National Microbiological Water Quality Guidelines for Marine Recreational Areas

Implications from a Review of Recent Research

Prepared for Envirolink

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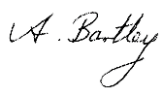	Formatting checked by:	Alison Bartley
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Contents

Executive summary

The National Microbiological Water Quality Guidelines for Marine and Freshwater Recreational Areas ['the guidelines', Ministry for the Environment (MfE) and Ministry of Health (MfE/MoH) 2003)] form a pivotal reference for water quality management in New Zealand. The guidelines underpin summer recreational water quality monitoring programmes undertaken by regional and unitary councils in collaboration and the Public Health Units of District Health Boards (PHUs). This monitoring is carried out to assess the microbiological water quality of freshwater and nearshore coastal areas commonly used for contact recreation. The guidelines also provide direction for monitoring the microbiological quality of recreational shellfish harvesting areas. The various threshold values identified in the guidelines are focused on safeguarding public health.

In recent years council practitioners have identified several concerns with the guidelines. The regional sector's Coastal Special Interest Group (Coastal SIG) secured MBIE Envirolink Tool funding in 2016/17 to review four issues relating to the marine component of the guidelines:

1. Whether the current enterococci values in the guidelines are still appropriate for managing human health risk, given the results of a series of recent overseas coastal swimming-and-health epidemiological studies.
2. Determining if the shellfish water quality component of the current guidelines could be enhanced.
3. To provide guidance as to the distance at which the guidelines are appropriate for use in waters adjacent or downstream of wastewater treatment plant discharges.[1]
4. Providing guidance on selection and use of appropriate indicator(s) to use in brackish water bodies for public health risk management (the guidelines are silent on this).

Appropriateness of enterococci guideline values

Over 100 papers of recent national and international studies were reviewed to determine if there is a case for modifying the numerical enterococci criteria in the guidelines for "swimmability". These studies used both epidemiological and Quantitative Microbial Risk Assessment (QMRA) approaches.

Surveillance thresholds

In general, the guidelines' surveillance requirements for marine waters should be retained, including re-sampling on consecutive days once the action value has been exceeded. The re-sampling requirement is important where sanitary surveys have indicated the possibility of ongoing contamination, such as can arise from leaking sewerage. However, some modifications are desirable:

(i) If logistical problems make sampling on consecutive days difficult, the repeat sample could be taken two days apart.

(ii) Consideration should be given to omitting the repeat-sample requirement for waters that have been graded as low quality (e.g., 'poor' or 'very poor' in terms of existing "Suitability for Recreational Grade").

[1] Conditions of use of the guidelines explicitly state they should not be directly applied to water impacted by nearby point source discharges of treated effluent. Despite this, no advice is provided as to the spatial extent where the guidelines may be suitable for use. The current report addresses this gap.

(iii) Because sample results are not available for at least one day, where predictors of faecal contamination are known, real-time models, based on local models and/or a semi-quantitative scoring approach (such as Auckland's SafeSwim package[2]), are preferable for indicating swimmability. These models could use swimmability surrogates, such as salinity or turbidity, provided a robust relationship can be demonstrated with indicator bacteria.

(iv) Improved advice on carrying out and interpreting the results of sanitary surveys would be helpful.

Grading of recreation sites

The grading component of the guidelines seem not to merit change at this time. However, a watching brief should be kept on recent developments, particularly in the USA, using: (i) pathogens; (ii) pathogen surrogates (e.g., coliphages); and (iii) more modern and epidemiologically-appropriate faecal indicator laboratory methods (especially qPCR—quantitative Polymerase Chain Reaction).

The US EPA approach (that uses the same numeric thresholds for freshwater and coastal water), should be kept under review—but in conjunction with a future review of the freshwater component of the guidelines.

Recreational shellfish-gathering waters

The shellfish component of the guidelines was examined for the possibility that it could be reformulated, based on a risk assessment approach using a new model. The model runs in Microsoft Excel, includes both microbial uptake and depuration, and potentially offers a better approach than the current or conventional bioaccumulation method. Comparisons were made between these two methods for the Motueka offshore shellfishery and to examine the possibility of replacing the current faecal coliform-based faecal indicator for shellfish-gathering waters with one based on enterococci.

We found the current shellfish-gathering component of the guidelines *could* be changed to one based on enterococci, with a requirement that the median is less than 7 enterococci per 100 mL and the maximum does not exceed 22 enterococci per 100 mL. Before such a change is considered, or before different microbes are adopted for measurement (making use of emerging enumeration techniques such as qPCR, or use of phages), the calculations should be checked in detail to examine model sensitivity. The implications of any guideline change also need careful consideration.

Consideration could also be given to revising the "shellfish-gathering season"-based approach in current use to one that recognises local practice, where gathering may occur over a longer "season" or even year-round.

Indicators near treated wastewater discharges

The results from over 20 recent coastal water QMRA projects were used to assess appropriate monitoring of human health risk in coastal waters near wastewater outfalls. We found sampling for enterococci is generally appropriate beyond 500 m of a discharge of *treated* wastewater, outside of which the individual illness risk is less than 1% (defined in the guidelines as the No Observable Adverse Effects Level, NOAEL). However, the appropriateness of this distance will depend on local hydrodynamics. For example, existence of a discharge plume may cause the risk to persist for some

[2] https://safeswim.org.nz/

distance downstream from the discharge point, whereas on the 'upstream' side of the discharge, the risk may be lower even though the site is closer to the point of discharge.

Quantitative Microbial Risk Assessments (QMRA), using chosen pathogens (especially Noroviruses for waters impacted by treated wastewater) should continue to be used to calculate potential health effects, for users of recreational water, of treated disinfected wastewater near outfalls—especially when informing an Assessment of Environmental Effects.

Brackish water indicators

The choice of faecal indicator for brackish waters is complicated. Our review used a simplified estuary model based on the understanding that the choice is determined by the *hydraulic residence time* of the brackish water body, rather than the water's salinity. We found that for long residence-time estuaries (greater than three days), enterococci should be chosen. For short residence-time estuaries, *E. coli* is the appropriate choice when near the inflowing river water, but enterococci should be chosen near the mouth. Between these locations, either indicator may be suitable. Accordingly, it appears wise to measure both indicators in low residence time systems and use the more stringent of the two test results for surveillance.

1 Introduction

The National Microbiological Water Quality guidelines for Marine and Freshwater Recreational Areas ['the guidelines', Ministry for the Environment (MfE) and Ministry of Health (MoH) 2003)] form a pivotal reference for water quality management in New Zealand. The guidelines underpin summer recreational water quality monitoring programmes undertaken by regional and unitary councils in collaboration with the Public Health Units of District Health Boards (PHUs). This monitoring is carried out to assess the microbiological quality of fresh- and nearshore coastal waters commonly used for contact recreation. The monitoring results are compared to 'trigger levels' in the guidelines which provide the basis for informing the public as to when risks of illness may be unacceptable. Monitoring data collated over time are also used by some councils to calculate a Suitability for Recreation Grade for each monitoring site or, more recently in the case of fresh water sites, to assess progress against water quality objectives set under the National Policy Statement for Fresh Water Management (NPS-FM).

In 2013, regional and unitary council staff responsible for recreational water quality monitoring programmes completed a discussion paper (Bolton-Ritchie et al. 2013) documenting issues that have arisen following the development, interpretation and implementation of the guidelines in the 10 years since they were published. These were further-elaborated by Milne et al. (2017). There has been concern that some aspects of the guidelines may be too precautionary, with potentially significant implications for regional and unitary councils (the regional sector), the agricultural sector and local communities, given that the guidelines underpin the mandatory "human health for recreation" attribute in the NPS-FM. There was also concern that substantial changes in New Zealand's land-use patterns have occurred since the 1998–2000 freshwater microbial study on which the *E. coli* freshwater guideline values were based. The effect of these changes on risk estimates is unclear.

In partnership with MfE and MoH, the regional sector – through its Environmental Monitoring and Reporting (EMaR) project – formulated packages of work relating to a review of the guidelines. In late 2015 the regional sector's Coastal Special Interest Group (Coastal SIG) formulated an MBIE Envirolink Tool proposal to fund four aspects of the review, primarily related to marine recreational waters.[3] These aspects, outlined in Section 1.3, are the focus of this report, funded under MBIE contract number C10X1610 to NIWA.

1.1 Contact recreation in marine waters

During use of surface waters for recreation, people can develop gastrointestinal or respiratory illnesses from microbial pathogens (e.g., viruses and protozoa) that may be present in the water. In New Zealand, concentrations of those pathogens are generally 'low'. Nevertheless, international studies (including New Zealand, McBride et al. 1988), have shown that illness may arise in waters with generally low pathogen concentrations.

To help manage risks to human health from microbial contamination, the Ministry for the Environment and the Ministry of Health jointly issued microbiological water quality guidelines for

[3] The original Envirolink Tool pre-proposal sought to update the data and procedures underpinning the guidelines' risk assessment for both fresh and marine recreational waters. However, the application was subsequently reduced in scope when MfE could not commit funding to support the fresh water component of the review. At the time of finalising this report, Phase 1 of the review of the fresh water component of the guidelines had been completed (Moriarty et al. 2018) and it was unclear when Phase 2 would proceed.

recreational areas in 2003.[4] A key feature of the guidelines is the use of faecal indicator bacteria (i.e., enterococci for marine waters), rather than the pathogens themselves. The one exception to this approach is where water recreation areas are impacted by nearby point sources of treated wastewater; in these instances, assessments of microbiological water quality must establish the relationship between indicator bacteria and key pathogens (MfE/MoH 2003).

The indicator-based approach uses information derived from epidemiological studies (discussed in subsection 2.3.1), most of which measured only indicators, not pathogens. Indicator bacteria are much more easily and cheaply measured than pathogens. Moreover, indicator bacteria also serve as an index of pathogenicity of the water; individual pathogens cannot act as an index of this nature because a pathogen that is not measured may be the cause of illness.

The approach taken in the guidelines follows that taken in the World Health Organization's guidelines (WHO 2003), where both long-term assessment of a coastal site's suitability for recreation (typically over a five-year period) and short-term (immediate) surveillance criteria (derived from the grading criteria, using a green-orange-red traffic light setup) are used. The numerical criteria that underpin the guidelines are based on a set of epidemiological studies carried out in the UK in the 1980s, elaborated in the next chapter. With the results of more recent overseas epidemiological studies now available, the Coastal SIG sought a review of the appropriateness of the existing enterococci numerical values used for managing human health risk in marine recreational waters. Having confidence in the guideline numbers is important – the suitability of natural waters for swimming is a topic that has a growing national profile, strengthened by improved public awareness of water quality issues and access to water quality data. For example, swimming water quality information presented on the regional sector's Land Water Air Aotearoa (LAWA) website attracted more than 30,900 visitors over the 2016/17 summer (Milne et al. 2017).

To better manage public health risks, the Coastal SIG also sought guidance on the selection of the most appropriate microbiological indicator to use in brackish waters such as estuaries. The guidelines are silent on this, leading to inconsistent practice between councils (Bolton-Ritchie et al. 2013).

1.2 Shellfish-gathering waters

The guidelines also address the suitability of shellfish-growing waters, especially when shellfish are eaten raw. Again, a faecal bacteria indicator is used – faecal coliforms – with numeric guideline values based on USEPA (1976) criteria. The guidelines provide a season-by-season ('medium term') suitability assessment, but do not identify short-term surveillance or long-term grading requirements. In addition, a shellfish-gathering season is not defined in the guidelines and councils have expressed concern that the numeric indicator bacteria values appear overly conservative, with no technical explanation of correlation between indicator bacteria and actual human health risk (Bolton-Ritchie et al. 2013). The Coastal SIG has therefore requested advice on other potential approaches to evaluating human health risks from recreational shellfish-gathering.

[4] The 2003 guidelines replaced earlier provisional or draft guidelines made public in 1992, 1998, 1999 and 2002—Department of Health (1992), MfE/MoH (1998, 1999, 2002).

1.3 Scope of this report

Four key issues identified by the Coastal SIG are addressed in this report:

1. Whether the current enterococci values in the guidelines are still appropriate for managing human health risk, given the results of a series of recent overseas coastal swimming-and-health epidemiological studies.

2. Determining if the shellfish water quality component of the current guidelines could be enhanced to better-reflect tolerable infection risks arising from consumption of raw shellfish flesh.

3. Guidance as to the distance at which the guidelines are appropriate for use in waters adjacent or downstream of discharges from wastewater treatment plants.

4. Guidance in the selection of an appropriate indicator(s) to use in brackish water bodies for 'State-of the Environment' reporting and for public health risk management.

1.4 Approach

An extensive review of recent national and international studies was performed to consider the first issue, i.e., whether there is a case for modifying the numerical criteria in the national microbiological water quality guidelines (MfE/MoH 2003).

For the second issue, the shellfish component of the guidelines is examined for the possibility that it could be reformulated, based on a risk assessment approach using a new shellfish uptake-and-depuration model.

The results from over 20 recent coastal water QMRA projects (Quantitative Microbial Risk Assessment) were used to address the third issue – selection of appropriate indicators near wastewater outfalls.

Finally, selection of appropriate indicators for use in brackish waters is addressed using a modelling approach that utilises information about differential inactivation rates for *E. coli* and enterococci in freshwater versus coastal water.

1.5 Report outline

This report comprises six chapters, with chapters 3 to 6 addressing each of the four issues outlined in Section 1.3:

- Chapter 2 outlines key aspects of the marine component of the current guidelines concerning 'grading' and 'surveillance'. The underpinning risk calculation procedures derived from epidemiological and risk assessment studies are also addressed.

- Chapter 3 summarises the main points from a review of more than 100 recent references relevant to numerical enterococci criteria for contact recreation in marine waters (Appendix A), addressing key findings in terms of surveillance, grading and sanitary inspection requirements. Appendix B provides explanations of the essentials of epidemiological and QMRA (Quantitative Microbial Risk Assessment) procedures. Appendix C contains key information on the consequences for children exposed to contaminated water.

- Chapter 4 discusses the development of possible enhancements to the current shellfish-gathering-waters guideline, using enterococci rather than faecal coliforms as the appropriate indicator, and a new, more sophisticated uptake-and-depuration model that incorporates simultaneous time-varying processes of virus uptake and virus depuration. Details of the bioaccumulation and uptake-and-depuration models are given in Appendices D and E, respectively.
- Chapter 5 addresses appropriate monitoring of faecal indicators in proximity to treated wastewater outfalls.
- Chapter 6 presents a simplified estuary model for *E. coli* and enterococci (detailed in Appendix F), leading to simple rules for indicator selection based on estuary hydraulic residence time.
- Chapter 7 summarises the findings of this project.

Some use is made of footnotes regarding technical details, to facilitate readability of the text. Some figures in the appendices are direct Microsoft Office Excel screen grabs, as they conveniently convey finer details of model input and output.

2 The guidelines – an overview of the marine component

The numerical requirements of the marine component of the MfE/MoH (2003) guidelines are advisory-only and lack the statutory recognition afforded to the freshwater component which have been included in the National Policy Statement for Freshwater Management 2014 (NZ Govt 2014; 2017). Recognition of this status is particularly relevant because many of the papers included in this review originate from the USA where the numerical requirements represent regulatory 'criteria' (or 'standards' in New Zealand terms).[5]

The guidelines define two key activities: short-term surveillance and long-term grading. Surveillance is ideally based on current microbiological water quality conditions ('is it safe to swim today'), determined through regular (typically weekly) water sampling over the summer bathing season. Grading is assessed using multiple years of microbiological data (typically five years), combined with an assessment of catchment sanitary risk (MfE/MoH 2003). Both surveillance and grading are outlined in this chapter, followed by an overview of two approaches used to underpin human health risk guidance in recreational waters: epidemiological studies and QMRA.

2.1 Surveillance

The surveillance 'traffic-light' criteria in the guidelines for marine waters are shown in Box 1 below. These criteria are used to manage recreational water quality on a 'day-to-day' basis, with councils typically carrying out water sampling on a weekly basis over the summer bathing season. The guidelines state that a bathing season will vary according to location but will generally extend from 1 November to 31 March, providing approximately 20 sample results per season. Several councils monitor for a shorter period, such as from December to February inclusive (Bolton-Ritchie et al. 2013, Milne et al. 2017).

Box 1:
Surveillance, alert and action levels for marine waters

Surveillance/Green Mode: No single sample greater than *140 enterococci/100 mL*.

- Continue routine (e.g. weekly) monitoring.

Alert/Amber Mode: Single sample greater than *140 enterococci/100 mL*.

- Increase sampling to daily (initial samples will be used to confirm if a problem exists).
- Consult the CAC to assist in identifying possible sources.
- Undertake a sanitary survey, and identify sources of contamination.

Action/Red Mode: Two consecutive single samples (resample within 24 hours of receiving the first sample results, or as soon as is practicable) greater than *280 enterococci/100 mL*.

