be considered more widely in bathing water profiling (iii). Research needs to include the identification of suitable candidate viral organisms and the development of standard methods suitable for bathing water use.

6.1.3. Secondary research question (2)

2. What are the current practices to minimise or manage this/these risk/s (of microbial contamination from diffuse and point sources in recreational waters)?

G3: EPA Victoria (2021). *Quantitative Microbial Risk Assessment (QMRA) for assessing risks to recreational users in Port Phillip Bay*

EPA Victoria (2021) noted that indicator organism testing typically assumes that 100% of the faecal material is of human origin. However, the source tracking study showed that human faeces only contributed an average of 13% of the total faecal contamination and the main contributors to faecal contamination were of avian and canine origin (contributions not quantified in report). The latter sources carry comparatively lower risks to human health. This suggests that considering the origin of the contamination should be a primary factor in assessing risks of water-based recreation in Port Phillip Bay, since it can significantly impact the outcome of the risk assessment. For example, human sources identified during the sanitary survey included bather shedding (release), toilet facilities, sewage treatment plant (STP) outfalls, STP by-passes, sewage overflows, sewage chokes, and boats.

[Reviewers note: The use of QMRA provided additional detail to support monitoring and sanitary assessment.]

G4: King et al. (2014). *The health risks of bathing in recreational waters. A rapid evidence assessment of water quality and gastrointestinal illness.*

The review contained no evidence to support a response to secondary research question (2).

G8: NHMRC (2008). *Guidelines for managing risks in recreational water*.

The guidelines contained no evidence to support a response to secondary research question (2).

G11: NSW DPIE (2020). *Protocol for Assessment and Management of Microbial Risks in Recreational Waters*.

Part 7 of the 7-part management framework for managing microbial risks in recreational water lists generic management actions to reduce the microbial risks to recreational water quality. Risk management recommendations are classified as follows:

- Actions to reduce likelihood
  - Pollution abatement sewage and stormwater controls
  - Use of microbial source tracking (MST) to assist in identifying sources and thus appropriate source controls
- Actions to reduce consequence
  - Beach closures
  - Informed personal choice based on media advisories
- Triggers for management actions
  - Water quality monitoring (based on enterococci)
  - Rapid responses to spill incidents (e.g. sewer overflows)

G13: US EPA (2017). 2017 Five-Year Review of the 2012 Recreational Water Quality Criteria.

Under the Science Review component, the US EPA considered accurate and reliable MST technologies could markedly improve future water quality management in the U.S., possibly allowing for the development of alternative site-specific criteria based on pollution sources present, strategic remediation planning based on faecal pollution levels from human sources.

Relevant findings under the Implementation Review component described by US EPA (2017) to the secondary research question (2) were:

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

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

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

G16: WHO (2018). WHO recommendations on scientific, analytical and epidemiological developments relevant to the parameters for bathing water quality in the Bathing Water Directive (2006/7/EC).

With respect to the secondary research question (2), under the heading of "Wider/Emerging Issues", the WHO recommended:

(i). At locations where swimmer's itch is known to occur, this should be included in the bathing water profile and information provided to members of the public.

(ii). Where cases of wound infection (e.g. caused by *Vibrio* spp.) have resulted from a recreational water exposure, this information should be communicated in the bathing water profile. In addition, on-site information should be provided including advice on bather hygiene measures to minimise risk and actions to take if a wound is sustained while bathing.

Table 6-3. Summary of guideline review results for Secondary Research Question 2: What are the current practices to minimise or manage this/these risk/s (of microbial contamination from diffuse and point sources in recreational waters)?

ID	Summary
G3: EPA Victoria (2021)	EPA Victoria's MST study of three Port Phillip Bay beaches showed that human faeces contributed on average of only 13% of the total faecal contamination and the main contributors to faecal contamination were of avian and canine origin which carry comparatively lower risks to human health. Human sources identified during the sanitary survey included bather shedding (release), toilet facilities, sewage treatment plant (STP) outfalls, STP by-passes, sewage overflows, sewage chokes, and boats. To be effective, management responses would need to be tailored to each source type, thus the authors recommended that the origin of the contamination should be a primary factor in assessing risks of water-based recreation, since it can significantly impact the outcome of the risk assessment.
G4: King et al. (2014)	The review contained no evidence to support a response to this question.
G8: NHMRC (2008)	The review contained no evidence to support a response to this question.
G11: NSW DPIE (2020)	Suggested risk management recommendations are helpfully classified by NSW DPIE (2020) as follows: • *Actions to reduce likelihood* o Pollution abatement sewage and stormwater controls o Use of microbial source tracking (MST) to assist in identifying sources and thus appropriate source controls • *Actions to reduce consequence* o Beach closures o Informed personal choice based on media advisories • *Triggers for management actions* o Water quality monitoring (based on enterococci) o Rapid responses to spill incidents (e.g. sewer overflows)
G13: US EPA (2017)	Findings from the US EPA review that are relevant to secondary research question 2 were: • *Further research on accurate and reliable MST technologies* should be undertaken to markedly improve future water quality management by: o Fostering development of alternative site-specific criteria based on local pollution sources and o Assisting strategic remediation planning to focus on faecal pollution from human sources.

ID	Summary
	• *Sanitary Surveys* should continue to serve as an important tool for informing site remediation, characterizing waters for QMRA and site-specific criteria development, and can be linked with integrated environmental modelling. • Predictive/Statistical Models offer a cheaper alternative for same-day notification and resulting public health protection than other rapid methods. • *Deterministic Process Models for Recreational Beach Site Assessment and Enhancement/Remediation* can include QMRA health-based models and can assist understanding of contaminant transport and development of support site-specific criteria and/or remediation options.
G16: WHO (2018)	With respect to the secondary research question (2) WHO recommended that information on the following dermal afflictions resulting from recreational water exposure should be conveyed to the public in the bathing water profile at sites they are known to occur: (i). Swimmer's itch; (ii). Wound infection (e.g. caused by *Vibrio* spp.). In addition, advice should be provided on bather hygiene measures to minimise risk and actions to take if a wound is sustained while bathing.

Table 6-4. Body of evidence summary for included grey literature

Guideline	Contribution to primary research question outcome?	Contribution to secondary research questions outcomes?	Outcomes relevant to Australian conditions?	Addresses target populations?	Identifies main factors impacting risk and its prediction?	Reviewer's comments	Overall Assessment
G3: EPA Victoria (2021)	Yes	Yes	Yes, the report describes a study conducted at 3 recreational beaches in Port Phillip Bay, a large 1,930 km2 shallow estuarine embayment around which greater metropolitan Melbourne is located.	No, the report does not address specific target populations such as children, immunocompromised or the elderly and how they may be impacted.	Yes. The mean p(ill) for primary contact recreation (PrCR) was 0.33% [95^{th} 1.07%] per exposure event, mostly due to norovirus 0.23% [0.82%]. Adenoviruses contributed the next highest proportion of total risks at 0.07% [0.23%], whilst bacteria and protozoa had p(ill) at or below 0.01%. Human sewage was identified as the most likely source of norovirus and adenovirus.	This was a large study with findings directly relevant to the primary and secondary research questions. The key findings demonstrate the broad utility of MST and QMRA approaches and are also important for informing local management responses.	Important Australian reference describing new methods for MST and innovative approach to QMRA. Published report does not appear to include all associated research.
G4: King et al. (2014)	Yes	Yes (Q.1)	Yes, the review focussed on the relationship between recreational water use and gastrointestinal illness (GI).	No, commented that "very little evidence on how the risk of GI varies with age."	Yes, states evidence suggests higher GI risk in bathers vs non-bathers at freshwater beaches but not marine beaches. Also increased risk of GI with higher exposure (e.g. head immersion, swallowing water) and time spent in water. Very high levels of pollution due to heavy rainfall and urban run-off or sewage contamination are associated with increased GI.	A concise although dated review. The authors were highly critical of the quality of the literature reviewed and suggested that the various results presented by the study authors could be an artefact of the range of methods used.	The review was thorough and conducted according to best practice at the time.
G8: NHMRC (2008)	Yes	No	Yes, Australian guidance document designed for Australian conditions.	No	Yes, discusses potential factors in general terms. Recommends a classificatory approach to the management of recreational water quality involving microbial-based categorisation of the water using a combination of sanitary inspection and microbial water-quality assessment combined with exposure controls at times of increased risk.	Although the purpose of this review is to support an update of Chapter 5 of the NHMRC guidelines, the classificatory approach to the management of recreational water quality described in the chapter could be considered to provide a baseline for reference.	The microbial-based classificatory approach described in the guidelines, although in need of updating, still describes an effective approach for management of recreational water quality.
G11: NSW DPIE (2020)	Yes	Yes	Yes, local guidance document designed for NSW conditions, but applicable nationwide.	No	Yes, the protocol provides detailed guidance and an improved assessment and management framework to support the implementation of	A noteworthy innovative aspect of the protocol is the provision of methods for assessing and scoring likelihoods of contamination from a wide variety of pollutions sources	The protocol provides a clear and detailed practical methodology for monitoring, assessing, and predicting risks from

Guideline	Contribution to primary research question outcome?	Contribution to secondary research questions outcomes?	Outcomes relevant to Australian conditions?	Addresses target populations?	Identifies main factors impacting risk and its prediction?	Reviewer's comments	Overall Assessment
					Chapter 5, Microbial Risks, of the 2008 NHMRC Guidelines	as well as templates for scoring such hazards in the appendices.	diffuse and point source microbial contamination in recreational waters.
G13: US EPA (2017)	Yes	Yes	Yes, contains appropriate recommendations relevant to research and implementation.	Yes, commentary stating that a growing body of evidence suggests that children can be disproportionately susceptible to health effects resulting from exposure to pathogens in recreational waters	Yes, evidence strongly suggests most illnesses in recreational waters are due to enteric viruses, thus development and implementation of viral indicators such as coliphage and use of qPCR methods is advocated.	The review was based on external and in-house reviews by EPA and industry consultation. The review provided general discussion under several headings. While the review was unable to draw any concise conclusions, several suggestions for further research are provided.	The review contains authoritative information; however, due to the heterogeneous subject matter, an effective synthesis was unable to be undertaken.
G16: WHO (2018)	Yes	Yes	Yes. Recommendations are structured in a manner that is relevant to the review of the Australian 2008 NHMRC Guidelines.	No	No. However, the main factors impacting risk are assumed and instead the review focuses on better characterisation of risk through recommendations for method standardisation and improved methods for laboratory analysis, statistical analysis, bathing water quality temporal and spatial variability, and predictive modelling,	The review recommends retention of: (i) enterococci and *E. coli* as FIB, (ii) current four level risk classification, and (iii) use of 95th percentiles to support the classifications. Whilst viruses are considered important bathing water profiling and for use in MST and QMRA, current evidence does not yet support the inclusion of viral indicators or pathogens, however, these items should be the focus of future research. Information on dermal afflictions due to recreational water exposure should be conveyed to the public in the bathing water profile at sites they are known to occur.	The review material is clearly presented and targets issues of relevance to the European Bathing Water Directive, but overall does not report on the expert analysis of the constituent research papers in detail. This may be intentional to keep the document concise.

6.2. Review of primary studies

How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?

J2: Ahmed, Hamilton, et al., (2018) Quantitative microbial risk assessment of microbial source tracking markers in recreational water contaminated with fresh untreated and secondary treated sewage. (Qualitative research)

Ahmed, Hamilton, et al., (2018) sought to develop a method for the use of qPCR markers to assess risks from untreated and treated sewage in beach water. The authors undertook an exploratory QMRA analysis based on the process limit of quantification (PLOQ) of five sewage-associated quantitative PCR (qPCR) MST markers [Bacteroides HF183 (HF183), *Methanobrevibacter smithii* nifH (nifH), human adenovirus (HAdV), human polyomavirus (HPyV) and pepper mild mottle virus (PMMoV)] in filter-sterilized beach water samples seeded with fresh untreated and secondary treated sewage samples. The objective of the study was to determine at what concentration these nucleic acid markers reflected a significant health risk from exposure to fresh untreated or secondary treated sewage in beach water.

Serial ten-fold dilution series of beach water samples dosed with either fresh untreated sewage or fresh secondary treated sewage were tested to determine the PLOQ of the 5 sewage biomarkers. The corresponding probability of GI illness from norovirus (NoV) or HAdV 40/41 for each biomarker PLOQ was also compared with the US EPA (2012b) tolerable benchmark for GI illness for recreational water of 36 GI Illnesses/1000 exposures or 0.036. This approach assumes that GI risks arise only from NoV and HAdV 40/41 and the only source of contamination is either fresh raw sewage or fresh secondary treated sewage.

Among all the markers tested, HF183 concentration needed to be greater in the water sample than other markers to correspond to an exceedance of the illness benchmark (Table 2 in paper). When NoV and HAdV 40/41 were used as reference pathogens, a median concentration of 3.22×10^3 GC of the HF183 in 100 mL of water sample represented a risk above the GI illness benchmark value when beach water was contaminated with fresh untreated sewage. Similarly, 3.66×10^3 GC of HF183 in 100 mL of water sample represented a risk above the benchmark for both NoV and HAdV 40/41, when beach water was contaminated with secondary treated sewage. Table 2 in the paper lists values for the other biomarkers tested.