- Increase sampling to daily (initial samples will be used to confirm if a problem exists).
- Consult the CAC to assist in identifying possible sources.
- Undertake a sanitary survey, and identify sources of contamination.
- Erect warning signs.
- Inform public through the media that a public health problem exists.

Notes: Either of the following methods may be used to enumerate enterococci: Enterolert™ or EPA Method 1600.* For national consistency it is recommended that accredited laboratories be used for microbiological tests (e.g. IANZ accreditation). Samples to test compliance should be over the bathing season appropriate to that locality (at least 1 November to 31 March) and sampling times should be restricted to between 0800 hours and 1800 hours.
* USEPA National Centre for Environmental Publications and Information (NCEPI), 11029 Kenwood Road, Cincinnati, OH45242, USA.

[5] The USA 'Criteria values' (USEPA 2012) are *recommendations*. States (and tribes) then need to adopt water quality standards that are regulatory in nature. Those recommendations become regulatory when adopted by a State.

Note that the Action mode does not get triggered until and unless two samples on consecutive days exceed 280 enterococci per 100 mL. In practice, there can be delays in collecting a second sample (e.g., bad weather) and we think that the Action mode should be triggered by a single exceedance. This is consent with both the application of the Action mode for fresh waters (based on *E. coli*) and recommendations made by MfE (2005) in a draft discussion paper on national reporting.

Derivation of the surveillance criteria is described at page I16 in the guidelines:

> *Neither the WHO (2003) nor the authors of the UK studies on which the WHO guidance is based give any guidance for deriving surveillance values. Accordingly, we have used results from previous uncontrolled epidemiological studies, in particular those of Cabelli (1983a), also used in previous versions of the guidelines. While this could be argued to be somewhat dislocated, it has the advantage of maintaining good continuity with past practice*.[6,7]

Accordingly, the green and red modes' boundary values were based on studies that formed the basis of previous New Zealand guidelines (Department of Health (1992), MfE/MoH (1998, 1999, 2002)].

2.2 Grading

The guidelines adopted the 'Annapolis Protocol', as promulgated by the World Health Organization (WHO 1989, 2001, 1993). This creates four long-term grading bands called 'Microbiological Assessment Categories' (MAC), ranging from 'A' to 'D'. Typically, long-term is taken as five years of weekly sampling in the bathing season (i.e., ~100 samples), although WHO (1993) states that 60 samples should be sufficient.

The three boundaries between the four bands are assessed as sample 95%iles (using the Hazen method), as in Table D1, reproduced below. These boundaries correspond to (maximum-average) HCGI (highly credible gastrointestinal illness) risks of 1%, 5% and 10%, and 0.3%, 1.9% and 3.9% risk of AFRI (Acute Febrile Respiratory Illness). These values (elaborated in MfE/MoH 2003, Table H1) have been promoted by WHO (2003, Table 4.7).

Table D1: Microbiological Assessment Category definitions for marine waters

A	Sample 95 percentile ≤ 40 enterococci/100 mL
B	Sample 95 percentile 41–200 enterococci/100 mL
C	Sample 95 percentile 201–500 enterococci/100 mL
D	Sample 95 percentile > 500 enterococci/100 mL

Source: WHO 2001.
Note: The Hazen method is used for calculating the 95 percentiles.[7]

The MAC value is combined with a Sanitary Inspection Category (SIC) to populate a 'Suitability for Recreation Grade' (SFRG) under which a site can be classified 'Very Good', 'Good', 'Fair', 'Poor', 'Very

[6] "Cabelli (1983a) is this report's Cabelli (1983).

[7] The Alert and Action thresholds for marine waters are 140 and 280 enterococci per 100 mL, respectively. These were derived by calculating 80%ile and 90%ile of Cabelli's results for waters at the borderline for compliance (i.e., a median of 35 enterococci per 100 mL, with respect to water quality criteria in force in the early 1980s). They are not risk-based, in that the choices of 80%ile and 90%ile were not based on any risk thresholds. For details of the percentile calculations, see page I16 of the guidelines. (Note that the enterococci logarithm in these calculations is to base 10, not to base 'e'.)

Poor' or 'Follow Up'.[8] (See Table D2 of the guidelines, reproduced on p14.) The SIC component, determined in the form of a 'Catchment Assessment Checklist' concerns local sanitary surveys to determine a site's potential risk of faecal contamination. Although the guidelines contain some detail on how these surveys can be performed (they should consider land use, water uses, microbiological hazards (e.g., wastewater discharges, waterfowl), river discharges and rainfall), there is scope for improvement (Bolton-Ritchie at al. 2013).

Table D2: Suitability for recreation grade for marine sites

Susceptibility to faecal influence		**Microbiological Assessment Category** Indicator counts (as percentiles – see Table D1)				**Exceptional circumstances** ***
		A ≤ 40 enterococci/ 100 mL	**B** 41–200 enterococci/ 100 mL	**C** 201–500 enterococci/ 100 mL	**D** > 500 enterococci/ 100 mL	
Sanitary Inspection Category	Very Low	Very Good	Very Good	Follow Up**	Follow Up**	
	Low	Very Good	Good	Fair	Follow Up**	
	Moderate	Follow Up*	Good	Fair	Poor	
	High	Follow Up*	Follow Up*	Poor	Very Poor	
	Very High	Follow Up*	Follow Up*	Follow Up*	Very Poor	
Exceptional circumstances						

Notes

* Indicates unexpected results requiring investigation (reassess SIC and MAC). If after reassessment the SFRG is still 'follow up', then assign a conservative grade (i.e. the first grade to the right of the 'follow up' in the same SIC row). This follows the precautionary principle applied in public health.

** Implies non-sewage sources of indicators, and this should be verified. If after verification the SFRG is still 'follow up', then assign a conservative grade (i.e. the first grade after 'follow up' in the same MAC column).

*** Exceptional circumstances: relate to known periods of higher risk for a graded beach, such as during a sewer rupture or an outbreak of a potentially waterborne pathogen in the community of the recreational area catchment. Under such circumstances a grading would not apply until the episode has abated.

(For example: if MAC = C and SIC = Moderate, then Suitability for Recreation Grade = Fair.)

2.3 Underpinning risk calculations

2.3.1 Epidemiological studies

In general, the marine (coastal) water component of the current guidelines (MfE/MoH 2003, WHO 2003) is based on 'controlled cohort' epidemiological studies. These studies involve recruiting adult volunteers, most of whom have not intended to go to a beach on a given week day. When taken to a beach they are divided into swimmers and non-swimmers. They all eat the same foods, to eliminate meal-related bias. The swimmer cohort is divided into further groups, each of which has a different exposure (e.g., fully immerse the head three times; do so only at chest depth, etc.). The health status of all volunteers is assessed after a few days, sometimes on two or three separate days over periods of days and weeks.

Water quality data are also collected for each survey day, particularly concentration data for the enterococci faecal indicator bacterium. Once all data are received, the association between water quality (measured as enterococci) and swimming is determined, using statistical modelling.

Further detail is given in Appendix B.

[8] 'Follow-up' arises when the MAC is inconsistent with the Sanitary Inspection Category (SIC). See Table D2. The SIC exercise is being applied, if at all, in a rather ad hoc manner. Ignoring it can confer a false sense of security, where contamination is intermittent.

The enterococci thresholds used in the guidelines for gastrointestinal and respiratory illnesses are based on a set of UK epidemiological studies (Kay et al. 2004) which were adopted in the WHO (2003) guidelines.

2.3.2 Quantitative Microbial Risk Assessments (QMRA)

A QMRA is the process of estimating the risk of infection or illness from exposure to microorganisms and underpins the freshwater component of the guidelines, principally because epidemiological studies had seldom been carried out for these waters.[9] A QMRA is briefly outlined here because QMRA studies form the basis of the assessment in Chapter 5, where the use of faecal indicators in proximity to treated wastewater outfalls is considered.

A QMRA focuses on the link between the number of pathogen units ingested or inhaled whilst swimming and the probability that infection and illness will occur, as given by a dose response relationship. Most of these relationships are based on clinical trials, using volunteers. Illness outbreak data can occasionally be used for this purpose (Teunis et al. 2005).

There are four main steps in a QMRA:

(i) selection of pathogens of concern;

(ii) assigning degrees of exposure of swimmers to those pathogens;

(iii) selecting appropriate dose-response information; and

(iv) calculating risk profiles and average human health risks. An iterative method is used, so that all values of variable quantities are captured (such as the duration of a swimming event), resulting in 'risk profiles'.

Further reading on the essentials of QMRA and the key information regarding exposure to contaminated water is given in Appendices A and B.

[9] Dufour (1984) is an exception. Since 2003, further epidemiological studies have been carried out for freshwater lakes, not rivers, as detailed in this project's literature review.

3 Adequacy of the current enterococci values

This chapter summarises the main points from a review of more than 100 recent references relevant to numerical enterococci criteria for contact recreation in marine waters, and considers international approaches in response to comments on the adequacy of the current surveillance, grading and sanitary inspection requirements in the MfE/MoH (2003) guidelines.

3.1 Key findings from the literature review

Appendix A presents summaries of the main findings of the literature review of more than 100 recent peer-reviewed papers from many countries dealing with advances in epidemiological (and related) studies regarding impacts of microbial contamination on the health of swimmers. The information is based on a report prepared by the US Environmental Protection Agency (via ICF Consultants, McBride (2018)) that considered:

- effect of ingestion of faecally-contaminated water on children's health;
- new information on health and enterococci;
- microbial source tracking;
- wet versus dry weather conditions, and impact on indicator organism concentrations; and
- miscellaneous topics (sediment disturbance, effect of multiple pathogens, pathogenic *E. coli* in coastal waters).

The principal findings from the review were:

- Culture-based enterococci assays continue to demonstrate an association between enterococci and swimmer's health risk in coastal waters when impacted by human sources, including treated wastewater.
- There are insufficient grounds to recommend abandoning culture-assayed enterococci, although there is a case for also monitoring enterococci assayed by qPCR[10] (as has been done by the USEPA).
- Enterococci by qPCR may also serve as a source tracking marker if a suitably expanded qPCR laboratory procedure could be implemented.[11] This would be a significant step forward. In general, risks posed by faecal sources from animals such as gulls, chickens and possums may pose a lesser risk relative to that created by human faecal material, but not so for risks to human health posed by faecal materials derived from bovine cattle[12] (and probably ovine ruminants (Soller et al. 2010a).
- Children are at higher risk than adults, because they generally ingest or inhale more water cf. adults, and/or they tend to be more susceptible, particularly in the 0-4 year-old age group (Arnold 2016, Supplementary Information).

[10] qPCR = quantitative Polymerase Chain Reaction, a recent laboratory test, targeting genetic sequences.
[11] This seems feasible, pers. comm. Dr Brent Gilpin, ESR, Christchurch.
[12] Schoen & Ashbolt 2010, Ehsan *et al.* 2015, Soller et al. (2010b, 2015), Brown et al. (2017).

- Dry-weather risk estimates have been found to be significantly lower than risks estimated for wet-weather conditions because pathogen concentrations tend to be higher during and immediately after rainfall.
- Development and use of alternative faecal indicators is a rich and evolving field, which suggests that it may be premature to promote their selection and use in revised guidelines.
- Future research efforts should look to complement traditional enterococci measurements with other microbial source-specific markers [especially the 'qPCR' method for enterococci, which Wade et al. (2008, 2010) found to be well-associated with swimmers' gastrointestinal illness]. Indeed, the USEPA's most recent criteria (2012) have included qPCR, along with traditional culture-based enterococci. They also have changed the HCGI (highly credible gastrointestinal illness) endpoint to just AGI (acute gastrointestinal illness).

3.2 Revisiting the surveillance and grading criteria

3.2.1 Should coastal and freshwater guidelines have the same numeric thresholds?

The USEPA 'Water Quality Criteria' (USEPA 2012) amalgamate their formerly separate freshwater and coastal water criteria, and use results of recent epidemiological studies to develop a single set of numeric criteria. These criteria use enterococci for both freshwater and coastal water, and *E. coli* for freshwater. They were recently endorsed in a formal review (USEPA 2018).

At first sight, amalgamated criteria could be an attractive option for New Zealand. However, it should be noted that:

- The pathogen content of New Zealand freshwaters, dominated by *Campylobacter*, is quite different to that in coastal waters, dominated by viruses. In contrast, the U.S. pathogen mix in both freshwater and coastal waters tend to be similar, with both predominantly impacted by contaminants of human origin (McBride et al. 2011,[13] 2013).
- The New Zealand guidelines do not include criteria for *E. coli* in coastal waters, so amalgamation of the freshwater and marine components of the guidelines would compromise human health protection.
- The USEPA has not adopted the Annapolis Protocol, and so fuses surveillance and grading together such that long-term and day-to-day information is combined (see Section 3.2.2).
- Adopting the USEPA approach would have implications for the freshwater component of the guidelines, currently under the first stages of review.

Accordingly, it is appropriate to continue to rely on the current approach for coastal waters, especially as it is still endorsed by the World Health Organization. However, the possibility of amalgamating criteria for both freshwater and coastal water should be kept in mind.

[13] https://www.foodsafety.govt.nz/elibrary/industry/examining-link-with-public-health/campylobacter-in-food-and-the-environment.pdf

3.2.2 Comparison with overseas surveillance criteria

New Zealand is alone in including distinct numerical surveillance requirements in its recreational microbiological water quality guidelines. Numerical surveillance criteria are absent from the European Bathing Directive.[14] In contrast, the USA (USEPA 2012) criteria essentially combine surveillance and grading together via its recommendations[15] on each *monthly* geometric mean value ('GM', 35 enterococci per 100 mL) and a 90th percentile statistical threshold value ('STV', 130 enterococci per 100 mL).[16] Generally in the USA short-term and long-term considerations are therefore amalgamated into the medium-term in the form of a monthly assessment, as follows:

> *The waterbody GM should not be greater than the selected GM magnitude in any 30-day interval. There should not be greater than a ten percent excursion frequency of the selected STV magnitude in the same 30-day interval.*[17]

However, for the usual (weekly) sampling frequency over a month, the STV is essentially a maximum value—because there would be insufficient data to calculate a 90th percentile (most percentile calculation formulae, including Hazen, require more than 4 data to facilitate prediction of a 90th percentile).[18]

The USEPA (2012) also contains a 'Beach Action Value' (BAV) of 70 enterococci per 100 mL. This is advisory-only. Its practical use is ([19]):

> *The BAV is simply the 75th percentile of the water quality distribution that the criteria are based on. Nothing needs to happen when a BAV is exceeded, but rather it is intended as a value that can be used as a precautionary beach management action value. For example, a manager may decide to resample to see if something unusual is happening (the chances of 2 in a row at that level or higher is 0.06). or it could trigger a beach notification if a manager wants to be very conservative (but as you understand, a single value could happen as often as 1/4 times). Whether or not they are too severe, I think depends on how they are applied. As I note above, for two samples in a row, perhaps not. For a single sample, I would think perhaps - unless, for example you have a beach with excellent water quality, then a single sample on this order may be adequate to tell you something different is happening.*

In summary, setting aside the advisory BAV, and assuming weekly sampling, the USEPA's STV (130 enterococci per 100 mL) is similar to the guidelines' Alert mode threshold (140 enterococci per 100 mL).

3.2.3 Should New Zealand's coastal water surveillance thresholds be changed?

The single sample numeric threshold used in freshwater surveillance for the Action and Alert modes of the guidelines are numerically equal to the B/C and C/D grades' MAC thresholds. For example, the freshwater C/D grading threshold is a 95th percentile of 550 *E. coli* per 100 mL, and the Action (red) mode is a single-sample maximum of the same concentration (i.e., 550 *E. coli* per 100 mL). This is not the case for coastal waters which have been derived from former studies (Cabelli 1983) – see page I16 of the guidelines and footnote (7). Were the same equality approach for freshwaters be taken for marine waters, the Alert threshold would rise from 140 to 200 enterococci per 100 mL and the

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[14] http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32006L0007

[15] States have a fair amount of latitude to determine their own criteria values. A State could propose a longer duration (for example, a 90 or 120 days bathing season), which would change some of the implementation issues identified above.

[16] These values apply to an estimated gastrointestinal risk of 3.6%. The Criteria also contain slightly lower GM and STV values for a risk of 3.2%.

[17] "GM" denotes the geometric mean.

[18] Note that Excel requires only one datum to compute *any* percentile. This is inappropriate.

[19] Pers. comm. Jeff Soller, Soller Environmental, San Francisco, CA.

Action threshold would rise from 280 to 500 enterococci per 100 mL (compare the Surveillance and Grading tables in sections 2.1 and 2.2), causing a substantial increase in threshold values.

At this point it becomes important to consider the effects of pathogen-sensitivity on children. The four-beaches epidemiological studies undertaken in the UK that underpin the marine water component of the current guidelines excluded children. Those studies (Kay et al. 2004)[20], were of the 'controlled cohort' type and, in the UK children cannot be recruited for such studies on ethical grounds. In contrast the uncontrolled cohort USA study of Cabelli (1983), and the more recent USA "NEEAR" studies, did include children).[21] Given that the current 140/280 Alert and Action thresholds of the guidelines are based on Cabelli's studies, for selected illness risks, there seems no case to relax them.

On the face of it, the USA STV value (130 enterococci per 100 mL) would suggest a case to make the surveillance criteria stricter. However, as explained in the previous section, under sampling more frequent than weekly over a month, the STV ceases to be a single-sample maximum.

We conclude that the current Alert and Action thresholds (140 and 280 enterococci per 100 mL) should remain (see also section 2.1 and footnote 7).

3.2.4 Comparison with overseas grading criteria

Here we contrast the guidelines' grading requirements with those of the USA (USEPA 2012) and the European Union.[22]

Grading requirements in all three jurisdictions are based on upper percentiles (90th and 95th). A summary is shown in Table 3-1, in which two 90th percentiles have been converted to 95th percentiles. It can be seen from Table 3-1 that the grades of the New Zealand guidelines:

- are stricter than the EU's 'Excellent' criterion
- are in harmony with both the EU and USEPA 'Good grades', and
- are more permissive than the EU 'Sufficient' grade.

Before considering the implications of the literature review for possible changes to the guidelines' grading thresholds, it is appropriate to consider the role of the burden-of-proof when considering compliance criteria for the guidelines.

The guidelines' marine water component is based on an even-handed approach to accounting for sampling variability, whereas the guidelines' approach for freshwater is precautionary.[23] If that approach were taken for the marine waters the guidelines' Microbiological Assessment Category's A/B, B/C and C/D thresholds would dramatically change—from 40, 200 and 500 to 2, 10 and 25 enterococci per 100 millilitres, respectively; concentrations that would be generally unattainable.

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[20] The four beaches were: (i) Langland Bay, near Swansea (120 bathers, 133 non-bathers), (ii) Moreton Beach near Wallasey on the north bank of the Wirral (101 bathers, 164 non-bathers), (iii) Southsea (172 bathers, 186 non-bathers) and (iv) Southend-on-Sea (155 bathers, 185 non-bathers). Mean bather age was 31.65 years versus 32.12 years for non-bathers. Fifty four percent of bathers were male while 46.5% were female. Beaches were selected to be a substantial distance apart, to maintain independence of beach conditions and cohorts of swimmers (Fleisher et al. 1996).

[21] https://archive.epa.gov/neear/web/html/index.html

[22] http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32006L0007

[23] If data are to be used to estimate the true value of some statistic (e.g., a median), that estimate will seldom coincide with the true value (this is called 'sampling error' by statisticians). In the even-handed approach (sometimes called 'face-value') the median of the data is taken as its true value. In a precautionary approach it is recognised that the median calculated from the data may return a result rather lower than the true value, in which case enterococci thresholds would be lower than the face-value result.

Table 3-1: International comparison of 95th percentile grading. Numbers are enterococci per 100 mL; NZ and EU assessment period is the bathing season, USEPA assessment period is monthly.

Jurisdiction	Grading and numeric value expressed as 95th percentile equivalent (N/100 mL)			
New Zealand (MAC)	'A'	'B'	'C'	'D'
	40	200	500	>500
EU Bathing Water Directive	'Excellent'	'Good'	'Sufficient'	
	100	200	~280[a]	
USEPA Criteria (STV)[24]		~200[a,b]		

[a] The EU 'Sufficient Grade' is expressed as a 90th percentile (185 enterococci per 100 mL), as is the USEPA Criteria's 'STV', (Statistical Threshold Value, 130 enterococci per 100 mL). We have converted these two values to 95th percentiles, using the ratio of those percentiles in earlier USEPA criteria (USEPA 1986, Table 4), i.e., multiplying the 90th percentile by 158/104 ≈ 1.52.

[b] The USEPA's single 'grade' is assumed to be equivalent to both the guidelines' Microbiological Assessment Category 'B' and to the EU Bathing Directive's 'Good'.

However, the rationale for the freshwater guidelines' component using the precautionary approach arose because the risk calculated for freshwater was derived from a nationwide QMRA study for campylobacteriosis, with *Campylobacter* concentrations correlated with concentrations of *E. coli*. However, because that correlation is moderate at best, a precautionary approach was taken to account for situations where the *E. coli* concentration is 'low' and *Campylobacter* concentrations are 'high'.[25] Coupled with that is the finding that for the four UK epidemiological studies on which the guidelines are based, higher risk profiles were produced than for any other relevant study (Prüss 1998). Because higher risk profiles were obtained, it is considered appropriate to be sufficiently precautionary.

Accordingly, no adjustment for a precautionary (or liberal) burden-of-proof seems warranted.

3.2.5 Should New Zealand's grades for coastal waters be changed?

The following comments are made in relation to the existing MAC grade boundaries:

A/B boundary (95%ile of 40 enterococci per 100 mL)
From MfE/MoH (2003, Table H1), this boundary corresponds to risks less than 1% for GI (Gastrointestinal Illness) and <0.3% for AFRI (Acute Febrile Respiratory Illness). Given that excellent water quality implies negligible risk, there seems no case to revise this boundary.

B/C boundary (95%ile of 200 enterococci per 100 mL)
There is no case to revise this boundary, given its harmony with overseas jurisdictions, as noted above.

C/D boundary (95%ile of 500 enterococci per 100 mL)
It could be argued from the U.S. Criteria that this threshold could be reduced, from 500 enterococci

[24] The EPA STV is not to be exceeded more than 10% of the time in any time period evaluated. But, if there are multiple time periods evaluated (as would be the case if 30-day durations were used), then not exceeding the STV in more than 10% of the samples in any time period is more stringent than not exceeding the STV 10% of the time in all samples collected. So, the USEPA criteria (as applied on a 30-day basis) is actually between the NZ "A" and "B" grades.

[25] Note that the freshwater guidelines did account for the fact that they are underpinned by a campylobacteriosis QMRA study, for *Campylobacter* alone, when in fact other pathogens may be at play. This was achieved by reducing the GI risks associated with the guidelines three thresholds, from 1%, 5% and 10% to 0.1%, 1% and 5% (see Page I17 of MfE/MoH 2003).

per 100 mL to about 300 enterococci per 100 mL. However, that would reduce the spread of the grading system and unduly penalise sites which may be affected in wet weather but which may be swimmable at other times (i.e., those with 95th percentile concentrations between 300 and 500 enterococci per 100 mL).

In terms of the Sanitary Inspection Categories (SIC), their inclusion in the guidelines reflects the site grading approach endorsed by WHO (2003) (refer Chapter 2). While we still strongly support the use of sanitary assessments to characterise the potential faecal contamination risks at a bathing site, better and more up-to-date guidance is needed to support the process of determining a SIC grade under the guidelines (see Bolton-Ritchie et al. 2013).

3.2.6 Key findings

Surveillance thresholds

In general, the guidelines' surveillance requirements for marine waters should be retained, including resampling on consecutive days once the action threshold has been exceeded. This resampling requirement is important where sanitary surveys have indicated the possibility of ongoing contamination, such as can arise from leaking sewerage. However, some modifications are desirable:

(i) If logistical problems make sampling on consecutive days difficult, the repeat sample could be taken two days apart.

(ii) Consideration should be given to omitting the repeat-sample requirement for waters that have been graded as low quality (e.g., 'poor' or 'very poor').

(iii) Because sample results are not available for at least one day, where predictors of faecal contamination are known, using real-time models, based on local models and/or a semi-quantitative scoring approach (such as Auckland's SafeSwim package) are preferable for indicating swimmability. These models could use swimmability surrogates, such as salinity or turbidity, provided a robust relationship can be demonstrated with indicator bacteria.

(iv) Improved advice on the conduct and use of sanitary surveys would be helpful (for surveillance and for grading.

Grading of recreation sites

The grading component of the guidelines seems not to merit change at this time. However, a watching brief should be kept on recent developments, particularly in the USA, using: (i) pathogens; (ii) pathogen surrogates (e.g., coliphages); and (iii) more modern and epidemiologically-appropriate faecal indicator laboratory methods (especially qPCR—quantitative Polymerase Chain Reaction).

The US EPA approach (that uses the same numeric thresholds for freshwater and coastal water), should be kept under review, but in conjunction with a review of the freshwater component of the guidelines.

4 Recreational shellfish-gathering waters

This chapter reviews guidance for managing human health risks from recreational shellfish-gathering. The faecal coliform thresholds in the existing MfE/MoH (2003) guidelines are outlined, including the origins of their development. The balance of the chapter is dedicated to investigating whether a new risk-based shellfish uptake-and-depuration model based on enterococci could be used to enhance the guidelines for marine waters. These guidelines could also apply to estuarine waters with a long residence time (refer to Chapter 6 on brackish water indicators).

4.1 Existing guidance

The guidelines contain human health-protection faecal coliform thresholds for waters overlying shellfish; it assumes that these shellfish can be taken by the public and eaten raw. The guidelines require that:

> *The median faecal coliform content of samples taken over a shellfish-gathering season shall not exceed a Most Probable Number (MPN) of 14/100 mL, and not more than 10% of samples should exceed an MPN of 43/100 mL (using a five-tube decimal dilution test).*

These criteria have their origins in the USEPA where the requirement for guidance regarding faecal coliform concentrations in shellfish gathering waters arose following a large outbreak of shellfish-related typhoid in 1924, simultaneously striking New York, Chicago and Washington D.C. The oysters, sourced from the Atlantic Seaboard oyster industry, were contaminated with *Salmonella typhi*. An estimated 150 individuals died. Seeking to minimise recurrence of such an outbreak, USA sanitation authorities eventually produced new microbiological standards for commercial harvesting waters, based on total coliforms[26] (Furfari 1968, see McBride 1990). The geometric mean of total coliforms was not to exceed 70 per 100 mL. This limit was developed by requiring that no more than 50% of 1 mL sample portions were positive for total coliforms, equivalent to an MPN of about 70/100 mL.[27] Subsequently, a study by the National Shellfish Sanitation Program collected coliform data from 15 States and two Canadian Provinces. From about 3,500 samples, the USEPA (1976) reported that

> *…70 coliform MPN per 100 ml at the 50th percentile was equivalent to a faecal coliform MPN of 14 per 100 ml. The data therefore indicate that a median value for a faecal coliform standard is 14 and the 90th percentile should not exceed 43 for a 5-tube, 3-dilution method...".*[28]

4.1.1 Assessing compliance

To determine whether a shellfish gathering site has complied with the guidelines, it is necessary to wait *until the season's end*, possibly creating the potential for health risk during the season. This would occur when high faecal coliform concentrations are found early in the season, but low concentrations are found toward its end.

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[26] It seems that faecal coliform tests were not available in the early part of the last century.

[27] Using Hoskins' MPN equations.

[28] For the USA (NSSP 2013), this is currently interpreted as: "Standard for the Approved Classification of Growing Areas Affected by Point Sources … Fecal Coliform Standard for Systematic Random Sampling. The fecal coliform median or geometric mean MPN or MF (mTEC) of the water sample results shall not exceed fourteen (14) per 100 mL and the estimated 90th percentile shall not exceed an MPN or MF (mTEC) of:
(a) 43 MPN per 100 mL for a five-tube decimal dilution test;
(b) 49 MPN per 100 mL for a three-tube decimal dilution test; or
(c) 28 MPN per 100 mL for a twelve-tube single dilution test; or
(d) 31 CFU per 100 mL for a MF (mTEC) test."
Item (c) is omitted for areas affected by nonpoint sources.

A simple resolution of this issue—enabling short-term health risk assessment—would be to follow the surveillance approach adopted in the guidelines' surveillance criteria (using single-sample maxima), and so change the 90th percentile to a maximum—making the guidelines more stringent. In that case the requirement would be stated as:

> *The median faecal coliform content of samples taken over a shellfish-gathering season shall not exceed a Most Probable Number (MPN) of 14/100 mL, and ~~not more than 10% of samples~~ no sample should exceed an MPN of 43/100 mL (using a five-tube decimal dilution test).*

In terms of the median value, "shellfish-gathering season" is not defined in the guidelines. While most councils monitor shellfish gathering waters for the summer bathing period only – so as to align with surveillance monitoring of marine waters – shellfish harvesting in some areas could occur year-round or at least on the seasons' shoulders. We therefore recommend that "season" be defined according to usage and in consultation with the community. Consideration also needs to be given to obtaining sufficient sample results to generate a robust median statistic (ideally at least 12).

Note that if the maximum approach is used (instead of the 90th percentile), a notification issue then arises: What should be reported to the public about the length of time to wait before being able to collect shellfish again? (The same issue arises, but less frequently, when more than 10 percent of water samples to be taken over a season exceed 43 faecal coliforms per 100 mL).

4.2 Using a model to make the shellfish guidelines risk-based

In this section we compare a new shellfish model with the 'standard' bioaccumulation model, utilising results for commercial shellfish harvesting impacts offshore from Motueka and Napier. The model of choice is then applied when considering possible changes to the guidelines for recreational shellfish-gathering waters, including changing the indicator from faecal coliforms to enterococci. A change in the indicator has the potential to deliver several advantages:

- laboratory costs may be reduced (but note that the method detection limit for Enterolert is 10 enterococci per 100 mL, so while many councils use this method for evaluating marine swimming waters, a membrane filtration method might be required for shellfish gathering risk assessment given that the required median would most likely be below 10 enterococci per 100 mL);
- the guidelines could be made risk-based by using an appropriate shellfish contamination model. In that case, both the swimming-related and shellfish-related microbial water standards would be risk-based; and
- the guidelines would become more precautionary, because enterococci can be expected to be inactivated more slowly than faecal coliforms in saline waters, i.e., faecal coliforms would increasingly under-indicate risk from hardier, true pathogens over time (Nelson et al. 2018).

4.2.1 Shellfish models

Two models are briefly described below. Both models consider two processes associated with the accumulation of pathogens in shellfish flesh: uptake and depuration. Despite differences in how these processes are represented, it is possible that the overall risk predicted by these two models is similar.

Bioaccumulation model

This model assumes that uptake and depuration are *instantaneous*, which assumes that:

- shellfish flesh is contaminated the moment that the overlying water is contaminated, and that
- shellfish flesh is contaminant-free the moment the water is also contaminant-free.

This method uses a shellfish bioaccumulation factor (Burkhardt and Calci 2000), denoted by 'BAF'. The pathogen dose ingested from consumption of a given mass of shellfish is taken as BAF × the number of pathogens present in the equivalent volume of seawater. For example, the virus dose ingested from consumption of 100 g of shellfish is taken as BAF × the concentration of viruses expressed as number per 100 mL of seawater (McBride 2005, Table 9.1).

Consequences of the underlying assumptions (bulleted above) include over-estimation of uptake, and under-estimation of depuration. Further details of the model are given in Appendix D.

Uptake-and-depuration model

The proposed model explicitly considers two *gradual* processes associated with accumulation of pathogens in shellfish flesh: uptake and depuration. Typical results are compared with the bioaccumulation model in Figure 4-1. Note that, when compared with the bioaccumulation model, the uptake-and-depuration model:

(i) has a lower maximum;

(ii) (ii) gradually accumulates viruses after water contamination starts; and

(iii) (iii) persists for some time after the contamination event has ceased.

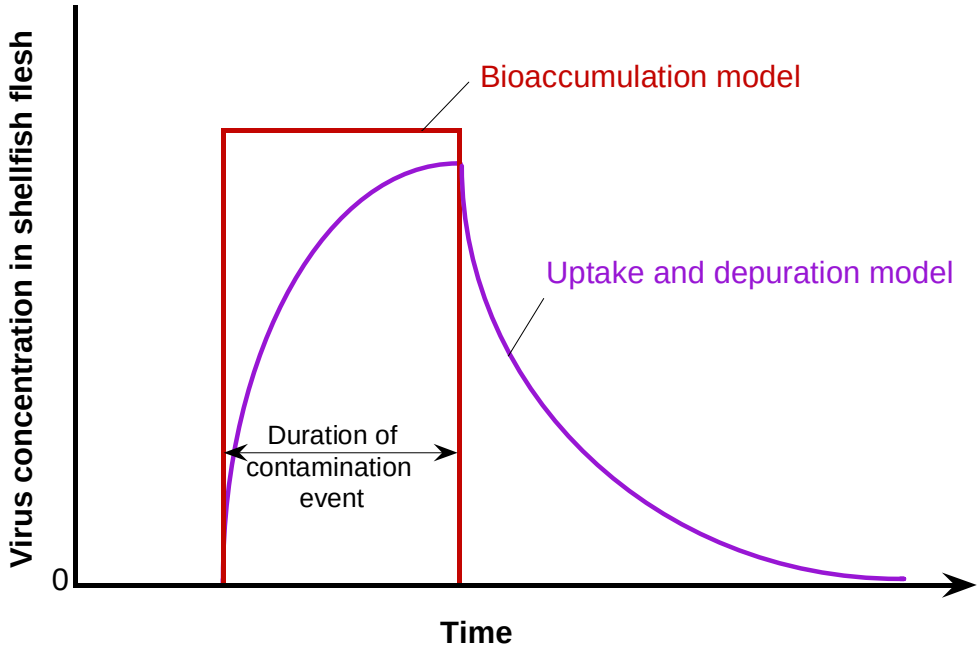

Figure 4-1: Idealised behaviour of the uptake-and-depuration model compared with the bioaccumulation model for a 'top hat' contamination event, i.e., the horizontal difference between the vertical red lines during which time the virus concentration is elevated and constant.