The authors note that the decay rates of markers especially HF183 or nifH, can be faster than HAdV. If enteric viruses persist longer in the environment and remain infective, the GI risk estimates generated by HF183 and nifH will markers need to be interpreted carefully. It was also assumed that all NoV and HAdV 40/41 quantified using qPCR are viable and infective.

J3: Ahmed, Payyappat, et al., (2019) Enhanced insights from human and animal host-associated molecular marker genes in a freshwater lake receiving wet weather overflows. (Qualitative research).

The impact of wet weather overflows of sewage to a freshwater swimming lake was assessed by Ahmed, Payyappat, et al., (2019). The authors used FIB and MST biomarkers to assess the magnitude of sewage and animal faecal contamination in Lake Parramatta water samples collected during a dry weather period and from two storm events that coincided with wet weather overflows of sewage to the lake.

Water samples were tested for a range of novel and established FIB and sewage-associated and animal faeces-associated MST marker genes [Sewage: Bacteroides HF183, crAssphage CPQ_056 and pepper mild mottle virus (PMMoV), Animal faeces:- Bacteroides BacCan-UCD, cowM2 and Helicobacter spp. associated GFD) along with the enumeration of culturable faecal indicator bacteria (FIB), namely *Escherichia coli* (*E. coli*) and *Enterococcus* spp.

The magnitude of general and source-specific faecal pollution was low in water samples collected during dry weather compared to storm events. The levels of HF183, crAssphage and PMMoV in water samples collected during storm events were as high as 6.39, 6.33 and 5.27 log10 GC/L of water, respectively. Moderate to strong positive correlations were observed among the quantitative occurrence of sewage-associated marker genes. The concentrations of HF183 and PMMoV in most storm water samples exceeded the risk benchmark threshold values established in the literature for primary contact recreators (see Table 3 in the paper). None of the samples tested was positive for the cowM2 (cow) marker gene, while BacCan-UCD (dog) and GFD (avian) animal-associated markers were sporadically detected in water samples collected from both dry weather and storm events.

Based on the results, the ongoing advice that swimming should be avoided for several days after storm events was considered appropriate. Further research to determine the decay rates of sewage-associated marker genes in relation to each other and enteric viruses would help refine current advice. Microbial source tracking approaches employed in the study provided insights into sources of contamination over currently used FIB.

J4: Ahmed, Payyappat, et al., (2020) Sewage-associated marker genes illustrate the impact of wet weather overflows and dry weather leakage in urban estuarine waters of Sydney, Australia. (Qualitative research).

Ahmed, Payyappat, et al., (2020) sought to determine the impacts of sewage network wet weather and dry weather overflows (WWOs and DWOs respectively) and associated sewage contamination at three estuarine sites receiving stormwater flows in Sydney, NSW.

Concentrations of culturable faecal indicator bacteria (FIB) and MST biomarker genes were measured in dry weather samples and samples following storm events from 6 sampling stations at each of the three estuaries. At each station samples were collected at (i) 0.5 m below the water surface, and (ii) 1 m above the bottom surface to investigate whether MST marker genes declined with depth.

MST biomarker genes used were Bacteroides HF183, pepper mild mottle virus (PMMoV), crAssphage CPQ_056, Lachnospiraceae (Lachno3), whilst FIB used were *E. coli* and enterococci. Water samples were also analysed for four animal faeces-associated biomarker genes targeting avian (GFD), dog (BacCan-UCD), cow (cowM2) and horse (HoF597) species to determine the extent of animal faecal contamination.

Analysis of sewage associated marker genes showed greater (i.e., 3–5 orders of magnitude) concentrations in water samples collected during the storm events compared to dry weather event.

Among the four animal faeces-associated marker genes, cow (cowM2) and horse (HoF597) could not be detected, while the avian (GFD) marker gene was consistently present, and the dog (BacCan-UCD) marker gene was occasionally detected. Overall results suggested that after rainfall, untreated sewage from wet weather overflows (WWOs) was present at sampling locations. In addition, microbial source tracking (MST) monitoring was able to distinguish the presence of a leaking sewer impacting on the recreational area during dry weather condition.

Depth profile analysis of FIB indicated overall greater concentrations at the surface compared to the bottom of the water bodies.

J5: Ahmed, Zhang, et al., (2019) Comparative decay of sewage-associated marker genes in beach water and sediment in a subtropical region. (Qualitative research).

The primary aim of this study was to investigate the decay of four sewage-associated bacterial and viral markers (HF183, HAdV, HPyV, and crAssphage), in relation to each other and qPCR FIB [*E. coli* (EC), and *Enterococcus* spp. (ENT) 23S rRNA genes], in sewage contaminated fresh and marine waters and accompanying sandy sediments.

Outdoor mesocosms containing water and sediment were inoculated with untreated sewage, and qPCR assays were used to quantify each target over 40 days to determine their decay rates.

Water samples were collected from two marine sites and one freshwater lake environment. Three replicate mesocosms (8-L plastic containers filled with sediment (4 - 5 cm depth) were constructed for each source of water giving nine mesocosms in total.

From each mesocosm, one water sample (100 mL) was collected on each of days 0, 1, 4, 8, 14, 24 and 40. On day 0, water in the mesocosms was sampled, using a 25mL pipette, within 30 min after sewage inoculation. Sediment core samplers, made by cutting the ends of plastic 10 mL pipettes, were used for sampling sediment from each mesocosm. One sediment sample (approximately 1 g) was collected from each mesocosm on days 0, 1, 4, 8, 14, 24 and 40. Water samples were collected prior to sediment samples to avoid sediment disturbance.

Decay rates of EC 23S rRNA, ENT 23S rRNA, and HF183 16S rRNA were significantly ($p < 0.05$) faster than the HAdV, HPyV and crAssphage markers in water samples from all mesocosms. In general, decay rates of bacterial targets were similar in the water columns of the studied mesocosms. Similarly, decay rates of viral targets were also alike in mesocosm water columns in relation to each other. The decay rates of FIB and sewage-associated markers were significantly faster in water samples compared to sediments in all three mesocosms.

Also in water mesocosm samples decay rates of bacterial targets (two FIB and HF183) were fast and similar across three mesocosms. Similarly, decay rates of viral targets (HAdV, HPyV and crAssphage) were similar in water samples, however, viral targets persisted significantly longer ($p < 0.05$) than did bacterial targets.

In sediment mesocosm samples decay rates of FIB and sewage-associated markers varied greatly across the marine and freshwater mesocosms. Decay rates of FIB and sewage-associated markers were significantly faster in water samples compared to sediment in all mesocosms.

FIB (EC and/or ENT) exhibited biphasic decay rates compared to MST markers in water and sediment samples from all mesocosms. Due to the nature of decay, the authors concluded that they may not be reliable markers to detect recent faecal pollution.

J6: Arnold, Schiff, et al., (2017) Acute Illness Among Surfers After Exposure to Seawater in Dry- and Wet-Weather Conditions. (Cohort study).

Arnold, Schiff, et al., (2017) sought to determine if exposure to seawater increased rates of incident illness among a longitudinal cohort of surfers in San Diego, California. Illness rates after surf periods were compared with periods after no surfing in order to determine whether exposure during or immediately after rainstorms increased rates more than did exposure during dry weather.

Beach water quality sampling was undertaken at two surf beaches receiving stormwater drainage. The study was undertaken over two winter periods presumably to capture sufficient rainfall events. A total of 654 individuals were enrolled who contributed on average 51 days of follow-up.

To examine illness rates separately for dry- and wet-weather exposures, the authors created a 3-level categorical exposure that classified each participant's follow-up time into unexposed, dry-weather exposure, and wet-weather exposure periods. Wet-weather exposure was defined as exposure to seawater within 3 days of 0.25 cm or more of rainfall in a 24-hour period and all other seawater exposure as dry-weather exposure. The wet weather criterion is the same rainfall criterion used by the local county (San Diego) for posting wet-weather beach advisories.

Findings related to illness associated with seawater exposure while surfing:

- Seawater exposure in the past 3 days was associated with increased incidence rates of gastrointestinal illness, diarrhea, sinus pain or infection, earache or infection, infection of open wound, skin rash, and fever, but not upper respiratory illness.
- With the exception of fever and skin rash, incidence rates increased from unexposed to dry-weather exposure to wet-weather exposure periods, a pattern also present on the risk scale.
- Compared with unexposed periods, wet-weather exposure led to the largest relative increase in:

- earaches/infections (adjusted incidence rate ratio (IRR) = 3.28, 95% confidence interval (CI): 1.95, 5.51); and
- infection of open wounds (adjusted IRR: 4.96, 95% CI: 2.18, 11.29).

Findings related to illness associated with faecal indicator bacteria levels:

- FIB *(Enterococcus)*, total coliform, and faecal coliform levels were positively associated with increased incidence of almost all outcomes during the study.
- Rainfall was a strong effect modifier of the association.
- During dry weather, there was no association between *Enterococcus* levels and illness except for infected wounds, but *Enterococcus* was strongly associated with illness after wet-weather exposure (e.g., for each log10 increase, gastrointestinal illness IRR = 2.17, 95% CI: 1.16, 4.03).
- There was evidence for excess risk of gastrointestinal illness at higher *Enterococcus* levels only during wet-weather periods: The predicted excess risk that corresponded to the current US EPA regulatory guideline of 35 colony-forming units per 100 mL was 16 episodes per 1,000 (95% CI: 5, 27).
- Negative control analyses showed no consistent association between faecal indicator bacteria and illness among participants during periods in which they had no recent seawater contact.

Surfing was associated with increased incidence of several categories of symptoms, and associations were stronger if surfing took place shortly after rainstorms. Higher levels of FIB were strongly associated with fever, sinus pain/infection, wound infection, and gastrointestinal symptoms within 3 days of rainstorms. The internal consistency between water-quality measurements, patterns of illness after dry- and wet-weather exposures, and incidence profiles with time since rainstorms lead the authors to conclude that seawater exposure during or close to rainstorms at beaches impacted by urban runoff in southern California increases the incidence rates of a broad set of acute illnesses among surfers. The authors concluded that the findings provide strong evidence to support the posting of beach warnings after rainstorms and initiatives that would reduce pathogen sources in urban runoff that flows to coastal waters.

J15: Gitter, Mena, et al., (2020) Human health risks associated with recreational waters: Preliminary approach of integrating quantitative microbial risk assessment with microbial source tracking. (Qualitative research).

The objective of the study was to conduct a QMRA estimating the risk for GI illness using MST data describing the primary sources contributing to a bacteria impairment in particular waterbody; using Walnut Creek, Brazos River Basin, Texas as an example. *E. coli* concentrations were used to predict reference pathogen concentrations from cattle (*Campylobacter*), human (Norovirus), and wildlife/domestic animals (*Cryptosporidium*), and thus the relative risk of GI illness from each source.

Known source faecal samples and *E. coli* isolates from water samples were used in MST analyses. The analyses combined ERIC-PCR and Riboprinting in which isolates from water samples were compared against the Texas MST Library and a local library to identify sources and enhance accuracy of results.

The three scenarios assessed the effects of changing the contributing percentages of different sources on the overall human health risk (assuming primary contact recreation). The simulated scenarios included:

(i). each faecal source contributes 100% to the FIB concentration;

(ii). each faecal source contributes based upon results: 10% human (combined with unidentified), 25% cattle/domestic animals, and 65% wildlife;

(iii). each faecal source contributes based upon modified MST results, separating cattle and domestic animals: 7% human, 20% cattle, and 73% wildlife/domestic animals/unidentified.

For each scenario, which included a mixture of faecal sources, the median risk for a GI illness was at least 0.31. The difference in total health risk when the human source contributed 10% as opposed to 7% (i.e. scenarios ii and iii) was negligible. High human infectivity of norovirus and the smaller ratio of faecal indicator bacteria to norovirus than for the other pathogens, resulted in the risk being relatively similar across each scenario. These findings indicate that the proportion of cattle/domestic animals and wildlife faecal loading had a much less substantial impact on the overall risk for a GI illness than human faecal sources.

J18: Henry, Schang, et al., (2016) Into the deep: Evaluation of SourceTracker for assessment of faecal contamination of coastal waters. (Qualitative research).

Henry, Schang, et al., (2016) undertook an observational study of sewage genetic marker decay rates in recreational freshwater and marine habitats.

The stated aim of the study was to use recreational water quality site microbial data (16S rRNA amplicon data) in high-throughput sequencing (HTS) and SourceTracker (a statistical tool for assessment of faecal contamination of coastal waters) to identify the main sources of contamination and thus assist water managers with achieving better understanding of the sources of contamination at several recreational beaches in the Port Philip Bay (Victoria) area.

Marine water sample collection in Port Phillip Bay was between December 2014 and March 2015 (42 samples). A further 83 samples of regional riverine, estuarine, greywater, stormwater, sewage and potable source waters, plus and faecal and sand samples (as a source of MST markers) were collected between Dec 2014 and Feb 2015.

The study applied artificial and in-laboratory derived bacterial communities to define the potential and limitations associated with the use of SourceTracker, prior to its application for faecal source tracking at three recreational beaches near Port Phillip Bay (Victoria, Australia).

The results demonstrated that at minimum multiple model runs of the SourceTracker modelling tool (i.e. technical replicates) were required to identify potential false positive predictions. The calculation of relative standard deviations (RSDs) for each attributed source improved overall predictive confidence in the results. In general, default parameter settings provided high sensitivity, specificity, accuracy, and precision. Application of SourceTracker to recreational beach samples identified treated effluent as major source of human derived faecal contamination, present in 69% of samples. Site-specific sources, such as raw sewage, stormwater and bacterial populations associated with the Yarra River estuary were also identified. Rainfall and associated sand resuspension at each location correlated with observed human faecal indicators. The results of the optimised SourceTracker analysis suggests that local sources of contamination have the greatest effect on recreational coastal water quality.

J20: Kelly, Feng, et al., (2018) Effect of beach management policies on recreational water quality. (Qualitative research).

Kelly, Feng, et al., (2018) utilised a large data set of FIB monitoring from Florida, USA beaches (Florida Healthy Beaches Program FHBP) plus a large survey of beach management agency regional offices with the aim of evaluating whether beaches characterized by a set of management policies are associated with lower FIB levels.

The study utilised a dataset of enterococci and faecal coliform data collected through the FHBP from July 31, 2000 to December 31, 2015.

Specific sources are not addressed, but risk factors including beach management practices are identified that are associated with increased FIB concentrations (e.g. animals such as dogs and birds, humans, availability of amenities, beach aspect and type (open ocean vs marshy areas), and beach grooming methods (disturbance of heavy wrack can increase FIB).

Analyses showed that beach geomorphology (beach type) was highly associated with exceedance of regulatory standards. Low enterococci exceedances were associated with open coast beaches (n = 211) that have sparse human densities, no homeless populations, low densities of dogs and birds, bird management policies, low densities of seaweed, beach renourishment, charge access fees, employ lifeguards, without nearby marinas, and those that manage stormwater.

Factor analysis and a linear regression confirmed beach type as the predominant factor with secondary influences from grooming activities (including seaweed densities and beach renourishment) and beach access (including charging fees, employing lifeguards, and without nearby marinas).

The study supported the work of researchers who found that:

- The presence of birds, humans, and dogs, cause an increase in FIB at recreational beaches.
- Beach sand can provide an area in which FIBs can proliferate.
- Grooming in areas with heavy beach wrack that involve disturbance of the wrack may actually increase concentrations.

The study also found that the availability of restrooms and showers; concession stands; solid waste management; and fees to access the beach can have an effect on FIBs.

Given the associations demonstrated between beach management and FIBs, the study results support the concept of sustainable beach management. Such management would streamline or even unify the operations of different agencies that manage beach erosion, wildlife, solid waste, beach patrol and law enforcement, amenities for beach visitors, water quality monitoring, and maintenance.

J23: Lugg, Cook, et al., (2012) Estimating 95th Percentiles from Microbial Sampling: A Novel Approach to Standardising their Application to Recreational Waters. (Qualitative research).

The study seeks to facilitate the application of recreational water guidelines that use the Wyer equation [Water Research 1999(33):715] for the purpose of allocating microbial water assessment categories (MACs). The paper describes development and application of the EnteroTester Excel tool, which facilitates the appropriate computation for 95th percentiles and corresponding classification for beach recreational water quality classifications (MACs) A, B and C, each with a defined range of infection risk.

The performance of the EnteroTester tool was assessed through Monte Carlo simulations and via practical application at c coastal beaches and freshwater sites in WA, NSW and the NT.

Monte Carlo simulation was used to evaluate different methods for 95th percentile calculations on randomly generated datasets from the reference lognormal distribution (95th%ile = 200, log10 SD = 0.81) with simulations run from sample sizes 8 to 100 - all values below 10 were treated as censored.

J30:Robins, Farkas, et al., (2019) Viral dispersal in the coastal zone: A method to quantify water quality risk. (Qualitative research).

The study aim was to investigate the relative importance of some of the key processes influencing viral dispersal through a river-estuary-coast system by applying a hydrodynamic model to simulate fluxes at a national monitoring site (Conwy estuary, North Wales, UK).

The authors developed a river-estuary-coast hydraulic model to simulate virus dispersal, driven by point source discharges and river flows in combination with tidal forcing. Viral inputs were based on measured wastewater adenovirus concentrations and the model was implemented with or without viral die-off.

The model was applied to the Conwy River through the estuary, to the Irish Sea coast where bathing waters and shellfisheries are known to be prone to viral contamination.

Five scenarios are described for modelling. These address factors such as dispersal, dilution, tide and include a worst case scenario. The scenarios were:

Run 1: annual simulation of viral dispersal
Run 2: virus dilution in space
Run 3: influence of hydrology on viral dispersal
Run 4: influence of the tide on viral dispersal
Run 5: worst case scenario

Using the suite of scenarios described above, the authors showed that river flow was the primary control of viral export to the coast.

Since the Conwy catchment is short and steep, and the estuary is small and river-dominated, short-duration high intensity 'flash floods' were shown to transport viruses through the estuary and out to sea, despite dilution or die-off effects.

Duplicating flow events (i.e., storm clustering) did not double the virus export since the virus re-entered the estuary on the flood tide.

The tidal magnitude and timing of high water relative to peak river flow were also important drivers regulating viral dispersal.

A worst case event simulation (i.e., combining high river flows with high viral loading and high spring tide) resulted in increased concentrations of virus at nearby coasts, although the spatial spread was similar to the previous scenarios.

The study applies to small, well-mixed estuaries (reviewer's note: these are common on the NSW coast).

J34: Russo, Eftim, et al., (2020) Evaluating health risks associated with exposure to ambient surface waters during recreational activities: A systematic review and meta-analysis. (Systematic review).

Russo, Eftim, et al., (2020) undertook a systematic review and meta-analysis to evaluate the current scientific evidence for differences in risk of illness between recreational activities typically associated with different levels of contact with water.

Three objectives were listed:

> (1) assess and summarize the scientific literature on the risk of illness associated with different types of recreational activities and different levels of water contact during recreation;
>
> (2) quantitatively estimate the pooled risk of illness associated with different categories of recreational activities and levels of water contact; and
>
> (3) evaluate risk of illness across activity and water contact categories to better understand illness risk associated with different types of recreation in ambient surface waters.

Systematic review of peer-reviewed publications published during or after 1950 as listed in the bibliographic databases PubMed, Web of Science, and TOXLINE located 8,618 potentially relevant studies which were then screened for quantitative measures of risk using inclusion/exclusion criteria established in advance.

The risk of illness associated with different categories of recreational activities and water contact was quantitatively evaluated by combining the results of multiple studies using meta-analysis.

Odds ratios (OR) were considered to be reasonable estimates of relative risk because the probability of infection due to water-based recreational activities is generally low.

Pooled risk estimates indicated:

- significant elevation of gastrointestinal illness with the recreational activity categories of swimming (OR 2.19, 95% CI: 1.82, 2.63) and sports-related contact (OR 2.69, 95% CI: 1.04, 6.92), and

- nonsignificant elevation of gastrointestinal illness with minimal contact (OR 1.27, 95% CI: 0.74, 2.16).
- significant elevation of respiratory illness with swimming (OR 1.78, 95% CI: 1.38, 2.29) and sports-related contact (OR 1.49, 95% CI: 1.00, 2.24), and
- no elevation of respiratory illness with minimal contact (OR 0.90, 95% CI: 0.71, 1.14)

The meta-analysis confirmed a strong association between swimming and both gastrointestinal illness (GI) and respiratory illness (RI). Swimming approximately doubles the risk of GI and increases the risk of RI by approximately 75% compared to no-contact controls.

J35: Schoen, Boehm, et al., (2020) Contamination scenario matters when using viral and bacterial human-associated genetic markers as indicators of health risk in untreated sewage-impacted recreational waters. (Qualitative research).

The aim of the study was to extend the QMRA approach to model the effects of sewage age on GI risk-based thresholds (RBT) using mixtures of sewage at different ages and genetic marker concentrations for human associated crAssphage, *Bacteroides* spp., and polyomavirus. Sewage samples were obtained from 49 wastewater facilities across the contiguous United States.

Reference enteric pathogens were *Salmonella enterica, Campylobacter, E. coli* O157:H7, *Cryptosporidium, Giardia,* norovirus, adenovirus.

Genetic markers were crAssphage (CPQ_056), human-associated *Bacteroides* spp. (HF183/BacR287 and HumM2) and human polyomavirus (HPyV).

QMRA was used to estimate the respective genetic marker concentration in untreated sewage-impacted recreational water that corresponds to the US EPA benchmark of ~32 illnesses per 1000 swimmers, referred to as an RBT in this study.

Risk-based threshold (RBT) estimates varied across different mixture and sewage age scenarios. Fresh sewage RBT estimates were not always protective when aged sewage was present, and aged sewage RBT estimates often fell below the marker lower limit of quantification. Conservative RBT estimates of 9.3×10^2 and 9.1×10^3 (copies/100 mL) for HF183/BacR287 and CPQ_056, respectively, were predicted when fresh sewage was greater (by volume) than aged at the time of measurement.

The human-associated genetic markers considered in the study varied in abundance in raw sewage and exhibit a range of reported decay rate constants. CrAssphage, a putative *Bacteroides* spp. bacteriophage, showed potential as an effective indicator since it had the lowest reported decay rate constant and was one of the most abundant human-associated genetic markers considered in the study.

Systematic QMRA analyses demonstrated the important relationship between these factors for selecting a robust human-associated genetic marker indicator for surface waters. As untreated sewage ages, differences in genetic marker abundance and decay rate constants become more pronounced, ultimately reducing indicator concentrations below typical analytical lower limit of quantification values, thus rendering an indicator ineffective.

J36: Shrestha and Dorevitch, (2019) Evaluation of rapid qPCR method for quantification of E. coli at non-point source impacted Lake Michigan beaches. (Qualitative research).

Shrestha and Dorevitch, (2019) evaluated a draft US EPA *E. coli* qPCR method, compared *E. coli* qPCR measurements with two established FIB (*E. coli* culture and enterococci qPCR) results, and explored potential strategies to establish *E. coli* qPCR Beach Action Value (BAV) criteria in the absence of an epidemiological study.

Conventional culture-based *E. coli* methods for monitoring FIB are used at Great Lakes recreational beaches. Cultivation methods require 18 or more hours to generate results. As a consequence, public notifications about beach action value (BAV) exceedance are based on prior-day water quality. Rapid qPCR monitoring of bacteria in beach water solves the 24-h delay problem, though the US EPA-approved qPCR method targets enterococci bacteria, while Great Lakes communities are familiar with *E. coli* monitoring. For an *E. coli* qPCR method to be useful for water quality management, it was important to systematically characterize method performance, and establish BAVs for public notification purposes.

Consequently, the authors evaluated rapid qPCR monitoring for *E. coli* in freshwater beach water samples to assess if it could solve the 24-h delay problem compared to culture techniques.

Based on analyses of 288 water samples collected from eight of Chicago's Lake Michigan beaches, the *E. coli* qPCR method demonstrated acceptable performance characteristics. The method is prone to low level DNA contamination, possibly originating from assay reagents derived from *E. coli* bacteria. Both *E. coli* and enterococci BAVs were exceeded in approximately 18% of the samples. *E. coli* qPCR values were correlated with both *E. coli* culture ($r = 0.83$; $p < 0.0001$) and enterococci qPCR ($r = 0.67$; $p < 0.0001$) values. These statistical results are for same day culture results. Correlations for 1 day lag between culture and qPCR results were not significant - indicating that *E. coli* culture results available to a beach manager on a given day (from samples cultured the prior day) was not predictive of current water quality.

The authors concluded that the finding that each method and reference FIB measurement yielded different *E. coli* qPCR BAV highlighted the need for further US EPA guidance for deriving new types of BAVs such as ones based on *E. coli* qPCR.

Table 6-5. Results summary for Primary research question, Outcome 1.

Study, Design, Quality	Population	Exposures	Location type	Outcome	Analysis	Results	Effect estimate	Significance
Research question: *How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?*								
Outcome 1. Study describes methods for assessing and predicting microbial risks in recreational waters from diffuse and point sources.								
J2: Ahmed, Hamilton, et al., (2018) Quantitative microbial risk assessment of microbial source tracking markers in recreational water contaminated with fresh untreated and secondary treated sewage. (Qualitative research)	Adults exposed to water during recreation.	Ingestion while swimming	Marine, beaches	Fresh untreated and secondary treated sewage dosed into filtered beach water samples. NoV and HAdV 40/41 were selected as reference pathogens as these viruses are known to cause swimming-associated illnesses in recreational waters. Genomic copies (GC) reported from qPCR - measured in triplicate and reported with standard deviations. Probability of GI illness compared to US EPA 2012 guideline risk of 36 GI Illnesses/1000 exposures or 0.036. Process limit of quantification (PLOQ) = smallest volume of sewage in which target pathogens could still be reliably quantified in 2/3 qPCR Reactions.	QMRA dose-response models based on log-normal probability distribution functions (pdfs) for concentrations of NoV and HAdV 40/41 in untreated sewage and secondary treated sewage. Genomic copies reported from qPCR - measured in triplicate and reported with standard deviations. Serial ten-fold dilution series of beach water samples dosed with either fresh untreated sewage or fresh secondary treated sewage were tested to determine the PLOQ of the 5 sewage biomarkers. The corresponding probability of GI illness from NoV or HAdV 40/41 for each biomarker PLOQ was also compared with the US EPA tolerable benchmark for GI illness for recreational water (0.036).	Among all the markers tested, HF183 concentration needed to be greater in the water sample than other markers to correspond to an exceedance of the illness benchmark (Table 2 in paper). When NoV and HAdV 40/41 were used as reference pathogens, a median concentration of 3.22×10^{3} GC of the HF183 in 100 mL of water sample represented a risk above the GI illness benchmark value when beach water was contaminated with fresh untreated sewage. Similarly, 3.66×10^{3} GC of HF183 in 100 mL of water sample represented a risk above the benchmark for both NoV and HAdV 40/41, when beach water was contaminated with secondary treated sewage. Table 2 in the paper lists values for the other biomarkers tested.	N/A as no statistical measure of effect undertaken. Concentrations (GC) of sewage-associated markers in 100 mL of beach water sample contaminated with either fresh untreated or secondary treated sewage that exceeded the median illness rate of 36/1,000 people at a single event for reference pathogens NoV and HAdV 40/41 are given in Table 2 of the paper.	N/A.