Detailed explanation of the models' development is given in Appendix E.

4.2.2 Analysis procedure

Analysis was done in six steps, considering Norovirus as the aetiological (causative) illness agent, which has been demonstrated to cause illness from consumption of oysters in France (Thebault et al. 2013), and as it so often is for shellfish generally (e.g., Schaeffer et al. 2013):

1. Describe and compare the performance of the new 'virus uptake-and-depuration' model with that of the standard bioaccumulation model, both of which have been used in a QMRA for offshore Motueka aquaculture.
2. Investigate the relative merits of the two models in considering revision of the guidelines.
3. Use the appropriate model to predict Norovirus concentrations in shellfish flesh.
4. Select a maximum tolerable health risk for a shellfish consumer.
5. Select a critical Norovirus concentration in the water at the shellfish bed(s) corresponding to the selected maximum health risk.
6. Use literature values for simple ratios of Norovirus concentrations to enterococci concentrations in receiving waters to devise new median and 90%ile values for proposed revised guidelines.[29]

4.2.3 Step 1: Applying the two models to offshore Motueka aquaculture

The aquaculture area is 4 km offshore from Motueka in the Tasman District, where improved wastewater treatment is in prospect. The possibility of health impacts arising from consumption of raw shellfish impacted by this treated wastewater was handled by the bioaccumulation QMRA method for shellfish risk prediction (McBride 2015). The model was used to calculate expected doses, given predictions of virus concentrations in the water at the aquaculture area by hydrodynamic models. These doses were used in a dose-response relationship to calculate risk profiles.

The uptake-and-depuration model was also applied to the water concentration data, to facilitate comparison between the models.

Both models use the same procedure for risk calculation, given a specified dose. Therefore, the two models can be compared using calculated doses alone, rather than infection/illness.

4.2.4 Step 2: Investigate the relative merits of the two models

Consider an extreme case where there is substantial illness in the Motueka community. Results for secondary treated wastewater effluent concentrations and a hydrodynamic model show that, in such a case, a typical value of virus concentration in the water overlying pipis can be taken as 10^{-2} per litre. We can also take typical values for the (dimensionless) bioaccumulation factor (50) and the shellfish meal size (50 g).[30,31] In this case we obtain an average received dose of 2.5 x 10^{-2} virus units.[32] The typical dose calculated using the uptake-and-depuration model is about 10^{-2}. This level of agreement is 'close', but can vary somewhat between cases (e.g., if adopting different bioaccumulation factors and meal sizes).[33]

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[29] Accounting for variability and uncertainty in these ratios follows later.

[30] As contained in unpublished spreadsheets supporting the published risk results in McBride (2015).

[31] In Appendix E the Norovirus concentration in the shellfish water is denoted by *c* (viruses per litre), the bioaccumulation factor is denoted by *b* (dimensionless), the shellfish meal size is denoted by *m* (grams) and the dose is denoted by *d*.

[32] Doses must be integers of course. So, a result of $d = 0.02$ for the average dose simply means that up to 2 people in 100 may ingest a dose of 1 (or more). The bioaccumulation model accounts for this, via random binomial sampling of input distributions.

[33] Note that this comparison uses 'typical' values rather than Monte Carlo risk percentiles, because there are substantial difficulties in using the new time-based model with the frequency-based bioaccumulation model (see the Appendix of Harper and McBride (2015).

It can be concluded from the above that the bioaccumulation model and the uptake-and-depuration models can give similar results. However, the bioaccumulation model is less attractive (even though it is simpler) because it does not mimic the actual processes occurring. It is essentially an empirical *frequency-based* approach, completely avoiding the time-history of contamination of shellfish flesh. This can give rise to some awkward outcomes, particularly for intertidal areas.[34] On the other hand, the uptake-and-depuration model is *time-based*, making it more appropriate for this investigation.[35]

4.2.5 Step 3: Predict Norovirus concentrations in shellfish flesh

Figure E-8 and Figure E-9 (of Appendix E) display the results for the uptake-and depuration model using default parameter settings, for two cases: (a) constant concentration of viruses in the water over a long period; (b) 'top hat' concentration of viruses in the water, in which viruses are present from hours 2–4, but absent at other times. Both the constant concentration and 'top hat' cases [(a) and (b)] are given maximum virus concentrations of 0.007 per litre in the overlying water—(for reasons revisited in section 4.2.8). The effects of uptake and depuration can be clearly seen on these results, especially for the 'top hat' case (Figure E-9): flesh can indeed continue to be contaminated after the pulse of contamination has departed.

The constant concentration case shows a maximum flesh concentration of approximately 15 viruses per (20 g) oyster whereas the 'top hat' case is two orders of magnitude lower, at approximately 0.01 viruses per shellfish.

4.2.6 Step 4: Select a maximum tolerable health risk

The adopted risk of shellfish-associated illness should be precautionary, in line with public health practice. In that case we could adopt 1% as the maximum tolerable illness risk, corresponding to the Lowest-Observable-Adverse-Effect Level (LOAEL) for swimming in marine waters (i.e., a Microbiological Assessment Category (MAC) of A). We say "could" because this choice begs the question: Should the same risk level for recreational water apply to shellfish consumption? That is a policy decision beyond the scope of this project.

4.2.7 Step 5: Critical virus concentration

Taking a precautionary approach, we assume that ingestion of one virus is sufficient to cause illness. Therefore, a tolerable health risk of 1% means that the *average* dose in any oyster should be 0.01. Eating more than one oyster, as implied by the adopted mean meal sizes, increases health risk. To get the corresponding virus concentration in the overlying water we assume that a collection of oysters is exposed to a virus-containing plume for one hour of a tidal cycle (flood or ebb tide, as appropriate). We run the uptake-and-depuration model with standard parameter settings, varying virus concentrations in the water until the predicted virus concentration in the shellfish flesh is 0.01 per gram. That concentration in the overlying water is 0.007 viruses per litre, as shown in Figure E-9 (in Appendix E). Examination of shellfish-associated QMRA results offshore from Napier (McBride 2016a), using the bioaccumulation approach, indicates a very similar result (0.01 Noroviruses per litre) for health protection.

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[34] In such a case, where the water is absent around low tide, the predicted virus concentration in the shellfish flesh is zero, so some rather arbitrary remedies must be employed.

[35] Future QMRA studies concerning risks associated with discharge of treated wastewater may replace the bioaccumulation model. In doing so some awkward issues to do with time-stepping within Monte-Carlo calculations will need to be resolved (see Appendix E.12).

4.2.8 New median and 90%ile enterococci values for shellfish waters?

If the ratio of enterococci to Norovirus concentrations in sewage translated through the treatment system is typically about 10,000 (as in Table 4-1), the required enterococci concentration in the water is 0.007x10,000 ≈ 70 per L ≈ 7 per 100 mL.

Table 4-1: Typical values of Noroviruses, faecal coliforms and enterococci in sewage (e.g., McBride 2012, Palliser et al. 2013).

Sewage	Average
Norovirus GII ('Norov'), #/L	10^4
Faecal coliforms ('Fc'), #/L	10^8
enterococci ('ent'), #/L	$5x10^7$
ent/Norov	10^4
ent/Fc	0.5

Preserving the 90%ile to median ratio in the current guidelines (43/14 ≈ 3.1), the modified "10% of samples" requirement would be 22 enterococci per 100 mL, (i.e., 7 × 3.1).

As with the current guidelines, these requirements will not apply when there is an illness outbreak in the local community. An example of such a case seems to have occurred in Napier in 2014/15 when Norovirus concentrations of consistently about 10^6 genome copies per litre were record in monthly samples of untreated wastewater (McBride 2016a).

Note that the health risk associated with a median of 7 enterococci per 100 mL is equivalent to that likely to arise following application of the current guideline. That is, from Table 4-1, the ratio of faecal coliforms to enterococci is about 2:1[36] and so this new guideline would correspond to a median of 14 and a sample 10%ile of 44 enterococci per 100 mL (i.e., 22×2).

4.3 Key findings

The current shellfish-gathering component of the guidelines could (not 'should)' be changed to one based on enterococci, with a requirement that the median is less than 7 enterococci per 100 mL and the maximum does not exceed 22 enterococci per 100 mL.

Before such a change is adopted, or before different microbes are adopted for measurement (making use of emerging enumeration techniques such as qPCR, or use of phages), the calculations should be checked in detail to examine model sensitivity. That would include assumed parameter values (e.g., shellfish filtration rate, length of exposure, indicator:pathogen ratios, translation of sewage concentrations to mixed coastal waters, etc.). This should include careful assessment of the implications such approaches may confer.

[36] See MfE/MoH (2003, page H12): "a count of 100 faecal coliforms / 100 mL equates to 42.7 enterococci per 100 mL". Note also that in the USA, the old recreational water criterion for faecal coliforms was 200/100mL as a median (or geometric mean) with a 90%ile of 400/100mL. The new enterococci recreational water criterion is median = 35/100mL with 90%ile of 104/100mL. These ratios are ~6:1 and 4:1.

5 Monitoring faecal indicators near treated wastewater outfalls

As noted in section 1.1, the MfE/MoH (2003) guidelines state that pathogens – rather than indicator bacteria – should be considered for assessing risks to human health at sites near to treated wastewater discharges. This recognises that the ratio between pathogens and indicators encountered in the underpinning epidemiological studies could be quite different from those that may occur at such sites—pathogens may survive wastewater treatment processes better than indicator organisms. However, in the absence of alternative guidance, the guidelines have still often been applied at sites near outfalls. This chapter therefore aims to provide advice on the typical spatial scale at which indicator bacteria guideline use is appropriate and to determine best practise methodology for determining risk within areas influenced by treated wastewater discharges.

Note that 'spatial scale' is somewhat misleading because it depends on local hydrodynamics. If a discharge plume passes along a route the risk may persist for some distance, whereas on the 'upstream' side that may be closer to the outfall the risk may be lower. Understanding local conditions is therefore critical.

5.1 Methods

We use calculated attributable health risk from 20 QMRA studies for marine sites affected by wastewater outfalls, tabled at various resource consent application hearings. The 'inappropriate' boundary (in the guidelines) is taken to be where the maximum attributable risk is 'tolerable' (MfE/MoH 2003, section A1). From the guidelines' Table H1, this maximum risk is 1% for gastrointestinal illness (MfE/MoH 2003, Table H1).

The studies cited in Table 5-1 cover a variety of wastewater treatment efficiencies and disinfection practices, both existing and likely future ones (a range of practices exist, some of which are likely to be more widely adopted in future). Many wastewater treatment systems provide at least secondary treatment, but often lack disinfection. Most resource consents authorise discharges derived following more-than-minimal disinfection, but many existing discharges have yet to go down that route.

5.2 Key findings

Table 5-1 indicates that use of the enterococci thresholds in the existing guidelines to assess health risk is generally appropriate beyond 500 m from a discharge of *treated* wastewater (many with current disinfection or proposed disinfection), beyond which the IIR (Individual Illness Risk) is less than 1%.

QMRA assessments, using chosen pathogens (especially Noroviruses), should continue to be used to predict the effect of treated disinfected wastewater near discharge outfalls, especially when informing an Assessment of Environmental Effects (AEE) to accompany a resource consent application. QMRA can also be used to assess 'aberrant discharges', such as overflows of sewage from wastewater infrastructure (Hudson et al. 2017).

Table 5-1: Details of 20 marine QMRA studies for mean and maximum health risk attributable to discharges of treated wastewater.

Site	City	Pathogen	Distance from outfall (km)	Individual Infection or illness Risk (IIR)		Reference
				Mean%	Max(%)	
Southshore	Christchurch	Adenovirus (GI)	2	0.004	0.02	McBride (2004), Table 1
Distance from diffuser	Gisborne[37]	Adenovirus (GI)	1.6	?	1	Davies-Colley et al. (2005), Table 11b
Wairau Estuary	Blenheim	Rotavirus	~1k, (site EM)	<0.01	1	McBride (2007), Table 5b
Tapu road	Kumeu	Rotavirus, Cryptosporidium	1k	0.023	1	McBride (2008), Table 4
Coast	Timaru	Rotavirus	1 km north and south	0.013	4	McBride (2008), Tables 2b
Cowans Bay	Warkworth	Rotavirus	4 km	0.008	1	Stott and McBride (2008), Table 2
Upstream of outfall	Helensville	Rotavirus	150 m	0.14	5	Palliser and McBride (2009a), Table 2a
South of outfall	Picton	Rotavirus	~1 km	0.14	?	Palliser and McBride (2009b), Table 2a
Ashore from diffuser	Napier	Norovirus	1.5 km	0.4	11	McBride (2011), Table 5 (children)
River	Wairoa	*Campylobacter*	0.5 km	0.823	39	McBride (2011a), Table 4-1[38]
Army Bay	Army Bay	Rotavirus	~1 km	0.005	1	Palliser (2011), Table A-4
Waipawa	Waipukurau/Waipawa	Rotavirus	~1 km	0.405	7	McBride (2011b), Table 4-2
Shoreline	Hokitika	Norovirus, adenovirus	3 km	0.18	8	Stott and McBride (2011), Table 3-2
Many shoreline sites	New Plymouth	Norovirus	1.5 km	0.35	0.35	McBride (2012)
Inshore	Snells/Algies	Rotavirus	0.5 km	0.04	2	Palliser and Pritchard (2012), Table B-4
Inshore	Whareroa	Norovirus	~1 km	0.011	11	Palliser et al. (2014), Table 7-4
Shoreline	Akaroa	Norovirus	1.5 km	0.3	1	McBride (2014)
Clarks Beach	Auckland	Norovirus and enterovirus	<1 km	0.036	0.7	McBride (2016), Tables 3-2 and 3-3
Warkworth	Warkworth	Norovirus and enterovirus	300 m	0.063	0.07	McBride and Hudson (2016), Table 3-9 (for child exposure to Noroviruses)
Bell Island	Nelson	Norovirus and enterovirus	300 m	<0.1	<0.1	McBride (2017)

[37] Adenovirus gastrointestinal risk calculated for July conditions with improved diffuser design. Adenovirus was the only virus assessed: maximum = 2 per litre. So, maximum ingested dose, given an ingested volume of 100 mL, is 0.2 viruses, of which 10% are respiratory. Maximum infection risk is $1 - e^{-rd}$, where r = effective dose = 0.02 viruses and $d \approx 0.41$ is the specific infection coefficient (McBride 2005). So, risk = 0.8%.

[38] Poorly treated meatworks effluent.

6 Brackish water indicators

This chapter provides advice regarding selection of the appropriate indicator/s to use in brackish (estuarine) water bodies, where salinity can range typically from 0.5 to 30 ppt. As noted in section 1.1, the MfE/MoH (2003) guidelines do not provide any commentary on this and often two, or sometimes even all three bacterial faecal indicators – faecal coliforms, *E. coli* and enterococci – are measured. This imposes costs on councils that may not be necessary and leads to confusion over which indicator measurement should inform health risk assessments.

6.1 Approach

We consider time and longitudinal variation of *E. coli* and enterococci down an idealised estuary, to examine the point at which the advantage of one microbe outweighs the other in terms of indicated illness risk. A simplified modelling approach is adopted, as described in Appendix F.[39] This conceptualises estuarine faecal contamination as a longitudinal cross-section average system, giving rise to a one-dimensional time-dependent model. The model has two boundary conditions:

(i) a specified concentration of *E. coli* for ebb tide at the model's upstream (river) boundary, and

(ii) a specified concentration of enterococci for flood tide at the estuary mouth.

Even a simplified model such as this is conceptually demanding, so we further simplify it to predict concentrations at mid-ebb and mid-flood tides, also explained in Appendix F. It is implemented in Microsoft Excel.[40]

6.2 Persistence of faecal indictors in brackish water

The model is driven fundamentally by four known features of inactivation of enterococci and *E. coli* as a function of salinity:[41]

a) Enterococci inactivation is independent of salinity but dependent on time (because UV from sunlight varies with time).
b) *E. coli* inactivation is dependent on time and salinity.
c) For freshwater at any given time, *E. coli* inactivation is less than the enterococci value.
d) The *E. coli* inactivation coefficient for coastal water is a constant multiple of the enterococci value at the same time.

Consequently, salinity cannot be used as a proxy to determine which indicator is appropriate—in brackish water, a particular value of salinity can arise from short-term or long-term residence time in an estuary or harbour, during which times different inactivation rates apply. The choice of faecal indicator bacterium is therefore dependent on estuary residence time and tidal state.

[39] This is a case where a simple model is likely to give more generally-applicable results cf. more elaborate and computationally demanding complex models. The former covers a wide range of possible scenarios in one sweep while the latter models each scenario in turn, each scenario being more demanding then the simple model.

[40] All Excel worksheets are written with named cells to facilitate clarity of understanding.

[41] The inactivation dependence on salinity (for *E. coli*), and the lack thereof for enterococci, has been observed experimentally: Davies-Colley et al. (1994), Sinton et al. (1994, 2002), Nelson et al. (2018). The driving mechanisms have yet to be completely understood.

6.3 Results

6.3.1 Long estuary residence time

In estuaries with long residence time (say, greater than 3 days),[42] we can expect that enterococci should be more appropriate than *E. coli* as pathogen indicators. In this case, the faecal indicators will have been in contact with brackish waters for some time, during which differential inactivation occurs, and we know that *E. coli* are inactivated more readily in coastal waters than enterococci.

6.3.2 Short estuary residence time

The estuary's compliance status is assumed to be completely marginal, in that for all tidal states the concentrations of *E. coli* in the inflowing river water is exactly at the median threshold in the 1992 provisional guidelines (DoH 1992)—126 per 100 mL. Likewise, the enterococci concentration at the mouth on the flood tide is constant—33 per 100 mL. The concentration on the ebb tide at the mouth is not specified, it is calculated by the model.

Perusal of these values shows that the choice of indicator is strongly distance-dependent (as are the indicator concentrations and risk), in contrast to long residence time estuaries in which the choice is driven by in-estuary contact time and so strongly favours enterococci.

6.4 Key findings

From these results we infer that:

- For long residence-time estuaries (greater than three days), use enterococci.
- For short residence time estuaries, *E. coli* is the appropriate choice when near the inflowing river water, but enterococci should be chosen near the mouth. Between these locations, either indicator may suffice. Accordingly, it appears wise to measure both indicators in low residence time systems.
- In surveillance mode, conflicting results may arise on occasion (e.g., enterococci above and *E. coli* below respective alert levels, and vice versa). In these cases, the more severe indicator result should be taken. One can expect that most of such cases will result in *E. coli* determining the Action mode, because any 'red' enterococci result would need to be confirmed by a repeat sample taken in the next day or so.

[42] This is 'Best Professional Judgement'. Various modelling exercises could be mounted to examine this estimate (i.e., 3 days), but the findings will be subject to particular assumptions, and so are unlikely to be of much assistance.

7 Conclusions

Appropriateness of the current enterococci values

Surveillance thresholds

In general, the MfE/MoH (2003) guidelines' surveillance requirements for marine waters should be retained, including resampling on consecutive days once the Action mode has been exceeded. That requirement is important where sanitary surveys have indicated the possibility of ongoing contamination, such as can arise from leaking sewerage. Some modifications are desirable:

(i) If logistical problems make sampling on consecutive days difficult, the repeat sample could be taken two days later.

(ii) Consideration should be given to omitting the repeat-sample requirement for waters that have been graded as low quality (e.g., 'poor' or 'very poor').

(iii) Because sample results are not available for at least one day, where predictors of faecal contamination are known, it would be preferable to develop and use real time models to indicate swimmability, based on local models and/or a semi-quantitative scoring approach (such as Auckland's SafeSwim package). These models could use swimmability surrogates, such as salinity or turbidity, provided a robust relationship can be demonstrated with indicator bacteria.

(iv) Improved advice on the conduct of sanitary surveys would be helpful.

Grading of recreation sites

The grading component of the guidelines seem not to merit change at this time. However, a watching brief should be kept on recent developments, particularly in the USA, using:

(i) pathogens;

(ii) pathogen surrogates (e.g., coliphages); and

(iii) more modern and epidemiologically-appropriate faecal indicator laboratory methods (especially qPCR).

The US EPA approach (that uses the same numeric thresholds for freshwater and coastal water), should be kept under review—but in conjunction with a review of the freshwater component of the New Zealand guidelines.

Recreational shellfish-gathering waters

Consideration could be given to revising the "shellfish-gathering season"-based approach in current use to one that recognises local practice, where gathering may occur over a longer "season" or even year-round.

Following use of two shellfish accumulation models, we found that the current shellfish-gathering component of the guidelines *could* be changed to one based on enterococci, with a requirement that the median is less than 7 enterococci per 100 mL and the maximum does not exceed 22 enterococci per 100 mL. Before such a change is considered, the calculations should be checked in detail to examine model sensitivity. The implications of any guideline change also need careful consideration.

Indicators near treated wastewater outfalls

Sampling for enterococci is generally appropriate beyond 500 m from a discharge of *treated* wastewater discharge, beyond which the individual illness risk is less than 1%. However, this scale will depend on local hydrodynamics. If a discharge plume passes along a route the risk may persist for some distance, whereas on the 'upstream' side that may be closer to the outfall, the risk may be lower. It would be unfortunate if these scales were applied in the absence of consideration of local conditions, especially those that may be identified when conducting sanitary surveys to inform a Sanitary Inspection Category (SIC) determination.

Quantitative Microbial Risk Assessments using chosen pathogens (especially Noroviruses for waters impacted by treated wastewater) should continue to be used to assess the potential health effects for users of recreational water near outfalls of treated disinfected wastewater—especially when informing an AEE.

Brackish water indicators

For brackish waters, the choice of faecal indicator is complicated. A simple model based on the understanding that the choice is determined by the residence time of the brackish water body, rather than the water's salinity, concluded that for long residence-time estuaries (greater than three days), enterococci should be chosen. For short residence-time estuaries, *E. coli* is the appropriate choice when near the inflowing river water, but enterococci should be chosen near the mouth. Between these locations, either indicator may suffice. Accordingly, it appears wise to measure both indicators in low residence time systems and use the more severe indicator result for surveillance determination.

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9 Glossary of abbreviations and terms

Aetiological agent	Microorganisms and microbial toxins that cause disease in humans.
AGI	Acute Gastrointestinal Illness.
AFGI	Acute Febrile Respiratory Illness.
BAF	Bio-accumulation factor
Beta-Poisson dose-response curve	A mathematically-derived infection approximate dose-response curve for variable infectivity, in which only mean doses are known.
Conditional illness probability	The probability of illness at a given dose given that infection has already occurred.
Conditional infection dose-response models	The (simpler) mathematical form of a dose-response equation that results when individual doses are known. (More complicated mathematical functions arise when individual doses are not known).
EPEC	Enteropathogenic *E. coli.*
ETEC	Enterotoxigenic *E. coli.*
GI	Gastrointestinal illness
GM	Geometric mean
HCGI	Highly Credible Gastrointestinal Illness.
Hypergeometric functions	Exact mathematical equations that defy simple calculation, yet are important in the analysis of clinical trial data and outbreak data for the infection response of a population exposed to a pathogen, and where individual doses are randomly distributed about a known mean value.
Illness ID_{50}	The dose required to cause illness in 50% of an exposed population, who are already infected.
Infection ID_{50}	The dose required to cause infection in 50% of an exposed population.
IIR	Individual's Illness Risk: The illness risk faced by a random person using the receiving waters on a random day, with no foreknowledge of microbial conditions.
MAC	Microbiological Assessment Category, used in the 2003 guidelines.
PCR	Polymerase Chain Reaction, a molecular technique for virus enumeration using DNA segment matching.
QMRA	Quantitative Microbial Risk Assessment.
qPCR	Quantitative PCR, a laboratory test (for enterococci) targeting particular genetic sequences.
SIC	Sanitary Inspection Category, used in the 2003 guidelines.
Simple binomial dose-response curve	A mathematically-derived infection dose-response curve for constant infectivity, in which individual doses are known.

Simple exponential dose-response curve	A mathematically-derived infection dose-response curve for constant infectivity, in which only mean doses are known.
SFRG	Suitability for Recreation Grade, used in the 2003 guidelines.
STEC/VTEC	Median Tissue Culture Infectious Dose: A laboratory culture technique measuring the amount of virus that produces a cytopathic effect in 50% of cell cultures inoculated.
STV	Statistical Threshold Value (used in the USEPA 2012 criteria)
$TCID_{50}$	Median Tissue Culture Infectious Dose: A laboratory culture technique measuring the amount of virus that produces a cytopathic effect in 50% of cell cultures inoculated.
Top hat	An artificial concentration distribution over time being zero at all times except for a limited period when a constant concentration occurs (used in testing the shellfish model's plausibility).
Virion	Shorthand for 'virus particle'.
VTEC/STEC	Verocytotoxin *E. coli.*

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Appendix A Detailed observations from the literature review

A.1 Overall

- Associations between gastrointestinal illness and both traditional and rapid methods for *Enterococcus* have been observed from epidemiological studies at marine beaches (Colford et al. 2012), and adopted in the USEPA's 'Water Quality Criteria 2012' (USEPA 2012).
- Epidemiological studies show that children appear to be at higher risk (cf. adults) when swimming/playing in water (Sanborn & Takoro 2013, de Man et al. 2014, Arnold et al. 2016).
- Risks associated with mixed sources are driven predominantly by the proportion of the contamination source with the greatest ability to cause human infection (potency), which is not necessarily the greatest source(s) of FIB (faecal indicator bacteria, Schoen & Ashbolt 2010, Soller et al. 2014).
- Norovirus is likely to be the most important pathogen for humans recreating in waters affected by discharges of treated sewage (Soller et al. 2010b).
- Many epidemiological studies report an increase in health risk for the exposed (e.g., swimmers) versus the non-exposed, but are reported as failing to find a relationship between some water quality variable and health risk.
- Epidemiological studies show a generally elevated risk of gastrointestinal illness in bathers compared to non-bathers but often no clear association with water quality as measured by faecal indicator bacteria; this is especially true where study sites are impacted by non-point source pollution (Fewtrell & Kay 2015).
- However, failure to establish such an association reflects the limited size of many studies; when 'large' datasets are gathered the association is revealed (Wade et al. 2010, Wyer et al. 2013).
- In contrast, QMRA models are built on pathogen dose-response curves which, in general, exhibit a monotonic increase in risk of infection or illness with increasing dose—so association of health risk and water quality is always evident from QMRA studies.
- Future studies should consider including more emerging pathogens, especially anti-biotic resistant pathogens (Leonard et al. 2015, Young 2016).
- Epidemiological studies and QMRA are quite different approaches to the task of setting health-related water quality guidelines, yet they are often complementary. In particular, their relative strengths vary from one location to another. For example, epidemiological studies capture the actual water ingestion or inhalation volumes during exposure events at its site(s), whereas QMRA has to estimate those volumes. On the other hand, epidemiological studies are restricted to the location(s) and times at which they were carried out, whereas QMRA can be applied to many other situations for which studies are scarce (such as locations not impacted by human wastes).
- Epidemiological studies can gain an extra advantage by including in their monitoring a selection of faecal indicators *and* pathogens, as do some of the reviewed documents. This includes different ways of measuring a given indicator, e.g., enterococci by culture and by PCR. In contrast, including several pathogens in a QMRA is relatively straightforward and less expensive than in epidemiological studies.

- It should always be recognised that environmental waters can contain a mix of pathogens, only some of which may be analysed in a given study. So, the calculated risk for those for selected pathogens may not capture the water's overall pathogenicity.
- Coupling QMRA with an epidemiological study at a single study site provides a unique ability to understand human health risk and illnesses, especially under conditions where water quality, as measured by traditional indicators, is good and/or average illness rates are lower than can be quantified via epidemiological methods (Soller et al. 2016).
- Dose-response models for Norovirus, as used in a number of QMRA models,[46] are remarkably dependent on the degree of aggregation of virions present in the low concentrations typical in environmental waters, a matter of some controversy.[47] For example, without loss of generality, take a simplified case where 10 people each ingest 100 mL of water from a container in which there are 10 Norovirus particles. If these pathogens are aggregated into one clump of ten, then only one of the ten people can be affected; the other nine are exposed to 'no dose'. At the other extreme, up to all ten people could be affected if there is no aggregation—each particle is independent of the other. Many, if not all, of the ten subjects then receives a 'low dose', thus increasing the average risk faced by these nine people. Studies of the dynamics and effects of aggregation phenomena on predicted health risk are therefore warranted (Soller et al. 2017 is an excellent start).

A.2 Effect on children's health

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

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

Regarding a), the innovative swimming pool studies reported by Dufour et al. (2006, 2017) show that children may ingest water at rates up to four times greater than adult rates. Increasingly, such data are being included in QMRA models. On the other hand, in some settings children may ingest at a lower rate (but still more than adults), depending on their swimming behaviour (Suppes et al. 2014). The choice of exposure data, particularly in terms of duration, has a substantial effect on risk predicted by QMRA.

Regarding b), it is commonly held in health risk modelling that children are born with inherent susceptibility that reduces over time as some immunity is developed and maintained (Pond 2005). Studies reported herein do not take explicit account of this aspect, yet it seems highly desirable to do so, especially as children appear to be the most at-risk group.

In reviewing many epidemiological studies, Prüss (1998) suggested that symptom rates were higher in lower age groups. The UK studies may therefore systematically underestimate risks to children.

A.3 New information on health and enterococci

- The faecal indicator bacterium *Enterococcus* spp., estimated by qPCR,[48] is well-associated with gastrointestinal illness (GI) among swimmers (Wade et al. 2010, Wyer et al. 2013).

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

[47] Teunis et al. (2008), Atmar et al. (2011, 2014), Messner et al. (2014), Schmidt (2014), McBride (2014b), van Abel (2016).

[48] qPCR is quantitative polymerase chain reaction, a DNA based genetic technique that does not include culturing.

- Statistically significant trends of increasing proportions of HAdV-positive (human adenoviruses) results in categories of increasing faecal indicator concentration were found in freshwater but not seawater samples (Wyer 2013).[49]

- Performance of a calibrated qPCR total enterococci indicator was compared to a culture-based assay to index infectious human enteric viruses released in treated human wastewater. Results illustrate that the pathogen source contributing the majority of risk in a mixture may be overlooked (when only assessing faecal indicators using a culture-based method (Schoen et al. 2011).

- At a beach with no known point sources (e.g., discharge of treated sewage), a dose-response relationship was observed between skin infections and enterococci enumerated using membrane filtration methods. No other significant dose-response relationships between reports of human illness and any of the other FIB or environmental measures were observed (Sinigalliano et al. 2010).

- Yau et al. (2014) noted that associations between GI illness incidence and FIB levels (*Enterococcus* EPA Method 1600) among swimmers who swallowed water were not significant when not accounting for submarine groundwater discharge, but were strongly associated when submarine groundwater discharge was high compared to when it was low.

These observations can be interpreted to imply that there are insufficient grounds to recommend abandoning culture-assayed enterococci, although there is a case for also monitoring enterococci assayed by qPCR.

A.4 Microbial source tracking

- Eventually, MST (Microbial Source Tracking) markers may support source apportionment as well as risk assessment, given additional epidemiological data and/or empirical descriptions of the pathogen-*Bacteroidales* relationships (Bambic et al. 2015).

- When the level of CAT (*Catellicoccus marimammalium*, a gull faeces marker) exceeds 7×10^3 copies/100 mL of water, the median predicted illness rate exceeds 3 illnesses/100 swimmers (Brown et al. 2017).[50]

- Kirs et al. (2016) argue for the inclusion of HF183 Taqman human faecal marker in future epidemiological studies (also being used in California).

- Nnane et al. (2011) report a 'very small' correlation coefficient between presumptive *E. coli* and phages of *Bacteroides* (GB-124).

- Viau et al. (2011) report that GI illness risks from viral exposures were generally orders of magnitude greater than bacterial exposures in Hawaiian waters impacted by stream discharges. The median risk associated with each stream was positively correlated with the concentration of *Clostridium perfringens* in the stream water.

- *Bacteroides* bacteriophages were considered potential markers of human sewage because they also survived for three days in fresh stream water and two days in marine water (Vijayavel et al. 2010).

[49] Once again 'not found' is unfortunate language; were the study to have been 'big enough' something would have been 'found'.
[50] The published paper has this limit as 4×10^6, but that is in error. See the reference section for details.

These observations show that development and use of alternative faecal indicators is a rich and evolving field. For example, the USEPA continues to pursue use of coliphages for human impacted waters. The main driver behind this is a sense that faecal indicator bacteria are not doing an adequate job and an indicator that more closely mimics human viruses would be better. Given that, it seems premature to promote their selection and use in revised guidelines.