Study, Design, Quality	Population	Exposures	Location type	Outcome	Analysis	Results	Effect estimate	Significance
Research question: *How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?*								
Outcome 1. Study describes methods for assessing and predicting microbial risks in recreational waters from diffuse and point sources.								
J3: Ahmed, Payyappat, et al., (2019) Enhanced insights from human and animal host-associated molecular marker genes in a freshwater lake receiving wet weather overflows. (Qualitative research).	Adults exposed to water during recreation.	Ingestion while swimming	Freshwater lake (Lake Parramatta)	FIB (*E. coli*, enterococci) and MST biomarkers used to assess magnitude of sewage and animal faecal contamination in Lake Parramatta water samples collected during a dry weather period and from two storm events coinciding with wet weather overflows of sewage to the lake. MST marker genes tested were: • Sewage: Bacteroides HF183, crAssphage CPQ_056 and pepper mild mottle virus (PMMoV), • Animal faeces:- Bacteroides BacCan-UCD, cowM2 and Helicobacter spp. associated GFD.	Box plots of FIB and MST marker genes concentrations in lake water samples categorised by sampling event and labelled for weather (wet vs dry). Pearson's correlation matrix among faecal indicator bacteria (FIB) and MST marker genes in pooled (n = 30) water samples The Pearson's product moment correlation with a two-tailed p value was used to establish the relationship between FIB and MST marker genes in lake water samples. Student's t-test used to identify significant relationships of the concentrations of FIB and MST marker genes between dry and wet weather events and between sampling depths (Reviewers note: 2-factor ANOVA would have been more appropriate here).	• Magnitude of general and source-specific faecal pollution was low in water samples collected during dry weather compared to storm events. • The concentrations of HF183 and PMMoV in most storm water samples exceeded the risk benchmark threshold values established in the literature for primary contact recreators (see Table 3 in the paper). • No samples positive for cowM2 (cow) marker gene • BacCan-UCD (dog) and GFD (avian) animal-associated markers sporadically detected in water samples collected from both dry weather and storm events.	Moderate to strong positive correlations observed among sewage-associated marker genes. Levels of HF183, crAssphage and PMMoV in water samples collected during storm events were as high as 6.39, 6.33 and 5.27 log10 GC/L of water, respectively.	P-values (significance) of correlations not given. P-values for multiple t-tests on HF183 concentrations given as follows: Storm event 1 v 2, $p > 0.05$, Depth (0.5 m vs 1 m above lakebed), $p > 0.05$ Storm event 1 v 2 at 0.5 m, $p < 0.05$ Storm event 1 v 2 at 1 m, $p > 0.05$

Study, Design, Quality	Population	Exposures	Location type	Outcome	Analysis	Results	Effect estimate	Significance
Research question: *How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?*								
Outcome 1. Study describes methods for assessing and predicting microbial risks in recreational waters from diffuse and point sources.								
J4: Ahmed, Payyappat, et al., (2020) Sewage-associated marker genes illustrate the impact of wet weather overflows and dry weather leakage in urban estuarine waters of Sydney, Australia. (Qualitative research).	Adults exposed to water during recreation.	Ingestion while swimming	Three urban estuarine recreational water sites in Sydney	Concentrations of culturable faecal indicator bacteria (FIB) and MST biomarker genes measured in dry weather samples and samples following storm events from 6 sampling stations at each of the three estuaries. At each station samples collected at (i) 0.5 m below the water surface, and (ii) 1 m above the bottom surface to investigate whether MST marker genes declined with depth.	MST biomarker genes used were Bacteroides HF183, pepper mild mottle virus (PMMoV), crAssphage CPQ_056, Lachnospiraceae (Lachno3), whilst FIB used were *E. coli* and enterococci. Water samples also analysed for animal faeces-associated biomarker genes targeting avian (GFD), dog (BacCan-UCD), cow (cowM2) and horse (HoF597) species to determine the extent of animal faecal contamination.	Analysis of sewage associated marker genes showed greater (i.e., 3–5 orders of magnitude) concentrations in water samples collected during the storm events compared to dry weather event; attributed to untreated sewage from wet weather overflows (WWOs). Animal faeces-associated marker genes, cowM2 (cow), HoF597 (horse) not detected, GFD (avian) consistently present, and BacCan-UCD (dog) occasionally detected. Depth profile analysis of FIB indicated overall greater concentrations at the surface compared to the bottom of the water bodies.	Student's t test performed to determine statistical significance of the concentrations of marker genes between dry and storm events as well as between two sampling depths. P values of < 0.05 were considered significant. Too many results to display here. See paper for details	Effects varied with FIB and MST marker gene but generally showed increased concentrations of both categories during storm events compared to dry weather.

Study, Design, Quality	Population	Exposures	Location type	Outcome	Analysis	Results	Effect estimate	Significance
Research question: *How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?*								
Outcome 1. Study describes methods for assessing and predicting microbial risks in recreational waters from diffuse and point sources.								
J5: Ahmed, Zhang, et al., (2019) Comparative decay of sewage-associated marker genes in beach water and sediment in a subtropical region. (Qualitative research).	N/A. No health assessment undertaken.	N/A. Study focuses on decay rates of FIB and bacterial and viral biomarkers	Outdoor mesocosms (8L plastic containers with 4-5 cm sediment). Water samples collected from 3 sites, (2 marine, 1 freshwater lake). 3 replicates for source; 9 mesocosms total.	Study investigated decay of 4 sewage-associated bacterial and viral markers (HF183, HAdV, HPyV, and crAssphage), in relation to each other and qPCR FIB [*E. coli* (EC), and *Enterococcus* spp. (ENT) 23S rRNA genes], in sewage contaminated fresh and marine waters and accompanying sandy sediments. Outdoor mesocosms containing water and sediment were inoculated with untreated sewage, and qPCR assays used to quantify each target over 40 days to determine decay rates.	1 water sample (100 mL) collected from each mesocosm on each of days 0, 1, 4, 8, 14, 24 and 40. 1 sediment sample (approximately 1 g) collected from each mesocosm on days 0, 1, 4, 8, 14, 24 and 40. Water samples were collected prior to sediment samples to avoid sediment disturbance.	In general, decay rates of bacterial targets were similar in the water columns of the studied mesocosms. Similarly, decay rates of viral targets were also alike in mesocosm water columns in relation to each other. The decay rates of FIB and sewage-associated markers were significantly faster in water samples compared to sediments in all three mesocosms. Decay rates of bacterial targets (two FIB and HF183) were fast and similar in water samples across three mesocosms. Similarly, decay rates of viral targets (HAdV, HPyV and crAssphage) were similar in water samples, however, viral targets persisted significantly longer ($p < 0.05$) than did bacterial targets.	Too many results to display here. See paper for details (Figure 1, Table 2).	Decay rates of EC 23S rRNA, ENT 23S rRNA, and HF183 16S rRNA were significantly ($p < 0.05$) faster than the HAdV, HPyV and crAssphage markers in water samples from all mesocosms. Decay rates of FIB and sewage-associated markers were significantly faster in water samples compared to sediment in all mesocosms.

Study, Design, Quality	Population	Exposures	Location type	Outcome	Analysis	Results	Effect estimate	Significance
Research question: *How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?*								
Outcome 1. Study describes methods for assessing and predicting microbial risks in recreational waters from diffuse and point sources.								
J6: Arnold, Schiff, et al., (2017) Acute Illness Among Surfers After Exposure to Seawater in Dry- and Wet-Weather Conditions. (Cohort study).	Adult surfers (>=18 yrs) (at San Diego, CA, USA).	Ingestion, inhalation, dermal exposure whilst surfing	Marine beaches, warm temperate, with adjacent urban stormwater sources	Cohort study undertaken to determine if exposure to seawater increased rates of incident illness among a longitudinal cohort of surfers in San Diego, California. Illness rates after surf periods were compared with periods after no surfing in order to determine whether exposure during or immediately after rainstorms increased rates more than did exposure during dry weather. Study was undertaken over two winter periods presumably to capture sufficient rainfall events. A total of 654 individuals were enrolled who contributed on average 51 days of follow-up	To examine illness rates separately for dry- and wet-weather exposures, the authors created a 3-level categorical exposure that classified each participant's follow-up time into unexposed, dry-weather exposure, and wet-weather exposure periods. Wet-weather exposure was defined as exposure to seawater within 3 days of 0.25 cm or more of rainfall in a 24-hour period and all other seawater exposure as dry-weather exposure. The wet weather criterion is the same rainfall criterion used by the local county (San Diego) for posting wet-weather beach advisories.	Surfing was associated with increased incidence of several categories of symptoms, and associations were stronger if surfing took place shortly after rainstorms. Higher levels of FIB were strongly associated with fever, sinus pain/infection, wound infection, and gastrointestinal symptoms within 3 days of rainstorms. FIB (including *Enterococcus*, total coliform, and faecal coliforms) levels were positively associated with increased incidence of almost all negative health outcomes during the study.	Compared with unexposed periods, wet-weather exposure led to the largest relative increase in: - earaches/ infections (adjusted incidence rate ratio (IRR) = 3.28, 95% confidence interval (CI): 1.95, 5.51); and - infection of open wounds (adjusted IRR: 4.96, 95% CI: 2.18, 11.29). *Enterococcus* strongly associated with illness after wet-weather exposure (e.g., for each log10 increase, gastrointestinal illness IRR = 2.17, 95% CI: 1.16, 4.03).	Detailed results tabulated in paper (Tables 3, 4). Tests of trend in the IRR between exposure categories were considered significant ($P < 0.05$) if the confidence interval for wet-weather exposure excluded 1.0

Study, Design, Quality	Population	Exposures	Location type	Outcome	Analysis	Results	Effect estimate	Significance
Research question: *How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?*								
Outcome 1. Study describes methods for assessing and predicting microbial risks in recreational waters from diffuse and point sources.								
J15: Gitter, Mena, et al., (2020) Human health risks associated with recreational waters: Preliminary approach of integrating quantitative microbial risk assessment with microbial source tracking. (Qualitative research).	Adults exposed to water during recreation.	Ingestion of water during primary contact recreation	Riverine freshwater, agricultural catchment	Objective was to conduct a QMRA estimating risk of GI illness using MST data describing the primary sources contributing to a bacteria impairment in particular waterbody; using Walnut Creek, Brazos River Basin, Texas as an example. *E. coli* concentrations were used to predict reference pathogen concentrations from cattle (*Campylobacter*), human (Norovirus), and wildlife/domestic animals (*Cryptosporidium*), and thus the relative risk of GI illness from each source.	MST analyses combined ERIC-PCR and Riboprinting in which isolates from water samples were compared against the Texas MST Library and a local library to identify sources and enhance accuracy of results.	Three scenarios assessed the effects of changing the contributing percentages of different sources on the overall human health risk (assuming primary contact recreation). The findings indicated that the proportion of cattle/domestic animals and wildlife faecal loading had a much less substantial impact on the overall risk for a GI illness than human faecal sources	For each scenario, which included a mixture of faecal sources, the median risk for a GI illness was at least 0.31. The difference in total health risk when the human source contributed 10% as opposed to 7% (i.e. scenarios ii and iii) was negligible.	High human infectivity of norovirus and the smaller ratio of faecal indicator bacteria to norovirus than for the other pathogens, resulted in the risk being relatively similar across each scenario.
J18: Henry, Schang, et al., (2016) Into the deep: Evaluation of SourceTracker for assessment of faecal contamination of coastal waters. (Qualitative research).	N/A. No health assessment undertaken.	N/A. The study is an observational study of sewage genetic marker decay rates in recreational freshwater and marine habitats	Locations sampled in Port Phillip Bay (marine) and regional riverine, estuarine, greywater, stormwater, sewage and potable source waters	Observational study of sewage genetic marker decay rates in recreational freshwater and marine habitats. Study aim was to use recreational water quality site microbial data (16S rRNA amplicon data) in high-throughput sequencing (HTS) and SourceTracker (a statistical tool for assessment of faecal contamination of coastal waters) to identify the main sources of contamination to assist in management of risks to recreational waters.	The study applied artificial and in-laboratory derived bacterial communities to define the potential and limitations associated with the use of SourceTracker, prior to its application for faecal source tracking at three recreational beaches near Port Phillip Bay (Victoria, Australia).	Application of SourceTracker to recreational beach samples identified treated effluent as major source of human derived faecal contamination, present in 69% of samples. Site-specific sources, such as raw sewage, stormwater and bacterial populations associated with the Yarra River estuary were also identified. Rainfall and associated sand resuspension at each location correlated with observed human faecal indicators.	The results of the optimised SourceTracker analysis suggests that local sources of contamination have the greatest effect on recreational coastal water quality.	N/A. Statistical significance testing not undertaken.