A.5 Wet/dry weather

- Fecal indicator bacteria measured in seawater (Enterococcus species, faecal coliforms, total coliforms) were strongly associated with incident illness only during wet weather. Urban coastal seawater exposure increases the incidence rates of many acute illnesses among Californian surfers, with higher incidence rates after rainstorms (Arnold et al. 2017).
- Swimming in natural swim environments and in pools following a recent faecal contamination event pose significant public health risks (Pintar 2010).
- Wet weather conditions contribute to elevated pathogen loads in the Chicago Waterways System (CAWS) to such an extent that disinfecting the effluents of three major WRPs that discharge to the CAWS would negligibly reduce the aggregate recreation season risk to incidental contact recreators (Rijal et al. 2011).
- Dry-weather risk estimates were found to be significantly lower than those predicted for wet-weather conditions (Sunger et al. 2016).

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

A.6 Outbreaks

- An outbreak among white-water rafters provides evidence of the changing epidemiology of leptospirosis and suggests consideration of a wider range of risk exposures, including those related to recreational activities of more affluent urban populations, in addition to the well-recognized occupational hazards of rural farming (Agampodi et al. 2014).
- The infection risks resulting from swimming in Belgian waters were above 50% for several days in waters near an accidental spillage of laboratory-cultured wild poliovirus type 3 from a vaccine production plant (Duizer et al. 2016).
- Approximately 5,700 outbreak-related cases were identified across the state of Utah in 2007. Of 1,506 interviewed patients with laboratory-confirmed cryptosporidiosis, 1,209 (80%) reported swimming in at least one of approximately 450 recreational water venues during their potential 14-day incubation period (Edwards et al. 2012).

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

A.7 Miscellaneous

- Activities that cause disturbance of sediments lead to elevated risk of infection to users of the river (South Africa, Abia et al. 2016).

- QMRA results reported by Corsi et al. (2016) highlight the importance of investigating multiple pathogens within multiple categories to avoid underestimating the prevalence and risk of waterborne pathogens.
- Potential EPEC strains were readily isolated from contaminated marine recreational water and may represent a public health risk to swimmers and beach users. The frequency of detection of potential EPEC strains varied considerably by sample. Neither STEC nor ETEC strains were detected (Hamilton et al. 2010).[51]
- During a seven-year period, illness outbreaks reported to the Australian OzFoodNet, were predominantly classified as being transmitted person-to-person or from an unknown source. Fifty-four (0.83%) outbreaks were classified as either 'waterborne' or 'suspected waterborne', of which 78% (42/54) were attributed to recreational water and 19% (10/54) to drinking water (Dale et al. 2010).

[51] EPEC is Enteropathogenic *E. coli*; ETEC is Enterotoxigenic *E. coli*; STEC is Verocytotoxin *E. coli*.

Appendix B Epidemiological and QMRA studies

B.1 Epidemiological study designs

Five types of epidemiological studies have been used to develop enterococci guidelines and standards internationally: two are *retrospective* and three are *prospective*, as shown on Table B-1.

Table B-1: Five types of epidemiological studies to develop microbiological guidelines and standards.

Type	Important examples
Retrospective	
1 Case-control—matching historical reported cases with similar individuals who did not report illness.	PHLS (1959), Zeigler et al. (2014).
2 Follow-up—comparison of public records of illness with likely environmental conditions	Ferley et al. (1989), Harrington et al. (1993), Edwards et al. (2012), Hall et al. (2017), Reddy et al. (2011), Schets et al. (2011a&b), Harder-Lauridsen et al. (2013), Zlot et al. (2015).
Prospective	
3 Uncontrolled cohort, multiple exposures—enrolment of individual water users who have already decided to attend	Stevenson (1953), Calderon et al. (1991), Soller et al. (2016).
4 Uncontrolled cohort, single exposure—restricting the enrolled to those who swam once in a given period (typically one week)	Cabelli (1983), Dufour (1984), Wade et al. (2006), Colford et al. (2007), Marion et al. (2010 & 2014), Papastergiou et al. (2011), Dorevich et al. (2012), Arnold et al. (2013), Wade et al. (2013a&b), Yau et al. (2014), Lampareilli et al. (2015), Griffith et al. (2016).
5 Controlled cohort, single exposure—deliberate enrolment of individuals who had not proposed to swim.	Kay et al. (1994), Fleisher et al. (1996), Sánchez-Nazario et al. (2014), Sinigalliano et al. (2010).

Adapted from McBride (2007).

'Follow-up' (retrospective) and 'Uncontrolled cohort' (prospective) studies have been the more common approaches, but the current guidelines rest on the 'controlled' prospective design. A classification of approaches is therefore warranted, as given in the Table.

These approaches are based on the well-established international understanding that a group swimming in waters containing faecal residues—from animal or human sources—in general face a higher health risk, compared to a similar group who don't swim (as reviewed by Prüss 1998). This enhanced risk is often found to be correlated with enterococci concentrations in those waters.

'Prospective controlled cohort' studies have been endorsed by the World Health Organization (WHO 1993). The relevant studies for the WHO guidelines were carried out for a set of four widely-separated beaches in the United Kingdom, from 1989 to 1992.[52] They involved recruiting eligible volunteers (having passed screening tests) and taking them to a selected beach on a given day. Participants were given the same food on the day. Some were directed to swim at a particular depth for at least 10 minutes, during which time they were to fully immerse their head at least three times. Others did not swim. The health status of each

[52] The four beaches were: (i) Langland Bay, near Swansea (120 bathers, 133 non-bathers), (ii) Moreton Beach near Wallasey on the north bank of the Wirral (101 bathers, 164 non-bathers), (iii) Southsea (172 bathers, 186 non-bathers) and (iv) Southend-on-Sea (155 bathers, 185 non-bathers). Mean bather age was 31.65 years versus 32.12 years for non-bathers. Fifty four percent of bathers were male while 46.5% were female. Beaches were selected to be a substantial distance apart, to maintain independence of beach conditions and cohorts of swimmers (Fleisher et al. 1996).

survey participant was subsequently assessed 7 and 21 days after attending the beach. Illness duration (for those who became ill) ranged from 4 to 8 days (Fleisher et al. 1998). The controlled cohort studies have tended to produce higher risks than uncontrolled prospective studies (Prüss 1998).

The second (more common) approach is the 'uncontrolled prospective' design, in which people having already decided to attend a beach and do as they want while there, are interviewed at the beach (if willing) and subsequently followed up to assess any illness episodes. Prior to 2002, this type of study formed the basis of New Zealand's microbiological recreational water quality guidelines. These were based on large epidemiological studies conducted at three coastal sites (Cabelli 1983, Cabelli et al. 1982 & 1983) and two inland lake sites (Dufour 1984), and were also influenced by results from a New Zealand study (Till et al. 2008). The USEPA 'NEEAR' studies underpinning that country's criteria (USEPA 2012) also follow this design as do the series of studies conducted in Southern California.[53]

The relative merits of epidemiological and QMRA approaches are given on Table B-2.

Table B-2: Comparing epidemiological and QMRA approaches for setting guidelines and standards.

Issue	Epidemiological studies	QMRA
Represents the whole community and their swimming patterns?	Yes. But only in uncontrolled studies.	No. Dose-response data are only available for healthy adults.
Minimises classification bias (Swimmer/non-swimmer)?	Yes. But only in controlled randomised trials. (In uncontrolled trials, non-swimmers tend to be less fit and older.)	Yes.
Reflects all pathogens present?	Yes. Swimmers are exposed to pathogens actually present.	No. Includes only the pathogens selected in the hazard assessment and dose-response steps. May ignore stirring of sediments (which may elevate concentrations of some pathogens in the water column).
Reflects all the strains of a pathogen?	Yes, because swimmers are exposed to the pathogens actually present.	No. Restricted to the few strains for which data are available[54] (usually a single strain).[55]
Reflects the whole range of exposures?	No. Restricted to the conditions present on the epidemiological survey.	Yes. Allows virtually unlimited exposure events. Can assist in interpretation of results of epidemiological studies.
Detects infection?	No.	Yes (calculates its prevalence).
Detects illness?	Yes.	Yes, but the translation from infection to illness may not be straightforward.[56]

Source: McBride (2007).

[53] http://sccwrp.org/Homepage.aspx

[54] These are: rotavirus, hepatitis virus, adenovirus 4, echovirus 12, coxsackie virus, *Salmonella*, *Shigella*, VTEC/STEC, *Campylobacter jejuni*, *Vibrio cholera*, *Entamoeba coli*, *Cryptosporidium (parvum and hominis), Giardia Lamblia*.

[55] *Cryptosporidium* is an exception, as there are now five *C. parvum*, trial results and another for *C. hominis*.

[56] In clinical trials, examples can be found for three possible alternatives: an increase in illness probability with increasing dose (e.g., salmonellosis), a decrease with higher doses (campylobacteriosis), and an illness probability (given infection) independent of the received dose. These alternatives may reflect different modes of interaction between pathogens and oocysts (Teunis et al. 1999). However, reconsideration using new data casts doubt on the pattern previously observed for campylobacteriosis, particularly for children (Teunis et al. 2005).

There has been considerable activity in the last ten years to see whether new laboratory microbiological tests could be used to complement (or even replace) enterococci as the appropriate microbiological water quality variable.[57]

B.2 Quantitative Microbial Risk Assessment (QMRA)

QMRA consists of four steps

1. Select the target pathogen (or pathogens).
2. Quantify pathogen exposure.
3. Select appropriate pathogen dose-response.
4. Quantify human health risk.

Computational details of the risk calculation are summarised by footnote[58] and need no further explanation. Discussion of steps 2 and 3 is warranted However.

Most importantly, three issues must be addressed when performing a QMRA.

- The selection of endpoint of the QMRA (infection versus illness) is more than a technical issue; wider concerns come into play (noting that infection rates are always at least as large as illness rates). The current freshwater guidelines (MfE/MoH 2003) use the infection endpoint, whereas their marine water component necessarily use illness [because they are based on epidemiological studies in which infection of swimmers is not (cannot be) measured].
- Dose-response functions inferred from clinical trials concern two probabilities: for infection, and then for illness (given that infection has already occurred). For many pathogens, there is more than one choice for these functions. All must be considered [many papers ignore these choices, not even mentioning the existence of the other(s)]. The rationale for the choice(s) made should be given.
- Understanding the choice of dose-response curves for nominated pathogens is often too casual: 'here's the formula, use it'. That's a trap for the unwary and ill-informed.

B.3 When infection data are not available

This often occurs when dose-response relationships are derived either from epidemiological studies or from outbreak studies. In such cases, doses cannot always be measured: (i) to do so in epidemiological studies is generally logistically impossible, (ii) by the time an outbreak occurs the contamination has probably abated.[59] Therefore, illness probabilities are inferred directly from these studies.

An exception: Milwaukee cryptosporidiosis outbreak (1993), when ice produced ten days before the outbreak arose was found to contain oocysts of *Cryptosporidium*.[60] The time-of-onset of these oocysts is on the order of ten days.

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57 For example, Bacteroidales HF183 as an indicator of human faecal material (Boehm et al. 2015) and CAT (*Catellicoccus marimammalium*) as an indicator of gulls' faeces (Brown et al. 2017).

58 Risk calculation demands an iterative Monte Carlo modelling, as explained by Haas et al. (1999) and McBride (2005), e.g., using the '@RISK' Excel plug-in (Palisade Corporation (2013).

59 Thebault et al. (2013) is an exception, where there was ongoing assessment of Norovirus contamination in coastal shellfish during Norovirus illness outbreaks.

60 http://www.nejm.org/doi/full/10.1056/NEJM199407213310304#t=article

Appendix C Exposure: Adults Versus Children

Swimmers' ingestion rates for children can differ markedly from adult rates, as summarised in ESR (2016). Also, surfers can ingest a greater volume than adult swimmers on any given exposure occasion (Tseng and Jiang 2012). QMRA exercises in New Zealand (more than 20) have therefore been based on child (not adult) swimmer's ingestion rate, assumed to be also appropriate to surfers' ingestion rates.[61]

C.1 Adults

Water ingestion rates by swimmers—a key component of dose-calculation, along with the pathogen concentration in that water—have been studied using novel biochemical procedures (Dufour et al. 2006, 2017) who report results of a clinical trial observing 53 volunteers involved in recreational swimming in an outdoor community swimming pool. Swimmers were assumed to ingest similar amounts of water during swimming in pools or in freshwater due to similar behaviours in each (frequently immersing their heads under the surface and remaining in the water for long periods of time). Cyanuric acid was used to trace water ingestion because it is present in outdoor swimming pools (as a decomposition product of chlorine-stabilising chloroisocyanurate) and passes through the human body unmetabolised. For each swimmer, the volume of water ingested during active swimming events lasting at least 45 minutes was calculated. It has become standard practice to apply these ingestion rates to coastal water recreation.[62]

C.2 Children

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

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

Regarding a), the innovative swimming pool studies reported by Dufour et al. (2006, 2017) show that children may ingest water at rates four times greater than adult rates. Increasingly, such data are being included in QMRA models. On the other hand, in some settings children may ingest at a lower rate (but still more than adults), depending on their swimming behaviour (Suppes et al. 2014). The choice of exposure data, particularly in terms of duration, has a substantial effect on risk predicted by QMRA.

Regarding b), it is commonly held in health risk modelling that children are born with inherent susceptibility that reduces over time as some immunity is developed and maintained (Pond 2005). Most studies reported herein do not take explicit account of this aspect, yet it seems highly desirable to do so, especially as children appear to be the most at-risk group.

[61] For example, Hokitika (Stott and McBride 2011), Napier (McBride 2011, 2016a), New Plymouth (McBride 2012), Akaroa (McBride 2014c), Motueka (McBride 2014d), South Manukau (McBride 2016b), Omaha (Stott and McBride 2016), Nelson (Hudson and McBride 2017, McBride 2017).

[62] Personal communication: Jeff Soller, Soller Environmental, California, USA (http://www.sollerenvironmental.com/env/main/Home.html).

Appendix D Bioaccumulation shellfish model

At the heart of this method is use of a (dimensionless) bioaccumulation factor (Burkhardt and Calci 2000), denoted by 'BAF'. The pathogen dose ingested from consumption of a given mass of shellfish is taken as BAF × the number of pathogens present in the equivalent volume of seawater: for example, the virus dose ingested from consumption of 100 g of shellfish is taken as BAF × the concentration of viruses expressed as number per 100 mL of seawater (McBride 2005, Table 9.1). Note that this assumes that this concentration has persisted long enough for that degree of accumulation to occur. It also needs data on shellfish meal size and (as above) virus concentrations in the overlying water. Therefore, we have

$$d = \frac{mbc}{V} \qquad (1)$$

where d is dose (#),[63] m is the meal size at a single sitting (g), b is the BAF (dimensionless), c is the virus concentration in the water (#/L) V (= 1,000 g/L) is needed to cancel units.

Note also that this model does not incorporate any numerical risk thresholds. Rather, it has been used in QMRA studies to calculate virus doses received by shellfish consumers and thence calculate human health risk, via a dose-response model.

[63] '#' denotes the number of viruses ingested, i.e., dose.

Appendix E Development of the uptake-and-depuration shellfish model

This appendix describes the development of a time-based uptake and depuration model.

E.1 Background

At present, the pathogen concentration in shellfish flesh is estimated directly from the surrounding water using a simple bioaccumulation factor approach (McBride, 2005) which, nonetheless, accounts for variable concentration of microbes in the overlying water. Available bioaccumulation data, however, are largely based on laboratory studies where shellfish are exposed to constant concentrations of pathogens over several days. Results drawn from these studies may not adequately reflect exposures to wastewater discharges which are likely to result in seawater concentrations with considerable temporal variability (McBride, 2007b). That variability arises because wastewater virus concentrations can vary over orders-of-magnitude (Hewitt et al. 2011) and also because of shifting patterns of coastal currents. Furthermore, the approach results in an *instantaneous* response in shellfish flesh to changes in the seawater concentration, neglecting the *gradual* processes of uptake and depuration. Consequently, flesh concentrations estimated using bioaccumulation factors may be systematically overestimated during uptake and underestimated during depuration.

Here we develop a mathematical model to reflect pathogen contamination in shellfish flesh in order to better understand the response to time-varying seawater concentrations. Ultimately, it is intended that the model will be used to inform the QMRA process, strengthening the basis on which health risk profiles for exposures due to consumption of raw shellfish are produced. At this stage, development of the model is focussed on Norovirus contamination in oysters, for which there is rather more information cf. mussels, cockles, etc. On the basis of current evidence (Greening & McCoubrey, 2010; Bellou et al. 2013), this combination reflects the greatest health risk to consumers of raw shellfish from areas potentially impacted by wastewater discharges. In addition, bioaccumulation data is available for Noroviruses in oysters (e.g., Greening et al. 2003; Le Guyader et al. 2006; McLeod et al. 2009a, Maalouf et al. 2011; Ventrone et al. 2013), whereas little information is available on Norovirus accumulation in other shellfish species.

E.2 Bioaccumulation factor

Published data with which to quantify bioaccumulation factors for viruses in shellfish is limited, and suggests a wide range of uncertainty between both viruses and shellfish species (Ball et al. 2008). Some of this uncertainty is accounted for in the QMRA Monte Carlo simulation by representing the bioaccumulation factor using a probability distribution.

The current set of values used to describe this distribution are based on experiments by Burkhardt & Calci (2000), who investigated F-RNA coliphages in Eastern oysters (*Crassostrea virginica*) over 12 consecutive months. Bioaccumulation rates were found to vary between 1 and 99 mL g^{-1}, with an overall average of 19 mL g^{-1}. From late autumn through to the end of winter however, bioaccumulation rates were observed to be significantly higher than at any other time of the year, with an average of 49.9 mL g^{-1}. This higher average value represents hyperaccumulation; defined as a period where the mean bioaccumulation rate is greater than one standard deviation above the mean for the entire dataset. Adopting these results, the bioaccumulation factor is described by a normal distribution truncated at 1 and 100 mL g^{-1}, with mean 49.9 and standard deviation 20.9 (McBride, 2005). Adopting the higher mean value (reflecting hyperaccumulation) represents a precautionary approach which is deemed appropriate in a risk management setting.

E.3 Literature review

A systematic review of shellfish-borne viral outbreaks worldwide (Bellou et al. 2013) identified Norovirus as the most commonly involved viral pathogen in reported outbreaks (83.7%), followed by hepatitis A virus (12.8%). The most frequently consumed shellfish in reported outbreaks was oysters (58.4%). A review of shellfish-associated Norovirus outbreaks in New Zealand (Greening & McCoubrey, 2010) also identified oysters (mainly Pacific oysters) as the shellfish species implicated in the majority of cases. Mussels and scallops have not been identified as causing Norovirus outbreaks; probably because of a lower contamination risk in these species due their occurrence mainly in deeper waters (Greening et al. 2009). On the basis of these studies, Norovirus contamination in oysters has been selected as the basis for model development. In addition to this combination reflecting the highest risk profile in shellfish-borne viral outbreaks, there is a limited amount of bioaccumulation data available to support model development, whereas information on Norovirus contamination in other shellfish species is almost non-existent. The following paragraphs summarize key aspects of the literature relating to model development, including the bio-kinetics of Noroviruses in oysters, information on uptake and depuration rates, and existing models.

E.4 Noroviruses

Noroviruses belong to the *Caliciviridae* family, a group of non-enveloped, icosahedral viruses with a single-stranded positive sense RNA genome. These viruses are highly diverse and are classified according to five genogroups, each of which is further divided into a number of genotypes (Atmar, 2010). The strains which infect humans are found in genogroups GI, GII and GIV. Both GI and GII strains have been observed in effluent from wastewater treatment plants around New Zealand, reflective of different-sized communities and treatment types (Hewitt et al. 2011). The majority of Norovirus outbreaks worldwide are associated with GII strains, particularly the GII.4 genotype (Maalouf et al. 2010; ESR, 2012); however, shellfish are frequently found to contain multiple strains, and the frequency of each genogroup detected is clearly distinct. GI strains, particularly the GI.1 genotype, are more frequently encountered in shellfish-related outbreaks than other Norovirus outbreaks, and the GII.4 genotype is not as dominant (Le Guyader et al. 2012). The reasons why GI strains are more frequently encountered in shellfish related outbreaks than other outbreaks are not yet well understood, but may be due to differences in accumulation efficiency as discussed below.

E.5 Norovirus biokinetics and localisation by oyster tissue

Oysters acquire their food by filter feeding using cilia on the gill surface to produce water currents to capture food particles in mucus on the gills. The gills then transport the food particles forward to the labial palps, where they are either guided to the mouth for ingestion or dropped into the mantle cavity and rejected as pseudofaeces (McLeod et al. 2009a). Ingested particles enter the stomach where they are subject to extracellular digestion by mechanical and chemical degradation. Finer particles are then guided towards the digestive diverticula where intracellular digestion is the main digestive process, whilst larger particles and small dense particles (e.g., sand) are channelled towards the intestine. Undigested remnants are stored in residual bodies within digestive cells. These cells eventually rupture, and the waste material is channelled back towards the stomach and ultimately to the intestine (Gosling, 2003). Transport of ingested food particles through the digestive tract occurs exclusively by ciliary motion. For large oysters, the time required for food to pass through the entire digestive tract varies from around 90 – 150 minutes (Galtsoff, 1964; Le Guyader et al. 2006). Of course, any viable virus particles so-ejected could become available to other nearby oysters.

Bioaccumulation experiments indicate that virus contamination is concentrated mainly in oyster digestive tissues (including the stomach, intestine and digestive diverticula) with lower amounts present in the gills,

mantle and labial palps (Schwab et al. 1998; Wang et al. 2008; McLeod et al. 2009a; Maalouf et al. 2010; Maalouf et al. 2011). Studies involving different genotypes suggest that GI strains are more efficiently bioaccumulated than GII (Maalouf et al. 2010; Le Guyader et al. 2012). For example, in analysing Norovirus concentrations in seawater samples and oyster digestive tissues collected over the course of a year, Le Guyader et al. (2012) found that GI Noroviruses required 30 viral RNA copies L^{-1} water to observe 1 viral RNA copy g^{-1} oyster tissue, whereas GII Noroviruses required approximately 1200. Though the exact mechanisms behind this difference in accumulation efficiency are not yet well understood, some research (Le Guyader et al. 2006; Tian et al. 2006) has demonstrated that Norovirus particles are capable of binding specifically to oyster tissues by attachment to carbohydrate ligands (similar to human histo-blood group antigens). Maalouf et al. (2010; 2011) observed that the binding of Norovirus particles to different tissue types is strain dependent; GI.1 virus particles bound only to digestive tissues (and not to the gills or mantle), whereas GII.4 virus particles bound to all three tissues. The authors suggest that binding of the GII.4 strain occurs through two distinct ligands, one primarily or exclusively present in digestive tissues and the other present in all three tissues, and that differential recognition of these ligands may lead to distinct outcomes in terms of the persistence of virus particles in the different organs. Le Guyader et al. (2006) also hypothesize that the binding of Norovirus particles to oyster tissues may provide a mechanism for these particles to avoid entering the digestive system and being degraded.

At a cellular level, studies indicate that after bioaccumulation Norovirus particles are present in the lumen of digestive tissues as well as in hemocytes in both the epithelium of digestive tissues and the surrounding connective tissue (Le Guyader et al. 2006; McLeod et al. 2009a). Virus particles were not detected in cells of the gills and labial palps (McLeod et al. 2009a); although this was probably due to low levels of virus contamination in these tissues and differences in the sensitivity of analysis techniques. Le Guyader et al. (2006) also noted differences in the localization of virus particles following bioaccumulation experiments vs. experiments with binding to different tissue sections. They suggest that particles found in hemocytes could reflect elimination of virus during digestion, though it is unknown if this is indeed the case or whether particles can escape digestion (e.g., via specific binding as discussed above). McLeod et al. (2009a) speculate that, while viruses within the oyster digestive tract lumen are likely to be depurated relatively rapidly when oysters are placed in clean water, elimination or inactivation of viruses that have been taken into cells may be much slower. This is consistent with a recent study by Provost et al. (2011) which implicated hemocytes as a site of Norovirus persistence within oysters. That study also proposes a possible alternate route of virus uptake that bypasses the digestive tract. In addition to their digestive function, hemocytes can engulf and phagocytize foreign pathogens present on the mucosal surfaces of oysters or within the hemolymph as part of their innate immune functions. As such, hemocytes may take up viruses directly from the water via association with the mucus-coated epithelial surfaces of the gills, mantle and other tissues. The relative contribution of this alternate uptake mechanism compared with the digestive route is unknown, however.

E.6 Uptake and depuration rates

Relatively few studies have measured Norovirus uptake rates during bioaccumulation. Maalouf et al. (2011) measured concentrations in the gills, mantle and digestive tissues of Pacific oysters exposed to constant concentrations of GI.1, GII.3 and GII.4 Noroviruses after 1 and 24 hours. Concentrations in the gills and mantle showed little variation between measurements, suggesting that these tissues may accumulate viruses to peak concentrations relatively rapidly. Concentrations in digestive tissues were much larger however, and continued to increase between 1 and 24 hours, indicating that peak concentrations were not achieved within this time.

After bioaccumulation of Pacific oysters with GII Noroviruses over a four-hour contact period, Neish (2013) observed that digestive tissue concentrations continued to increase up to four days after the initial contamination. Furthermore, Ventrone et al. (2013) found that oyster digestive tissue concentrations progressively increased over nine days of exposure to constant concentrations of GI and GII Noroviruses, and that no competition occurred when oysters were exposed to both strains. Together, these uptake studies suggest that Noroviruses can accumulate in oyster tissues in as little as an hour, but that the time to reach peak concentrations may be several days. Ventrone et al. (2013) also observed no difference in the levels of contamination resulting from the same amount of virus administered as a single high dose versus repeated low doses. This finding raises important questions over how the risk profiles may differ (if at all) for shellfish growing in coastal waters that are subject to frequent small contamination events compared to infrequent large contamination events.

Viruses are known to be retained by bivalve shellfish for significantly longer periods of time than bacterial indicators such as *E. coli* and faecal coliforms (Richards et al. 2010). While bacteria are generally eliminated within 2 – 3 days, viruses in shellfish may persist for several weeks depending on factors such as virus type, shellfish species, seawater temperature and salinity (Richards et al. 2010). Table E-1 contains a summary of published data on Norovirus persistence in oysters during depuration experiments. There is a clear temperature influence, with virus contamination persisting for longer periods of time in colder temperatures. All studies are also consistent in indicating that Noroviruses are retained in oyster tissues for significantly longer than 48 hours, even at warmer temperatures. The longest-running of the depuration experiments (Greening et al. 2003) demonstrated virus persistence for up to 4 and 9 weeks in separate experiments under natural conditions, though the results of the second experiment may have been influenced by contamination with a 'wild' strain sometime between day 0 and week 2.

It should also be noted that there is no current method for assessing the infectiousness of Norovirus particles in shellfish (Greening, 2007). Thus, although viruses may persist in shellfish tissues for several weeks, their infectivity status is generally not known. McLeod et al. (2009b) found that most poliovirus present in oyster gut samples was not infectious, whilst the converse was true for hepatitis A virus. Either way, this uncertainty is taken account of in the QMRA calculations which report on the risk of 'infection' rather than the risk of 'illness' (McBride, 2007).

E.7 Existing modelling approaches

To the best of our knowledge, there are no existing mathematical models for Norovirus contamination in oysters (or any other shellfish species). In fact, contamination of shellfish by enteric viruses in general is not well studied from a mathematical perspective. Models have been applied to other contaminants however; including shellfish poisoning toxins (Blanco et al. 1997; Moroño et al. 2003; Li et al. 2005) as well as heavy metals (Luoma & Rainbow, 2005; Wang & Rainbow, 2008).

A comprehensive review of modelling approaches is given by Landrum et al. (1992). The types of models used to predict toxin accumulation can be broadly divided into steady-state versions (such as the current bioaccumulation factor approach used in QMRA calculations), and kinetic versions that describe the changes in toxicant concentrations over time. Landrum et al. note that organisms can attain steady state if both the exposure and the environmental / physiological factors affecting the uptake and loss of contaminants remain constant for a sufficiently long time. Such models may be inadequate, however, in predicting the accumulation of contaminants resulting from spatially and temporally varying exposures.

Table E-1: Summary of depuration studies for Noroviruses and oysters.

Reference	Species	Genotype	Depuration conditions	Summary
McLeod et al. (2009b)	Pacific oysters	GII.4	20 °C	No significant reduction in contamination after 23 hours.
Schwab et al. (1998)	Eastern oysters	GI.1	22 °C	7% reduction in contamination after 48 hours.
Ueki et al. (2007)	Pacific oysters	GII.6	10 °C	No significant reduction in contamination after 10 days.
Neish (2013)	Pacific oysters	GII	8 °C; 16 °C	No significant reduction in contamination after 14 days at 8 °C; approximately 41% reduction after 14 days at 16 °C.
Nappier et al. (2008)	Suminoe oysters (*C. ariakensis)* and Eastern oysters	GI.1	20 – 23 °C; 8, 12 and 20 ppt salinity	Suminoe oysters remained positive for Norovirus after 29 days at all salinities; Eastern oysters did not bioaccumulate Norovirus at 8 ppt salinity, and remained positive for Norovirus for up to 22 and 25 days at 12 and 20 ppt salinity.
Greening et al. (2003)	Pacific oysters	Norwalk-like virus	12 °C – 18 °C	Virus detected for up to 4 and 9 weeks in separate experiments under natural conditions, although the results of the second experiment may have been influenced by contamination with a 'wild' Norwalk-like virus strain sometime between day 0 and week 2.

Kinematic models generally fall into two categories: compartment-based models and models based on organism physiology. In the former approach, the compartments do not necessarily represent physical entities; rather they group quantities of contaminants with the same dynamics (i.e., that share the same uptake and elimination rates). Examples include Blanco et al. (1997) and Moroño et al. (2003), who applied 1- and 2-compartment models to predict accumulation of paralytic and diarrheic shellfish poisoning toxins in mussels. The 2-compartment models represent fast and slow detoxification rates. Blanco et al. (1997) reported that the detoxification kinetics of paralytic shellfish poisoning (PSP) toxins could not be correctly described by a 1-compartment model, however the 2-compartment model seemed to offer an adequate description even when the actual situation may be much more complex. In contrast, Moroño et al. (2003) found that a 1-compartment model was sufficient to describe the intoxication-detoxification processes for diarrhoeic shellfish poisoning (DSP) toxins.

Models based on organism physiology (bio-kinetic models) generally separate an organism into anatomical compartments representing different organs or groups of kinetically-related tissues. Differential mass-balance equations are written to describe the uptake, elimination and transfer of contaminants between anatomical compartments based on real physiological processes (Landrum et al. 1992). Li et al. (2005) derived a bio-kinetic model for PSP toxins in mussels based on five tissue compartments: the viscera, gills, hepatopancreas, adductor muscle and foot. Their model contributes to the understanding of toxin dynamics within mussels, and allows the validity of assumptions made in 1- and 2-compartment models to be checked. Landrum et al. (1992) note that bio-kinetic models can better represent reality in the sense that they are focused on the mechanistic nature of organisms and not just rate processes. This may not always be the case however, if there is insufficient understanding of the physiological processes involved. Bio-kinetic models may also incorporate more detail than is necessary, or at least feasible, to include in some cases.

All of the kinetic models mentioned above (as well as others surveyed but not discussed here) are based on the underlying assumptions that the transfer mechanisms between compartments (anatomical or otherwise) can be represented by simple first-order processes, and that the rate constants for these processes are independent of time. These assumptions result in models that may be solved analytically in some cases, or at least using relatively simple numerical techniques. The major difficulty in using kinetic models, however, is the number of parameters that must be determined; some of which are not directly measurable.

E.8 Mathematical model formulation

We formulate a bio-kinetic model for Norovirus contamination in oysters based on the physiology and virus kinetics as discussed in Section E.5. The resulting model structure is more complex than is perhaps ideal for incorporating into the QMRA process; the motivation, however, is to facilitate a better understanding of the processes involved and to provide a foundation on which simpler structures may be built. Since the model is physically-based, it could also be used to explore the impact of different management techniques (e.g., depuration methods to try to reduce virus persistence). This section describes the conceptual formulation of the model, simple case solutions, and best estimates of parameters based on comparison with published bioaccumulation data.

E.9 Conception model formulation

Our conceptual bio-kinetic model for Norovirus contamination in oyster flesh is illustrated in Figure E-1. We represent the flesh using three main tissue compartments: the gills (including labial palps), digestive tissue (including the mouth, oesophagus, stomach, intestine and digestive diverticula) and non-digestive tissue. The non-digestive tissue primarily includes the mantle and the connective tissue surrounding the digestive organs, and excludes the adductor muscle. McLeod et al. (2009a) cite preliminary experiments that indicate the adductor muscle does not accumulate significant amounts of virus, and concentrations for this tissue type are not reported elsewhere in the literature.

The tissue compartments in the model are chosen to reflect both oyster physiology and the localization of virus particles observed after bioaccumulation experiments (Section E-5). As the oyster feeds, food particles are transported in water currents across the gills towards the mouth. We assume that a portion of virus particles (either suspended in the water or attached to food particles) may be retained in the gill tissue. After ingestion, virus particles enter the digestive tract in a so-called 'free' phase and are transported towards the anus by ciliary motion. Whilst in the digestive tract, these 'free' virus particles may enter the 'bound' phase by specific binding to digestive tissue cells, or be absorbed into the cells of non-digestive tissue. This formulation assumes that the main uptake route is digestive, and that direct uptake of virus particles by hemocytes is negligible. Viruses in the 'free' phase are subject to both excretion and elimination by die-off or degradation during digestion. Viruses in the 'bound' phase or those that have been taken into cells are also subject to eventual degradation; however, it is anticipated that the elimination rates in these compartments may be much slower.