Study, Design, Quality	Population	Exposures	Location type	Outcome	Analysis	Results	Effect estimate	Significance
Research question: *How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?*								
Outcome 1. Study describes methods for assessing and predicting microbial risks in recreational waters from diffuse and point sources.								
J20: Kelly, Feng, et al., (2018) Effect of beach management policies on recreational water quality. (Qualitative research).	N/A. No health assessment undertaken.	N/A. Study focus is on beach management practices.	Marine and estuarine beaches of Florida state, USA.	The primary objective of the study was to evaluate whether beaches characterized by a set of management policies are associated with lower FIB levels. The study utilised a large data set of FIB monitoring from Florida, USA beaches (Florida Healthy Beaches Program FHBP) plus a large survey of beach management agency regional offices. FIB data considered were enterococci and faecal coliforms.	Application of inclusion criteria (minimum of 120 samples during the 15-year period of record; 2000-2015) resulted in 316 beaches for evaluation. Beach types included open coast, bay, inlet-channel situated, man-made structure-protected, marsh surrounded, and back-reef beaches. The FIB data for each beach were converted to a percent exceedance value to track the fraction of times that the beaches exceeded regulatory guidelines. A beach management survey was developed to collect data on management policies. The results were then compared to FIB data to determine which management condition corresponded to lower bacteria levels. The observed influence of beach morphology determined how each of the responses in the beach management survey were analysed.	Analyses showed that beach geomorphology (beach type) was highly associated with exceedance of regulatory standards. Low enterococci exceedances were associated with open coast beaches (n = 211) that have sparse human densities, no homeless populations, low densities of dogs and birds, bird management policies, low densities of seaweed, beach renourishment, charge access fees, employ lifeguards, without nearby marinas, and those that manage stormwater.	Factor analysis and a linear regression confirmed beach type as the predominant factor with secondary influences from grooming activities (including seaweed densities and beach renourishment) and beach access (including charging fees, employing lifeguards, and without nearby marinas).	P-values for significance tests are given in Tables 1 to 6 in the paper. There are too many to list here.

Study, Design, Quality	Population	Exposures	Location type	Outcome	Analysis	Results	Effect estimate	Significance
Research question: *How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?*								
Outcome 1. Study describes methods for assessing and predicting microbial risks in recreational waters from diffuse and point sources.								
J23: Lugg, Cook, et al., (2012) Estimating 95th Percentiles from Microbial Sampling: A Novel Approach to Standardising their Application to Recreational Waters. (Qualitative research).	N/A. No health assessment undertaken.	N/A. Study focus is on statistical methods.	Desktop study. Assumes marine, estuarine, freshwater environments.	The paper describes development and application of the EnteroTester Excel tool, which facilitates the appropriate computation for 95t^{h} percentiles and corresponding classifications for beach recreational water quality microbial assessment categories (MACs).	The authors describe the statistical methodology and results of trialling the EnteroTester Excel Tool on coastal beaches and freshwater sites in WA, NSW and the NT. Tool performance assessed via Monte Carlo Simulations (MCS) and practical application at coastal beaches and freshwater sites at several Australian jurisdictions.	MCS used to evaluate different methods for 95th percentile calculations on randomly generated datasets from the reference lognormal distribution with simulations run from sample sizes 8 to 100. The EnteroTester template proved rapid, convenient, reliable, efficient in terms of data requirements, and more accurate in estimating infection risk and in placing recreational waters in their correct MACs than other methods of calculating 95th percentiles.	N/A as no statistical measure of effect undertaken.	N/A.

Study, Design, Quality	Population	Exposures	Location type	Outcome	Analysis	Results	Effect estimate	Significance
Research question: *How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?*								
Outcome 1. Study describes methods for assessing and predicting microbial risks in recreational waters from diffuse and point sources.								
J30:Robins, Farkas, et al., (2019) Viral dispersal in the coastal zone: A method to quantify water quality risk. (Qualitative research).	N/A. No health assessment undertaken.	N/A. Study focus is on hydraulic modelling of virus dispersal in an estuary.	Estuary (Conwy estuary, North Wales, UK)	Study aimed to investigate key processes influencing viral dispersal at bathing and shellfish harvesting sites along a river-estuary-coast system via hydraulic modelling. The model simulated virus dispersal, driven by point source discharges and river flows in combination with tidal forcing. Viral inputs were based on measured wastewater adenovirus concentrations. Model implemented with or without viral die-off.	The hydraulic model was a vertically averaged hydrostatic ocean model. For all simulations, key parameters (depth, velocity, salinity, virus concentration) were output every 15 min [creating time series of flows, depths and predicted Adenovirus concentrations in within the estuary, for each scenario]. Five scenarios addressing dispersal, dilution, tide, including a worst-case scenario.	River flow was the primary control of viral export to the coast. Since the Conwy catchment is short and steep, and the estuary is small and river-dominated [Reviewers note: common attributes of NSW estuaries], short-duration high intensity 'flash floods' were shown to transport viruses through the estuary and out to sea, despite dilution or die-off effects. Duplicating flow events (i.e., storm clustering) did not double the virus export since the virus re-entered the estuary on the flood tide. The tidal magnitude and timing of high-water relative to peak river flow were also important drivers regulating viral dispersal.	N/A as no statistical measure of effect undertaken.	N/A.

Study, Design, Quality	Population	Exposures	Location type	Outcome	Analysis	Results	Effect estimate	Significance
Research question: *How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?*								
Outcome 1. Study describes methods for assessing and predicting microbial risks in recreational waters from diffuse and point sources.								
J34: Russo, Eftim, et al., (2020) Evaluating health risks associated with exposure to ambient surface waters during recreational activities: A systematic review and meta-analysis. (Systematic review).	Adults and children exposed to water during recreation.	Ingestion of water during primary contact recreation	Marine, estuarine, freshwater recreational water sites	The study consisted of a systematic review and meta-analysis to evaluate the current scientific evidence for differences in risk of illness between recreational activities typically associated with different levels of contact with water.	The risk of illness associated with different categories of recreational activities and water contact was quantitatively evaluated by combining the results of multiple studies using meta-analysis. Odds ratios (OR) were considered to be reasonable estimates of relative risk because the probability of infection due to water-based recreational activities is generally low.	Too many OR results are present in the paper to discuss here. Key findings however were the pooled risk estimates showing: • significant elevation of gastrointestinal illness with the recreational activity categories of swimming (OR 2.19, 95% CI: 1.82, 2.63) and sports-related contact (OR 2.69, 95% CI: 1.04, 6.92), and • nonsignificant elevation of gastrointestinal illness with minimal contact (OR 1.27, 95% CI: 0.74, 2.16). • significant elevation of respiratory illness with swimming (OR 1.78, 95% CI: 1.38, 2.29) and sports-related contact (OR 1.49, 95% CI: 1.00, 2.24), and • no elevation of respiratory illness with minimal contact (OR 0.90, 95% CI: 0.71, 1.14) Also note were the pooled risk estimates for GI for adults and children were significantly greater than 1, whilst only estimates for adults were greater than 1 for RI. Both GI and RI risk estimates were not significantly different from each other.	Formal significance tests were limited to Egger's test for publication bias coefficient (too many to show here, see Figs 5 & 7 in paper). The effect of swimming was summarised by the meta-analysis which confirmed a strong association between swimming and both gastrointestinal illness (GI) and respiratory illness (RI). *Swimming approximately doubles the risk of GI and increases the risk of RI by approximately 75% compared to no-contact controls.*	Significance tests (bias coefficient in Egger's test) applied for publication bias (Funnel Plots) used $p < 0.10$ as the threshold for rejecting the null hypothesis. Any meta-analysis result derived from fewer than three studies was regarded as inconclusive.

Study, Design, Quality	Population	Exposures	Location type	Outcome	Analysis	Results	Effect estimate	Significance
Research question: *How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?*								
Outcome 1. Study describes methods for assessing and predicting microbial risks in recreational waters from diffuse and point sources.								
J35: Schoen, Boehm, et al., (2020) Contamination scenario matters when using viral and bacterial human-associated genetic markers as indicators of health risk in untreated sewage-impacted recreational waters. (Qualitative research)	Adults exposed to water during recreation.	Ingestion of water during primary contact recreation	Marine, estuarine, and freshwater.	The aim of the study was to extend the QMRA approach to model the effects of sewage age on GI risk-based thresholds (RBT) using mixtures of sewage at different ages and genetic marker concentrations for human associated crAssphage, *Bacteroides* spp., and polyomavirus. Sewage samples were obtained from 49 wastewater facilities across the contiguous United States.	Reference enteric pathogens were *Salmonella enterica, Campylobacter, E. coli* O157:H7, *Cryptosporidium, Giardia,* norovirus, adenovirus. Genetic markers were crAssphage (CPQ_056), human-associated *Bacteroides* spp. (HF183/BacR287 and HumM2) and human polyomavirus (HPyV). QMRA was used to estimate the respective genetic marker concentration in untreated sewage-impacted recreational water that corresponds to the United States Environmental Protection Agency (US EPA) benchmark of ~32 illnesses per 1000 swimmers, referred to as an RBT in this study.	Risk-based threshold (RBT) estimates varied across different mixture and sewage age scenarios. Fresh sewage RBT estimates were not always protective when aged sewage was present, and aged sewage RBT estimates often fell below the marker lower limit of quantification (LLOQ). Conservative RBT estimates of 9.3×10^2 and 9.1×10^3 (copies/100 mL) for HF183/BacR287 and CPQ_056, respectively, were predicted when fresh sewage was greater (by volume) than aged at the time of measurement. The human-associated genetic markers considered in the study varied in abundance in raw sewage and exhibit a range of reported decay rate constants. CrAssphage, a putative *Bacteroides* spp. bacteriophage, showed potential as an effective indicator since it had the lowest reported decay rate constant and was one of the most abundant human-associated genetic markers considered in the study.	N/A as no statistical measure of effect undertaken. Systematic QMRA analyses demonstrated the important relationship between these factors for selecting a robust human-associated genetic marker indicator for surface waters. As untreated sewage ages, differences in genetic marker abundance and decay rate constants become more pronounced, ultimately reducing indicator concentrations below typical analytical LLOQ values, thus rendering an indicator ineffective.	N/A.

Study, Design, Quality	Population	Exposures	Location type	Outcome	Analysis	Results	Effect estimate	Significance
Research question: *How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?*								
Outcome 1. Study describes methods for assessing and predicting microbial risks in recreational waters from diffuse and point sources.								
J36: Shrestha and Dorevitch, (2019) Evaluation of rapid qPCR method for quantification of *E. coli* at non-point source impacted Lake Michigan beaches. (Qualitative research).	N/A. No health assessment undertaken.	N/A. Study focus is on effectiveness of *E. coli* qPCR for determining Beach Action Values (BAVs).	Very large freshwater lake (Lake Michigan)	The study evaluates a draft US EPA *E. coli* qPCR method, compared *E. coli* qPCR measurements with two established FIB (*E. coli* culture and enterococci qPCR) results, and explored potential strategies to establish *E. coli* qPCR Beach Action Value (BAV) criteria in the absence of an epidemiological study. Rapid qPCR monitoring for *E. coli* freshwaters was assessed as a potential solution to the 24-h delay problem compared to culture techniques.	Based on analyses of 288 water samples collected from eight of Chicago's Lake Michigan beaches, the *E. coli* qPCR method demonstrated acceptable performance characteristics. The method is prone to low level DNA contamination, possibly originating from assay reagents derived from *E. coli* bacteria.	Both *E. coli* and enterococci BAVs were exceeded in approximately 18% of the samples. *E. coli* qPCR values were correlated with both *E. coli* culture ($r = 0.83$; $p < 0.0001$) and enterococci qPCR ($r = 0.67$; $p < 0.0001$) values. These statistical results are for same day culture results. Correlations for 1 day lag between culture and qPCR results were not significant - indicating that *E. coli* culture results available to a beach manager on a given day (from samples cultured the prior day) was not predictive of current water quality.	Given the availability of a qPCR method (enterococci) developed by US EPA, along with criteria values and BAVs calibrated directly to observed health risk in epidemiological studies, the authors stated that there was little reason to attempt developing BAV criteria for other testing methods like *E. coli* qPCR in the absence of guidance from the US EPA about how results of such water testing methods predict health risk.	Significance tests used $p < 0.05$ as the threshold for rejecting the null hypothesis.

6.3. Assessment of the certainty in the body of evidence

6.3.1. Grading the certainty of evidence of primary studies

As described in the research protocol (O'Connor, 2020), a process based on the OHAT approach to using the GRADE framework was used to assess the certainty of the body of evidence for the 13 primary studies used to answer the primary research question (OHAT, 2019).

As noted in Section 2.3.2, at the direction of the Committee, secondary research questions were addressed through the review of existing guidelines and reviews only, rather than through review of the primary studies and thus were not included in the GRADE assessment.

Initial confidence ratings

Each evidence stream was assigned an initial certainty rating similar to that described in the OHAT Handbook (OHAT, 2019). The systematic review did not include a GRADE assessment but was given an initial rating of "moderate certainty" based on limitations of the meta-analysis noted by the review authors. Cohort studies are categorised in the OHAT Handbook as "low to moderate certainty"; however, based on the types of comparison groups and efforts taken to determine pre-exposure for the included cohort study, this was assigned an initial certainty of "moderate". Qualitative studies were given the same initial rating as observational studies ("low certainty").

Risk of bias

While none of the studies reviewed were designed as randomised control trials or similar clinical trials, there was a general low risk of bias across the included studies.

Unexplained inconsistency

A large amount of heterogeneity was observed across the body of evidence; however, this can be explained by the inconsistent nature of the exposure scenarios for recreational water exposure (different recreational water exposures, durations, locations and types) and study designs. This resulted in a rating of 'not serious' across all study types and outcomes.

Indirectness

Most of the included studies were relevant to the primary research question and the populations and recreational exposure types could be assessed for Australian settings. This resulted in a rating of 'not serious' across all study types.

Imprecision

Reasonable efforts were made to assess the statistical significance of the findings across the body of evidence. This resulted in a rating of 'not serious' across all study types.

Publication bias

Publication bias was not detected except in the systematic review. However, this did not appear to be a concern to the review authors and was therefore not used as a reason to downgrade the certainty in the evidence.

Reasons for upgrading

There was insufficient information to determine if there were any further reasons to upgrade the certainty of the overall body of evidence using the GRADE system.

Overall certainty rating

The GRADE assessment of the overall quality of the primary studies body of evidence was undertaken for the outcome identified for the primary research question which was "*How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?".*

The overall certainty rating was "moderate" for the systematic review and cohort study, leading to a final certainty rating of "*moderately confident in the reported associations*". The overall certainty rating was "low" for the 11 qualitative studies (Table 6-6). This led to a final certainty rating of "*limited confidence in the reported associations*". This result stems from the high degree of heterogeneity in study focus of the 13 studies that made it through the screening and quality assessment stages of the literature review. The broad nature of the primary research question resulted in a wide range of study types being eligible for inclusion and consequently it was not possible to apply gradings for some the categories in the GRADE assessment (e.g. magnitude of effect). None of the factors that could influence a change in the grading of certainty of the body of evidence (Figure 2-1, Table 2-8) were identified (Table 6-6).

It is worth noting that methods and approaches for systematic reviews of environmental health evidence is still an area of research and development, and further modification of the available frameworks and tools is beyond the scope of services required for this review. Further analysis and evaluation of the primary studies by the Committee can be undertaken if required.

Table 6-6. GRADE report for presence of significant human health risks due to microbial risks in recreational water

Body of evidence *Evidence stream or study type (# studies)* *Initial certainty rating (OHAT,2019)*	**Risk of bias** *Serious, not serious, unknown* Describe trends, key questions, issues	**Unexplained inconsistency** *Serious, not serious, not applicable (NA)* Describe results in terms of consistency, explain apparent inconsistency	**Indirectness** *Serious or not serious, NA* Discuss use of upstream indicators or populations with less relevance, any time-related exposure considerations	**Imprecision** *Serious, not serious, unknown, NA* Discuss ability to distinguish treatment from control, describe confidence intervals (if available)	**Publication bias** *Detected, undetected* Discuss factors that might indicate publication bias (e.g., funding, lag)	**Magnitude of effect** *Large, not large, unknown, NA* Describe magnitude of response or strength of association	**Dose Response** *Yes, no, unknown* Outline evidence for or against dose response	**Residual confounding** *Yes, no, unknown* Address whether there is evidence that confounding would bias toward null	**Consistency across species/model** *Yes, no, NA* Describe cross-species, model, or population consistency	**Other reason to increase confidence?** *Yes or no* Describe any other factors that increase confidence in the results	**Final certainty rating** *High, moderate, low or very low* List reasons for down-grading or upgrading
Research question: *How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?*											
Outcome 1. Study describes methods for assessing and predicting microbial risks in recreational waters from diffuse and point sources.											
Systematic review (1) (Assesses 92 primary studies of mixed study type) Moderate certainty	Not serious. Overall risk of bias is probably low (85/92 studies low risk of bias, 7/92 high risk of bias)	Not serious	Not serious[2].	Not serious	Detected but not downgraded	N/A[3]	N/A[4]	No[5]	N/A[6]	No	⊕⊕⊕O **Moderate certainty[7]**
Cohort study (1) Moderate certainty	Not serious. Risk of bias is probably low	N/A	Not serious[2].	Not serious	Undetected	N/A[3]	N/A[4]	No[5]	N/A[6]	No	⊕⊕⊕O **Moderate certainty**
Qualitative studies (11) Low certainty	Not serious Overall risk of bias is probably low	Not serious[1].	Not serious[2].	Not serious	Undetected	N/A[3]	N/A[4]	No[5]	N/A[6]	No	⊕⊕OO **Low certainty**

[1].Substantial (high) heterogeneity present. However, this is due to the included studies (including those in the systematic review) each focussing on different aspects of microbial risks to recreational water users.
[2].Indirectness of evidence is not serious. No changes to the nature of the proposed risks, or their settings would be expected to have occurred since the publication of the studies.

[3]. Due to heterogeneity of studies and, in many cases, the multitude of effects tested, no single class of effect could be isolated with respect to the primary research question.
[4]. With respect to microbial risks, specific pathogen dose response relationships were not reported in the selected literature therefore no grading against this category can be made.
[5]. No evidence of confounding of evidence was identified in any of the studies.
[6]. In most cases the studies addressed human health effects via reference to guideline values, whilst the remainder did not specifically address health impacts, thus no grading against this category can be made.
[7]. GRADE assessment was not reported by the authors of the systematic review; however, this certainty rating attempts to capture the findings and limitations of the reported meta-analyses.

Key to GRADE quality of evidence:
⊕⊕⊕⊕ = High; We are very confident in the reported associations;
⊕⊕⊕◯ = Moderate; We are moderately confident in the reported associations;
⊕⊕◯◯ = Low; Our confidence in the reported associations is limited;
⊕◯◯◯ = Very Low; We are not confident about the reported associations.

7 Discussion

7.1. Primary research question

How can we monitor, assess, and predict risks from diffuse and point source microbial contamination in recreational waters?

In response to the primary research question evidence was sought from quality grey literature and primary studies that addressed monitoring, assessment, and prediction of risks from microbial diffuse and point sources in recreational waters. Given the broad nature of the research question, there was substantial heterogeneity in the literature which made it challenging to distil the review findings. Similarly, there was some overlap with the secondary research questions, particularly with respect to the use of indicators and/or surrogates of microbial risk. Some broad themes, consistent with the primary research question, were present in the selected literature. These themes are listed in Table 7-1 and discussed further in the following text. The findings of the critical appraisal or certainty assessment of the underpinning evidence are also described.

Table 7-1. Major themes relevant to the primary research question in the reviewed literature.

Sources	Critical appraisal or certainty assessment of included evidence	Themes
G3, G16	Suitable to adopt/adapt	The use of QMRA to evaluate alternative pollution events and management scenarios.
J2, J35	Low certainty: limited confidence in the reported associations	
G4, G8, G11, G16	Suitable to adopt/adapt	Systematic review findings broadly supportive of current paradigms of sources of microbial risks to recreational waters, types of situations or events increasing risk of GI, and classificatory approaches to management of GI risk.
J23	Low certainty: limited confidence in the reported associations	
G13	Suitable to adopt/adapt	Limited but emerging recognition of the greater susceptibility of children to health effects from exposure to pathogens in recreational waters.
G16	Suitable to adopt/adapt	Retention of existing FIB *E. coli* and enterococci for monitoring and assessing the extent of faecal contamination of recreational waters and thus GI risk.
J2, J35, J36	Low certainty: limited confidence in the reported associations	Availability of qPCR enterococci criteria values to trigger implementation of beach management actions and enterococci calibrated directly to observed health risk in epidemiological studies.
G13, G16	n/a	Further research opportunities for MST, QMRA, AMRB, standardisation of methods.
J15, J18	Low certainty: limited confidence in the reported associations	A positive relationship in recreational waters between the relative contribution of human faecal matter among pollution sources and greater health impacts on users compared to animal sources.
J6, J34	Moderate certainty: moderate certainty in the reported associations	Positive association between increased exposure rates in marine waters and higher levels of FIB and in GI and RI in swimmers including surfers.
J20	Low certainty: limited confidence in the reported associations	Recognition that certain beach geomorphology attributes and certain beach management practices greatly influence the probability of compliance with regulatory standards.
J3, J4, J30	Low certainty: limited confidence in the reported associations	Positive relationship between rivers flows and increased concentrations of FIB, biomarkers and pathogens in estuarine recreational waters.

Suitable to adopt/adapt (guidelines) or limited confidence in the associated findings (primary studies): *The use of QMRA to evaluate alternative pollution events and management scenarios.*

The following guidelines were found to be relevant and suitable to adopt/adapt based on an assessment of administrative and technical processes.

EPA Victoria (2021) used QMRA methods to compare the probability of illness from water-based recreational activities at the three beach locations within Port Phillip Bay, Victoria Bay, and to provide an example QMRA application. They showed that for primary and secondary contact recreational events the probabilities of contracting an illness were very low compared to the rates of illness expected at local regulatory criteria and were comparable to those reported in epidemiological studies with similar water body-types and pollution sources.

In recognition of the growing role of QRMA in public health risk assessment and management and drawing on an extensive literature review in support of scientific recommendations for the EU Bathing Water Directive, WHO (2018) recommended that a review of QMRA be undertaken to provide standardised information and advice on their practical application to Member States.

There is low certainty in the following primary studies based on their risk of bias assessments.

Ahmed, Hamilton, et al., (2018) combined the use of exploratory QMRA analysis with the development of a method for the use of qPCR MST markers to assess risks from untreated and treated sewage in beach water. Similarly, Schoen, Boehm, et al., (2020) extended the QMRA approach to model the effects of sewage age on GI risk-based thresholds (RBT) using mixtures of sewage at different ages and genetic marker concentrations for human associated crAssphage, Bacteroides spp., and polyomavirus.

The latter study was a rigorous and well-designed study with valuable and locally relevant observations on the use of human associated phage, FIB, human infectious virus genetic markers and QRMA methods for assessment of risks to recreational water quality. The study opens the way for the use of genetic marker concentrations to assess recreational water quality by linking marker concentrations to risk of illness.

Suitable to adopt/adapt (guidelines) and limited confidence in the associated findings (primary studies): *Systematic review findings were broadly supportive of current paradigms of sources of microbial risks to recreational waters, types of situations or events increasing risk of GI, and classificatory approaches to management of GI risk.*

The following guidelines/reviews were found to be relevant and suitable to adopt/adapt based on an assessment of administrative and technical processes.

The review by King et al. (2014) of epidemiological literature published between 2003 and 2014 sought to examined the relationship between recreational water use GI. Their findings broadly supported the current paradigms that GI risk increases with the level of exposure and time spent in the water although curiously they reported a significantly greater risk of GI in bathers compared to non-bathers at freshwater sites but a lack of any relationship between GI in bathers compared to non-bathers at marine beaches. This difference between marine and freshwater sites could be due to the problems with the literature King et al. reviewed as they were highly critical of the quality of the literature.

The current 2008 NHMRC Guidelines advocates a microbial-based categorisation of the water using a combination of sanitary inspection and microbial water-quality assessment. The NSW DPIE (2020) protocol for assessment and management of microbial risks in recreational waters builds on the 2008 NHMRC Guidelines and contains further additional high-quality guidance and templates for assessing and scoring likelihoods of contamination from a wide variety of pollution sources.

WHO (2018) supported the continued use of the EU's current four level classification system (excellent, good, sufficient, and poor) of bathing water quality which shares some similarities with the Australian system advocated by NHMRC (2008). WHO's recommendation that EU bathing water quality classification system be based on 95th percentile water quality standards would bring the EU into alignment with the existing Australian approach and is an endorsement of such an approach.

There is low certainty in the following primary study based on their risk of bias assessment.

The paper by Lugg, Cook, et al., (2012) describes an Excel tool (EnteroTester) which facilitates the appropriate computation for 95th percentiles and corresponding classifications for beach recreational water quality classifications (MACs) A, B and C, each with a defined range of infection risk. The tool has been demonstrated to be useful in an Australian context and provides an example of the types of tools that could be made available to support effective beach management classifications consistent with the 2008 NHMRC Guidelines.

Suitable to adopt/adapt: *Limited but emerging recognition of the greater susceptibility of children to health effects from exposure to pathogens in recreational waters.*

The following guideline was found to be relevant and suitable to adopt/adapt based on an assessment of administrative and technical processes.

In its five-year review of the 2012 recreational water quality criteria US EPA (2017) concluded that there was a growing body of evidence suggesting children are disproportionately susceptible to health effects resulting from exposure to pathogens in recreational waters. The susceptibility may be due to physiological (e.g. immunological, digestive tract) or behavioural (e.g. longer duration exposures, greater rates of ingestion, etc.) differences compared to adults. The US EPA commented that there were opportunities for further resolution of epidemiological relationships, especially around children's health protection and wider application of *Enterococcus* spp. qPCR techniques for monitoring.

Suitable to adopt/adapt: *Retention of existing FIB* E. coli *and enterococci for monitoring and assessing the extent of faecal contamination of recreational waters and thus GI risk.*

The following guideline was found to be relevant and suitable to adopt/adapt based on an assessment of administrative and technical processes.

In its recent EU bathing water review, WHO (2018) recommended the retention of intestinal enterococci and *E. coli* as indicators of bathing water microbial risks. With respect to enterococci, WHO cited the presence of sound epidemiological data supporting its continued use.

Limited confidence in the reported associations: *Availability of qPCR enterococci criteria values to trigger implementation of beach management actions and enterococci calibrated directly to observed health risk in epidemiological studies.*

There is low certainty in the following primary studies based on their risk of bias assessments.

Ahmed, Hamilton, et al., (2018) measured the concentration of several sewage-associated nucleic acid markers in samples dosed with either untreated or treated sewage and diluted to have concentrations of norovirus and adenovirus (NoV and HAdV 40/41) in genomic copies (GC) per 100 mL consistent with the US EPA benchmark 0.036 for GI. The authors reported the concentrations of human-specific HF183 Bacteriodes 16S rRNA genetic marker at the GI benchmark were 3.22 x 10^3 and 3.66 x10^3 GC/100 mL in waters contaminated with raw and treated sewage respectively.

Schoen, Boehm, et al., (2020) considered the age of sewage in sewage impacted recreational waters and estimated values of 9.3 × 10^2 and 9.1 × 10^3 (GC/100 mL) for HF183/BacR287 and CPQ_056, respectively, when fresh sewage was greater (by volume) than aged at the time of measurement. Given the differences in biomarker concentrations at the predicted GI thresholds between the two studies, such methods require closer evaluation to understand the causes of such differences. For example Ahmed, Hamilton, et al., assumed that all NoV and HAdV 40/41 quantified using qPCR were viable and infective, whilst Schoen, Boehm, et al., pointed out that no recreational water epidemiology study had measured the recently updated HF183/BacR287 DNA targets (nor the biomarker, crAssphage which was also considered) and attempted to relate these genetic markers to swimmer GI illness.

Shrestha and Dorevitch, (2019) evaluated a rapid qPCR method for quantification of *E. coli* for use at Lake Michigan beaches. Whilst the methodology showed merit, the authors concluded that the finding that each method and reference FIB measurement yielded different *E. coli* qPCR Beach Action Values (BAV) highlighted the need for further US EPA guidance for deriving new types of BAVs such as ones based on *E. coli* qPCR.

Further research opportunities for MST, QMRA, AMRB, standardisation of methods

US EPA (2017) identified the need for more research to better understand the role the environment plays in transferring antimicrobial resistant bacteria (AMRB) to primary contact recreators, whilst WHO (2018) considered that a detailed state of the art review of MST techniques and QMRA, to provide standardised information and advice on their practical application would be of value to EU Member States.

Although WHO released general guidance for QMRA in 2016 (WHO, 2016), the recommendation in relation to bathing water appears to be more focussed on the recreational water contexts and the potential for use of QMRA within a regulatory framework and the development of site-specific faecal indicator levels.

WHO (2018) expresses some important views on the use of MST and further research. However, since this particular topic aligns closely with secondary research question 1, it is addressed under that question in Section 7.3.1.

Limited confidence in the reported association: *A positive relationship in recreational waters between the relative contribution of human faecal matter among pollution sources and greater health impacts on users compared to animal sources.*

There is low certainty in the following primary studies based on their risk of bias assessments.

To demonstrate the potential of QMRA combined with MST data to determine the primary sources of contamination, Gitter, Mena, et al., (2020) estimated risk of GI illness rates in swimmers in an example waterbody (Walnut Creek, Brazos River Basin, Texas). The authors used *E. coli* concentrations to predict reference pathogen concentrations from cattle (*Campylobacter*), human (Norovirus), and wildlife/domestic animals (*Cryptosporidium*), and thus the relative risk of GI illness from each source.

MST analyses combined ERIC-PCR and Riboprinting in which isolates from water samples were compared against the Texas MST Library and a local library to identify sources and enhance accuracy of results. The findings indicated that the proportion of cattle/domestic animals and wildlife faecal loading had a much less substantial impact on the overall risk for a GI illness than human faecal sources.

Gitter, Mena, et al.'s study is innovative and easy to read, despite the complexity of analyses involved. The innovation of using the ratios of *E. coli* to reference pathogen concentrations in faecal sources to predict the reference pathogen concentration in the recreational water given the measured *E. coli* concentration is a clever approach. This approach does ignore the potential for *E. coli* arising from other sources (probably a low likelihood), and also ignores the likelihood of differential environmental decay rates between the *E. coli* and reference pathogens. With respect to differential decay, the ingested dose equation (equation 1 in the paper) follows the assumption that faecal pollution is fresh and directly deposited into the water, and not aged. The authors note that while these conditions may not be as realistic of actual environmental conditions, they serve as a worst-case scenario.

An Australian study, Henry, Schang, et al., (2016), described sampling of locations in Port Phillip Bay (marine) and in regional riverine, estuarine, greywater, stormwater source water tributaries to the bay for genetic evaluation of microbial communities. Sewage and potable waters that could contribute to sources were also sampled. The study aim was to use recreational water quality site microbial community data (as measured by 16S rRNA amplicon data) in high-throughput sequencing (HTS) and SourceTracker (a statistical tool for assessment of faecal contamination of coastal waters) to identify the main sources of contamination. SourceTracker to recreational beach samples identified treated effluent as major source of human derived faecal contamination, present in 69% of samples. Site-specific sources, such as raw sewage, stormwater and bacterial populations associated with the Yarra River estuary were also identified.

Moderate confidence in the reported association: *Positive association between increased exposure rates in marine waters and higher levels of FIB and in GI and RI in swimmers including surfers.*

There is moderate certainty in the following cohort study and systematic review based on their risk of bias assessments.

In a longitudinal cohort study illness rates in surfers in San Diego, California, Arnold, Schiff, et al., (2017) found that surfing was associated with increased incidence of several categories of symptoms, and associations were stronger if surfing took place shortly after rainstorms. Higher levels of FIB were strongly associated with fever, sinus pain/infection, wound infection, and GI within 3 days of rainstorms. Levels of FIB (including *Enterococcus*, total coliform, and faecal coliforms) were positively associated with increased incidence of almost all negative health outcomes during the study.

The magnitude of the statistical effects observed in Arnold, Schiff, et al.'s study are greatly dictated by the local recreational water quality environment of the study area. However, they should be relevant to any metropolitan surf beaches in temperate and subtropical regions of Australia, particularly in the vicinity of stormwater discharges. The overall findings of the study - urban coastal seawater exposure increases the incidence rates of many acute illnesses among surfers, with higher incidence rates after rainstorms - is consistent with the general epidemiological literature on water recreation at coastal beaches. The study findings support the continued use of FIB as a tool for assessing health risks to recreational water users at metropolitan surf beaches.

Russo, Eftim, et al., (2020) undertook a systematic review and meta-analysis to evaluate the current scientific evidence for differences in risk of illness between recreational activities typically associated with different levels of contact with water. The meta-analysis confirmed a strong association between swimming and both gastrointestinal illness (GI) and respiratory illness (RI). Swimming approximately doubles the risk of GI and increases the risk of RI by approximately 75% compared to no-contact controls.

Limited confidence in the reported associations: *Recognition that certain beach geomorphology attributes and certain beach management practices greatly influence the probability of compliance with regulatory standards.*

There is low certainty in the following primary study based on the risk of bias assessment.

Kelly, Feng, et al., (2018) evaluated whether beaches characterized by a set of management policies are associated with lower FIB levels. The study utilised a large data set of FIB monitoring from Florida, USA beaches (Florida Healthy Beaches Program FHBP) plus a large survey of beach management agency regional offices. FIB data considered were enterococci and faecal coliforms.

Analyses showed that beach geomorphology (beach type) was highly associated with exceedance of regulatory standards. Low enterococci exceedances were associated with open coast beaches (n = 211) that have sparse human densities, no homeless populations, low densities of dogs and birds, bird management policies, low densities of seaweed, beach renourishment, charge access fees, employ lifeguards, without nearby marinas, and those that manage stormwater.

Limited confidence in the reported association: *Positive relationship between rivers flows and increased concentrations of FIB, biomarkers and pathogens in estuarine recreational waters.*

There is low certainty in the following primary studies based on their risk of bias assessments.

Ahmed, Payyappat, et al., (2019) used FIB (*E. coli*, enterococci) and MST biomarkers to assess the magnitude of sewage and animal faecal contamination in Lake Parramatta water samples collected during a dry weather period and from two storm events coinciding with wet weather overflows of sewage to the lake. The study found that concentrations of the sewage-associated MST marker genes HF183 and PMMoV in most storm water samples exceeded the risk benchmark threshold values established in the literature for primary contact recreators.

In a similar study, Ahmed, Payyappat, et al., (2020) measured concentrations of culturable FIB and MST biomarker genes in dry weather samples and samples following storm events from 6 recreational water sampling sites at each of three Sydney estuaries. Analysis of sewage associated marker genes showed greater (i.e., 3–5 orders of magnitude) concentrations in water samples collected during the storm events compared to dry weather event; attributed to untreated sewage from wet weather overflows.

A UK study of the key processes influencing viral dispersal at bathing and shellfish harvesting sites along a river-estuary-coast system Robins, Farkas, et al., (2019) used hydraulic modelling to show that river flow was the primary control of viral export to the coast. It is noteworthy that study catchment (Conwy, Wales, UK) was short and steep, and the estuary was small and river-dominated which common attributes of NSW estuaries. The hydraulic modelling showed that short-duration high intensity ‘flash floods’ were shown to transport viruses through the estuary and out to sea, despite dilution and die-off effects.

7.2. GRADE assessment of primary studies

The GRADE quality assessment for the primary studies literature was “moderate” for the systematic review and cohort study (Russo, Eftim, et al., (2020) and Arnold, Schiff, et al., (2017)). This led to a final certainty rating of “*moderately confident in the reported associations*”.

The overall certainty rating was “low” for the 11 quantitative studies and this led to a final certainty rating of “*limited confidence in the reported associations*”. These results stemmed from the high degree of heterogeneity among the 13 studies selected which arose principally as a result of the broad nature of the primary research question. None of the factors that could influence a change in the grading of certainty of the body of evidence were identified.

It is worth noting that methods and approaches for systematic reviews of environmental health evidence is still an area of research and development, and further modification of the available frameworks and tools is beyond the scope of services required for this review. Further analysis and evaluation of the primary studies by the Committee can be undertaken if required.

7.3. Secondary research questions

Evidence for the secondary research questions was limited to the grey literature to make the workload manageable as set out in the research protocol , with different suites of grey literature documents relevant depending on the research question.

7.3.1. (i) What are the indicators or surrogates of contamination from diffuse and point sources in recreational waters?

Convention indicators for microbial risk commonly refer to the faecal indicator bacteria (FIB) *E. coli* and enterococci. Developments in genetic technologies have led to the more widespread use of genetic markers for faecal microorganisms, typically as part of MST studies. WHO (2018) notes that the idea behind MST is that genetic markers within certain faecal microbes are strongly associated with specific hosts (e.g. humans, livestock, dogs and gulls) (Table 7-2) and that certain identified attributes of those microbes can be used as markers for faecal contamination from that host.

WHO further states that while the concept behind MST is conceptually clear, the application of techniques and interpretation of results is a work in progress. Ideally, MST would provide clear source apportionments (e.g. percentages of faecal indicator organisms are derived from humans, birds, domestic animals, and other unspecified sources). WHO notes that such quantification currently relies on a number of assumptions which often are not fully met or are untested, including:

- host-specific markers are host-specific and do not cross react with other species;
- host-specific markers have similar environmental survival rates, fate and transport;
- the species of interest shed a similar amount of its host-specific markers;

- the faecal indicator organism: marker relationship is similar between species and markers;
- each host-specific marker has a similar prevalence and proportional distribution among individuals within the species.

Such issues need to be clarified and some level of standardisation of methods is required to assist in the incorporation of MST methods within regulatory frameworks.

Table 7-2. MST targets and associated hosts WHO (2018).

Human	Cow/ruminant/pig	Gull	Dog
Human viruses:	CowM2	Gull2	DogBac
Enterovirus - EV	CowM3	LeeSeaGull	BacCan
Adenovirus - AdV	BacCow	Gull4	
Norovirus (GI) - NovGI	BacR		
Norovirus (GII) - NoVGII	Rum2Bac		
Polyomavirus JC – PyV-JC	Bovine AdV - BAdV		
Polyomavirus BK – PyV-BK	Bovine PyV - BPyV		
HF183	Pig2Bac		
BacHum	Porcine AdV - PAdV		
HumM2			
Lachno2			
HB			

Under the Science Review component described in the 2017 Five-Year Review of the 2012 Recreational Water Quality Criteria, US EPA (2017) concluded that development and use of viral indicators, such as coliphage, may yield advances in public health protection, since evidence strongly suggests most illnesses in recreational waters are due to enteric viruses. US EPA concluded that despite their contribution to improved beach monitoring programs, opportunities remain for further refinement of qPCR methodologies.

US EPA noted that *Enterococcus* spp. measured by qPCR is a better predictor of swimming-associated GI illness and more timely than current culturable bacterial indicators. Furthermore, although observations show that development and use of alternative faecal indicators is a rich and evolving field, US EPA considered that no strong case was made for changing the indicators currently.

In the Australian context, EPA Victoria (2021) used MST techniques to identify sources of human sewage, canine and avian microbial contamination and assess risks to recreational waters. The study involved measured of qPCR Bacteroides marker HF183/BacR287 as an indicator of human sewage, as well as enterococci concentrations, and reported a significant correlation between proportion of the microbial communities were like human sewage microbial communities and the qPCR marker ($p=0.008$) and enterococci ($P<0.001$).

Similarly a significant relationship was reported between enterococci concentrations and the total proportion of faecal microbial communities ($p<0.001$), perhaps confirming that enterococci provide an estimate of the overall level of faecal contamination.

Several primary studies, including a number of Australian studies, described above under the primary research question also make use of qPCR methods, indicating an increasing interest amongst researchers in the use of such indicators.

In the literature included in this review, there is little distinction between diffuse and point sources of microbial contamination, however, such a distinction is commonly used in studies of water supply catchment protection and may warrant further discussion in the revised NHMRC Guidelines.

7.3.2. (ii) What are the current practices to minimise or manage contamination from diffuse and point sources in recreational waters?

As noted above under the discussion for the primary research question, the 2008 NHMRC Guidelines describe a classificatory approach to the management of recreational water quality. The approach involves microbial-based categorisation of the water using a combination of sanitary inspection, microbial water-quality assessment and prevention of exposure at times of increased risk. No publications describing applications of the current guidelines made it through the screening and quality assessment process. However a recent document, the NSW Department of Planning, Industry and Environment (NSW DPIE) *Protocol for Assessment and Management of Microbial Risks in Recreational Waters* (NSW DPIE 2020) builds on the classificatory framework of the guidelines. In particular, Part 7 of the 7-part management framework for managing microbial risks in recreational water lists generic management actions to reduce microbial risks to recreational water quality. Suggested risk management recommendations are helpfully classified as follows:

- *Actions to reduce likelihood*
    - Pollution abatement sewage and stormwater controls
    - Use of microbial source tracking (MST) to assist in identifying sources and thus appropriate source controls
- *Actions to reduce consequence*
    - Beach closures
    - Informed personal choice based on media advisories
- *Triggers for management actions*
    - Water quality monitoring (based on enterococci)
    - Rapid responses to spill incidents (e.g. sewer overflows)

Additional information in support of each subheading is contained in Part 7 of the Protocol.

Under the Science Review component described in the 2017 Five-Year Review of the 2012 Recreational Water Quality Criteria, US EPA (2017) concluded that further research on accurate and reliable MST technologies should be undertaken to markedly improve future water quality management by (i) fostering development of alternative site-specific criteria based on local pollution sources and (ii) assisting strategic remediation planning to focus on faecal pollution from human sources.

Under the Implementation Review component US EPA (2017) concluded that

- *Sanitary Surveys* should continue to serve as an important tool for informing site remediation, characterizing waters for QMRA and site-specific criteria development, and can be linked with integrated environmental modelling.
- *Predictive/Statistical Models* offer a cheaper alternative for same-day notification and resulting public health protection than other rapid methods.
- *Deterministic Process Models for Recreational Beach Site Assessment and Enhancement/Remediation* can include QMRA health-based models and can assist understanding of contaminant transport and development of support site-specific criteria and/or remediation options.

With respect to management of microbial contamination from diffuse and point sources WHO (2018) contained only minor recommendations focussing on dermal afflictions. For the following dermal afflictions resulting from recreational water exposure WHO stated that information should be conveyed to the public in the bathing water profile at sites they are known to occur:

(i) Swimmer's itch;

(ii) Wound infection (e.g. caused by *Vibrio* spp.). In addition, advice should be provided on bather hygiene measures to minimise risk and actions to take if a wound is sustained while bathing.

EPA Victoria 's MST study of three Port Phillip Bay beaches showed that human faeces contributed on average of only 13% of the total faecal contamination and the main contributors to faecal contamination were of avian and canine origin which carry comparatively lower risks to human health. Human sources identified during the sanitary survey included bather shedding (release), toilet facilities, sewage treatment plant (STP) outfalls, STP by-passes, sewage overflows, sewage chokes, and boats. To be effective, management responses would need to be tailored to each source type, thus the authors recommended that the origin of the contamination should be a primary factor in assessing risks of water-based recreation, since it can significantly impact the outcome of the risk assessment.

7.4. Other questions from the Committee

Since the publication of the 2008 NHMRC Guidelines, the field of risk assessment (in particular QMRA) has become well established and new technologies to monitor indicators and pathogens have been developed. Therefore, in preparing our responses to the main research questions listed above, the Committee suggested that the narrative review should consider a number of additional questions based on the scientific evidence produced since 2003 (Table 7-3). These questions were addressed based on evaluation of the available body of evidence included in this review. Further analysis and evaluation of the available body of evidence or additional studies by the Committee can be undertaken if required to answer these questions.

Table 7-3. Additional questions for supporting main research questions for the narrative review.

Additional Questions	Responses
(i) What are drawbacks of the interpretation of risks provided by the previous guidelines when applied to the Australian context?	There was no evidence from the included studies to indicate that there are drawbacks to the interpretation of risks provided by the 2008 NHMRC Guidelines. While there are concerns within the Committee that current advice is not always appropriate for interpreting microbial risks when applied to the Australian context, there was no relevant information on this identified from the included studies. The Committee may like to review this further at a later stage; however, it is likely that further research is required in this area. Based on guidance from other jurisdictions, the Committee could consider potential opportunities for additional information in the Guidelines that would assist end users in risk interpretation. For example the Committee could consider including additional guidance on source identification and associated pathogen profiles which would assist in tailoring risk management to focus on the most important sources. This could be achieved by providing short case studies giving examples of the use of appropriate techniques to assist in risk identification and interpretation, for example: • Advocating the use of MST methods to permit better identification of sources and some quantification of source loads; • Use of QMRA to identify high risk periods, quantify potential impacts during such periods, and by working back from tolerable risk thresholds to identify locally relevant tolerable limits for contributing factors (e.g. flows in local waterways, rainfall) or triggers for management actions (e.g. beach closures)
(ii) What happens when pollution is from non-point sources or when pollution is mainly associated with sources other than human?	As noted in the major themes above (Section 7.1), there was evidence from the studies and guidance included in this review that indicated a positive relationship in recreational waters between the relative contribution of human faecal matter among pollution sources and greater health impacts on users compared to animal sources. Several studies indicated that human sewage is the greatest risk source, and viruses are the most problematic pathogen for causing GI, particularly norovirus, e.g. Gitter, Mena, et al., (2020) estimated risk of GI illness rates in swimmers in a Texas creek and use PCR and riboprinting matched to a local MST library to identify sources. The findings indicated that the proportion of cattle/domestic animals and wildlife faecal loading had a much less substantial impact on the overall risk for a GI illness than human faecal sources. A study in PPB (Henry, Schang, et al., 2016) used microbial community 16S rRNA data from recreational beach samples combined with HTS and the statistical tool SourceTracker to show that treated effluent was major source of human derived faecal contamination, present in 69% of samples. Site-specific sources, such as raw sewage, stormwater and bacterial populations associated with the Yarra River estuary were also identified. EPA Victoria (2021) reported that human sources identified during the sanitary survey included bather shedding (release), toilet facilities, sewage treatment plant (STP) outfalls, STP by-passes, sewage overflows, sewage chokes, and boats. Thus the available evidence suggests that when pollution is from non-point sources or when pollution is mainly associated with sources other than human, that microbial risks to recreational users is significantly less than when it is from point sources of human waste. A possible exception to this view may be the impact of bather shedding in high density recreational areas where there may be some confusion as whether the source is a point source or diffuse source.
(iii) Can a new framework be developed to take into account these variations and truly reflect potential health outcomes in different settings (including in freshwaters)?	There was no evidence in the included literature supporting major changes to existing frameworks. However further research opportunities were identified for MST, QMRA and standardisation of methods. For example, US EPA (2017) concluded that sanitary surveys should continue to serve as an important tool in management of microbial risks to recreational water by characterizing waters for QMRA and site-specific criteria development, and linking with integrated environmental modelling (e.g. predictive statistical and deterministic models). When drafting a new framework the Committee could consider potentially adapting sections of guidance from the recently published NSW DPIE Protocol for assessment and management of microbial risks in recreational waters (NSW DPIE, 2020). The protocol is closely aligned with and builds on the 2008 NHMRC Guidelines and describes an improved framework for managing microbial risks in recreational water with the most innovative components being methods for: • Selection of sites for assessment • Sanitary inspections • Microbial water quality monitoring • Microbial assessment and beach classification • Reporting

Additional Questions	Responses
	Important elements of the protocol that could support framework improvements are: • The inclusion of an initial site prioritisation step which prioritises beaches to provide a basis for determining resource allocation. • A detailed Sanitary Inspection process with 5 steps; • A microbial water quality monitoring program with specific guidance for sampling design and documentation, quality control aspects including sampling procedures, lab methods and accreditation, data management and work health and safety. • A microbial assessment and beach classification program which describes the methodology for determining beach suitability grades • Reporting of: Annual Classifications, Weekly Star Ratings, Advisories following rainfall events, daily beach pollution forecasts, communication planning and methods of communication. • Appendices with high quality templates for data collection and reporting for such items as: Sanitary inspection major attributes (e.g. pollutions sources), and water quality sample log sheets. A further noteworthy innovative aspect of the Protocol is the inclusion of methods for assessing and scoring likelihoods of contamination from a wide variety of pollution sources. Templates for scoring are also provided in the appendices. Part 7 of the 7-part management framework also lists helpful generic management actions to reduce the microbial risks to recreational water quality. The study by Kelly, Feng, et al., (2018) utilised a large data set of FIB monitoring from Florida, USA, beaches plus a large survey of regional beach management agency offices identified the beach geomorphology attributes and beach management practices that support compliance with regulatory standards. For example risk factors including beach management practices are identified that are associated with increased FIB concentrations (e.g. animals such as dogs and birds, humans, availability of amenities, beach aspect and type (open ocean vs marshy areas), and beach grooming methods (disturbance of heavy wrack can increase FIB). Conversely low enterococci exceedances were associated with open coast beaches that have sparse human densities, no homeless populations, low densities of dogs and birds, bird management policies, low densities of seaweed, beach renourishment, charge access fees, employ lifeguards, without nearby marinas, and those that manage stormwater. Whilst none of the above findings are surprising they act to reinforce our understanding of microbial risks and can be used to support improved guidance information for risk management of recreational beaches. Many of the above factors can also be applied to estuarine or freshwater settings, with the exception of those only present in marine settings.
(iv) Can the previous values be retained as default values in absence of a risk assessment process?	No evidence was identified to suggest change is necessary on the existing guidance on single-sample water quality triggers for short-term water quality assessment. However, US EPA (2017) noted that *Enterococcus* spp. measured by qPCR is a better predictor of swimming-associated GI illness and more timely than current culturable bacterial indicators. Furthermore, although observations show that development and use of alternative faecal indicators is a rich and evolving field, US EPA considered that no strong case was made for changing the indicators currently. However, US EPA did suggest that development and use of viral indicators, such as coliphage, may yield advances in public health protection, since evidence strongly suggests most illnesses in recreational waters are due to enteric viruses. US EPA concluded that despite their contribution to improved beach monitoring programs, opportunities remain for further refinement of qPCR methodologies. On a related theme, WHO 2018 concluded that current evidence does not support the inclusion of a viral indicator (or viral pathogen) as a regulatory parameter within EU Bathing Water Directive.
(v) Can source tracking be a part of this framework in identifying sources of contamination?	There was limited evidence identified to suggest source tracking should or should not be used in microbial risk assessment frameworks. WHO (2018) states that while the concept behind source tracking is conceptually clear, the application of techniques and interpretation of results is a work in progress. WHO notes that such quantification currently relies on a number of assumptions which often are not fully met or are untested. Notwithstanding the caveats on the use of MST identified by WHO, a number of MST studies were evaluated and included as part of this narrative review. With the exception of the study by Gitter, Mena, et al., (2020), the studies were characterised by methodological complexities, excessive detail and limitations regarding the interpretation of results particularly with the statistical analysis. Such outcomes possibly stem from the crossover of disciplines between

Additional Questions	Responses
	laboratory-focussed genetic analyses and environmentally-focused field sampling and public health regulatory frameworks. Drawing on the above observations on MST studies as well as those by WHO it is apparent that some level of standardisation of methods is required to assist in the incorporation of MST methods within the NHMRC framework for managing microbial risks in recreational waters.

7.5. Concluding comments

An evaluation of evidence contained in six grey literature documents and 13 primary research studies provided satisfactory evidence to support detailed responses to the primary and secondary research documents. The findings from each study are described in the text and summarised in tables. This is followed by thematic summaries based on the major themes identified relevant to each of the three research questions (1 primary and 2 secondary). Finally a section responding to a series of additional questions posed by the Committee is included which draws on the available evidence identified for the review.

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