The exact mechanisms behind the elimination of virus particles from cells are as yet unknown. Following the approach of other kinetic models (Section E.7), we assume that the elimination of virus particles from the tissue compartments (as well as the transfer between tissue compartments) can be described by simple first-order processes with constant rate coefficients. Under these simplifying assumptions, the flux diagram corresponding to the conceptual model is as shown in Figure E-2. The parameters, along with their physical meanings, are summarized in Table E-2. We anticipate that the conceptual framework of the model may be applied to both GI and GII strains, and this appears to be the case when the model predictions are compared with measured data for both GI.1 and GII.3 (see Section E.10.2).

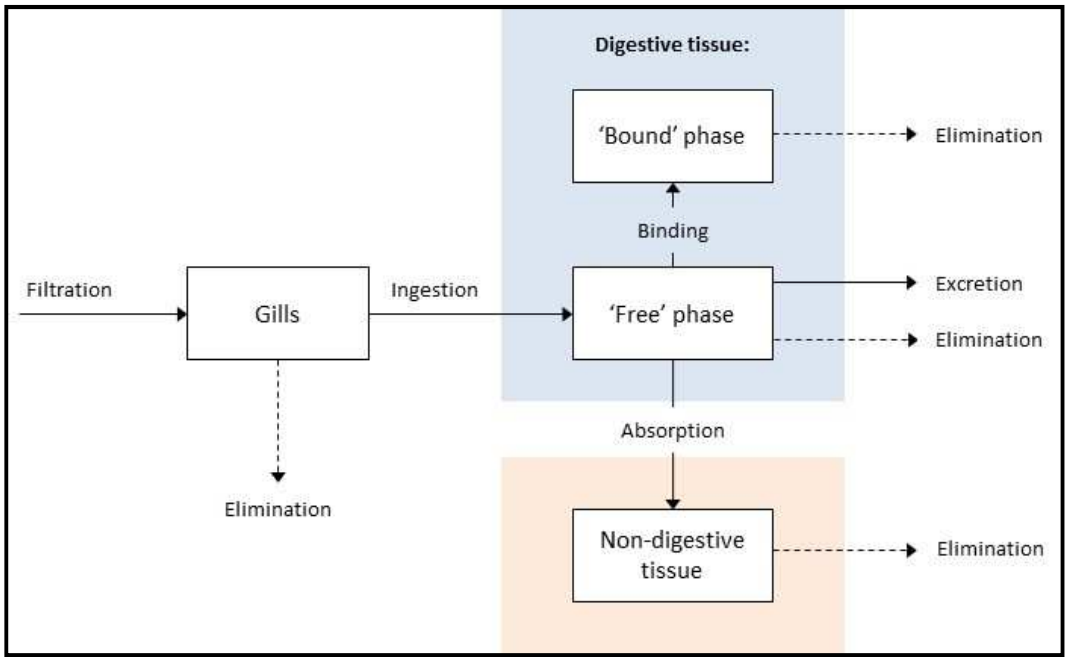


Figure E-1: Conceptual bio-kinetic model for Norovirus contamination in oyster flesh.

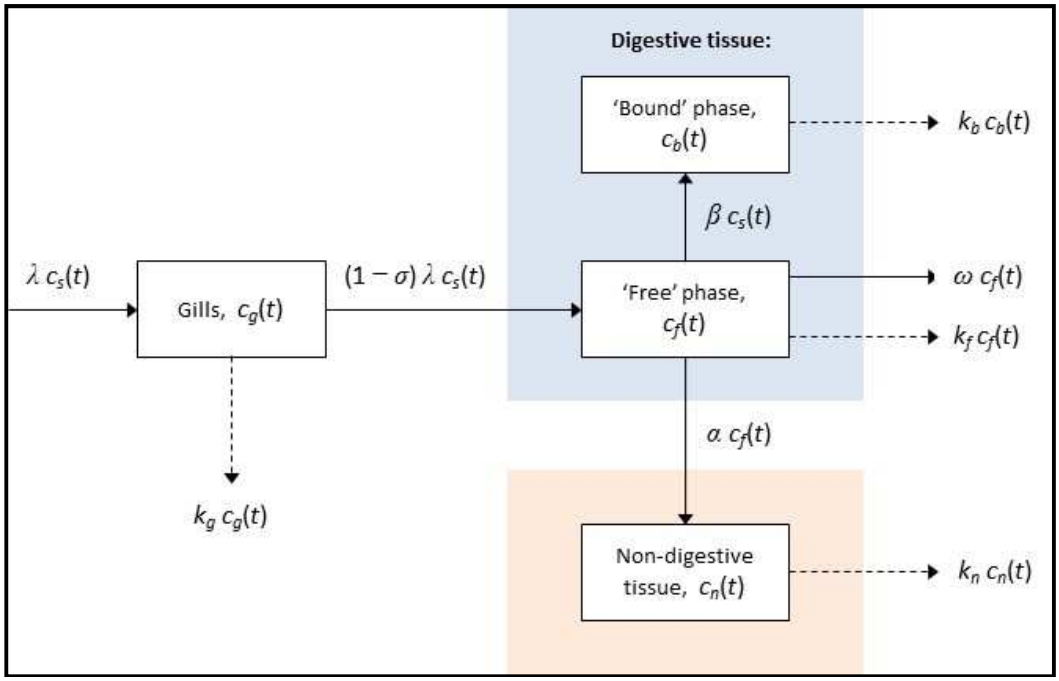


Figure E-2: Flux diagram showing the concentration of Norovirus particles in each tissue compartment and the transfer rates between compartments. Parameters values and units are described in Table E-2.

Table E-2: Parameters used to describe the distribution and transfer of Norovirus particles between oyster tissue compartments according to the conceptual model in Figures E-1 and E-2.

Parameter	Description	Unit
$c_b(t)$	Virus concentration in the 'bound' phase in digestive tissue	count g^{-1}
$c_f(t)$	Virus concentration in the 'free' phase in digestive tissue	count g^{-1}
$c_g(t)$	Virus concentration in gill tissue	count g^{-1}
$c_n(t)$	Virus concentration in non-digestive tissue	count g^{-1}
$c_s(t)$	Virus concentration in seawater	count L^{-1}
k_b	Elimination rate from the 'bound' phase in digestive tissue	hr^{-1}
k_f	Elimination rate from the 'free' phase in digestive tissue	hr^{-1}
k_g	Elimination rate from gill tissue	hr^{-1}
k_n	Elimination rate from non-digestive tissue	hr^{-1}
α	Absorption rate from the 'free' phase in digestive tissue to non-digestive tissue	hr^{-1}
β	Binding rate from the 'free' phase in digestive tissue to the 'bound' phase	hr^{-1}
λ	Specific filtration rate (filtration rate per unit body wet weight)	L hr^{-1} g^{-1}
σ	Proportion of filtered virus particles retained in gill tissue	-
ω	Proportional excretion rate	hr^{-1}

The system of differential equations that describes the distribution of virus particles (as derived from a formal mass balance between the tissue compartments) is given by

$$\frac{dc_g}{dt} = \frac{\sigma\lambda}{\phi_g} c_s - k_g c_g, \tag{2}$$

$$\frac{dc_f}{dt} = (1-\sigma)\frac{\lambda}{\phi_d} c_s - \eta c_f, \tag{3}$$

$$\frac{dc_b}{dt} = \beta c_f - k_b c_b, \tag{4}$$

$$\frac{dc_n}{dt} = \frac{\alpha\phi_d}{\phi_n} c_f - k_n c_n, \tag{5}$$

where φ_g, φ_d and φ_n represent the masses of the gills, digestive and non-digestive tissues, respectively, relative to the oyster body weight [-]. Note that these fractions do not sum to one, as the non-digestive tissue does not include the adductor muscle. The physical descriptions for the remaining parameters are given in Table E-2; the exception being η which represents the combined removal rate of virus particles from the 'free' phase, i.e.,

$$\eta = \alpha + \beta + \omega + k_f \tag{6}$$

Equations (E.1) – (E.4) may be readily solved depending upon the mathematical functional form selected for the virus concentration in seawater. For the general case, assuming an arbitrary, time-varying seawater concentration and arbitrary initial conditions at time $t = 0$, the solutions can be written in integral form[64] as:

$$c_g(t) = c_g(0)\mathrm{e}^{-k_g t} + \frac{\sigma\lambda}{\varphi_g}\int_0^t c_s(\tau)\mathrm{e}^{-k_g(t-\tau)}\,\mathrm{d}\tau, \tag{7}$$

$$c_f(t) = c_f(0)\mathrm{e}^{-\eta t} + (1-\sigma)\frac{\lambda}{\varphi_d}\int_0^t c_s(\tau)\mathrm{e}^{-\eta(t-\tau)}\,\mathrm{d}\tau, \tag{8}$$

$$c_b(t) = c_b(0)\mathrm{e}^{-k_b t} + \beta\int_0^t c_f(\tau)\mathrm{e}^{-k_b(t-\tau)}\,\mathrm{d}\tau, \tag{9}$$

$$c_n(t) = c_n(0)\mathrm{e}^{-k_n t} + \frac{\alpha\varphi_d}{\varphi_n}\int_0^t c_f(\tau)\mathrm{e}^{-k_n(t-\tau)}\,d\tau. \tag{10}$$

The bulk number of viruses in the oyster is then readily calculated as

$$C(t) = M\left[\varphi_g c_g + \varphi_d\left(c_f + c_b\right) + \varphi_n c_n\right]. \tag{11}$$

where *M* is the shellfish mass (g). Analytic forms are available for the special cases of uptake and depuration under constant conditions, which are used in comparison with published bioaccumulation data in order to estimate values for the model parameters are given below.

E.9.1Uptake under constant conditions

For the special case where the virus concentration in seawater is held constant [$c_s(t) = \bar{c}_s$], with some non-zero initial contamination of the shellfish at time $t=0$, the general solutions in Equations (E.6)–(E.9), derived analytically, reduce to:

$$c_g(t) = c_g(0)\mathrm{e}^{-k_g t} + \frac{\sigma\lambda\bar{c}_s}{\phi_g k_g}\left(1-\mathrm{e}^{-k_g t}\right), \tag{12}$$

$$c_f(t) = c_f(0)\mathrm{e}^{-\eta t} + (1-\sigma)\frac{\lambda\bar{c}_s}{\phi_d\eta}\left(1-\mathrm{e}^{-\eta t}\right), \tag{13}$$

$$c_b(t) = c_b(0)\mathrm{e}^{-k_b t} + \left(\frac{\beta}{k_b-\eta}\right)c_f(0)\left(\mathrm{e}^{-\eta t} - \mathrm{e}^{-k_b t}\right) + \frac{\beta(1-\sigma)\lambda\,\bar{c}_s}{\phi_d\eta k_b}\left(1 - \frac{k_b\mathrm{e}^{-\eta t} - \eta\mathrm{e}^{-k_b t}}{k_b-\eta}\right), \tag{14}$$

$$c_n(t) = c_n(0)\mathrm{e}^{-k_n t} + \frac{\alpha\phi_d}{\phi_n(k_n-\eta)}c_f(0)\left(\mathrm{e}^{-\eta t} - \mathrm{e}^{-k_n t}\right) + \frac{\alpha(1-\sigma)\lambda\,\bar{c}_s}{\phi_n k_n\eta}\left(1 - \frac{k_n\mathrm{e}^{-\eta t} - \eta\mathrm{e}^{-k_n t}}{k_n-\eta}\right). \tag{15}$$

[64] These solutions were derived using Mathematica®.

Given enough time, the virus distribution between tissue compartments will reach a steady-state. The steady-state concentrations, determined by setting the derivatives in Equations (E.1) – (E.4) equal to zero, are given by:

$$c_g^* = \frac{\sigma \lambda \bar{c}_s}{\phi_g k_g}, \tag{16}$$

$$c_f^* = \frac{(1-\sigma)\lambda\, \bar{c}_s}{\phi_d\, \eta}, \tag{17}$$

$$c_b^* = \frac{\beta(1-\sigma)\lambda\, \bar{c}_s}{\phi_d\, k_b\, \eta}, \tag{18}$$

$$c_n^* = \frac{\alpha(1-\sigma)\lambda \bar{c}_s}{\phi_n k_n \eta}. \tag{19}$$

Note that these expressions for the steady-states are identical to the parameter groupings before the parentheses in the final terms on the right-hand side of Equations (E.11) – (E.14), as expected (as $t \rightarrow \infty$).

E.9.2 Depuration under constant conditions

For the special case where contaminated oysters are placed in clean seawater [$c_s(t) = 0$] at time $t = \tau$, the reduction in viral loads over time is described by

$$c_g(t) = c_g(\tau) \mathrm{e}^{-k_g(t-\tau)}, \tag{20}$$

$$c_f(t) = c_f(\tau) \mathrm{e}^{-\eta(t-\tau)}, \tag{21}$$

$$c_b(t) = c_b(\tau) \mathrm{e}^{-k_b(t-\tau)} + \frac{\beta c_f(\tau)}{k_b - \eta}\left(\mathrm{e}^{-\eta(t-\tau)} - \mathrm{e}^{-k_b(t-\tau)}\right), \tag{22}$$

$$c_n(t) = c_n(\tau) \mathrm{e}^{-k_n(t-\tau)} + \frac{\alpha \phi_d c_f(\tau)}{\phi_n (k_n - \eta)}\left(\mathrm{e}^{-\eta(t-\tau)} - \mathrm{e}^{-k_n(t-\tau)}\right), \tag{23}$$

where $c_g(t)$, $c_f(t)$, $c_b(t)$, $c_n(t)$ represent the initial virus concentrations in the respective tissue compartments when depuration begins.[65] Assuming that ε represents the proportion of virus particles in the 'bound' phase in digestive tissues, the combined digestive tissue concentration $c_d(t) = c_f(t) + c_b(t)$ may be written as

$$c_d(t) = c_d(\tau)\left((1-\varepsilon)\left(1 + \frac{\beta}{k_b - \eta}\right)\mathrm{e}^{-\eta(t-\tau)} + \left(\varepsilon - \frac{\beta(1-\varepsilon)}{k_b - \eta}\right)\mathrm{e}^{-k_b(t-\tau)}\right). \tag{24}$$

If the binding rate $\beta = 0$ then all virus particles in digestive tissues will be in the 'free' phase ($\varepsilon = 0$). In that case, the time to eliminate 90% of the initial digestive tissue contamination will be

$$t_{90} = -\frac{1}{\eta}\ln(0.1). \tag{25}$$[66]

[65] This does not include any loss of infectivity; it is strictly amounts being depurated.

[66] Obtained by setting $c_d(t) = 0.1 \times c_d(\tau)$ in Equation (24) and solving for $t_{90} = (t - \tau)$.

On the other hand, if all virus particles in digestive tissues are in the 'bound' phase ($\varepsilon = 1$), then the time to eliminate 90% of the initial digestive tissue contamination will be

$$t_{90} = -\frac{1}{k_b}\ln(0.1). \tag{26}$$

E.10 Parameterisation and comparison with experimental data

Since there is little precedent for this type of bio-kinetic model applied to Norovirus contamination in oysters, limited information is available with which to parameterize the model and published data suitable for calibration and / or validation are scarce. Suggested values adopted for the physiological parameters, including typical oyster body weights, relative tissue weights and water filtration rates are summarized in Table E-3 – Table E-5. The remaining virological parameters have no prior basis in the literature, and are estimated by fitting the model to measured tissue concentrations as reported by Maalouf et al. (2011) and Ventrone et al. (2013).

Maalouf et al. (2011) carried out bioaccumulation experiments using Pacific oysters exposed to three different Norovirus strains (GI.1, GII.3 and GII.4) for 24 hours at concentrations of approximately 10^6, 10^7 and 10^8 RNA copies L^{-1} and temperatures of 8 and 12 °C. Measurements were made of virus concentrations in the gills, mantle and digestive tissues after 1 and 24 hours. Over a longer time period, Ventrone et al. (2013) conducted experiments with oysters exposed to two different Norovirus strains (GI.1 and GII.3) at approximately 10^5 RNA copies L^{-1} and 8 °C. Measurements were made of virus concentrations in digestive tissues after 1, 3, 6 and 9 days. Attempting to parameterize the model against the reported data is problematic because of the limited data points, the number of parameters and the lack of any prior knowledge on parameter values. Nevertheless, we draw estimates as discussed below using the GI.1 data set, reserving the GII.3 data set for comparison.

E.10.1 Parameterisation

The measured concentrations from both sets of experiments are reported as $\log_{10}$ RNA copies g^{-1}. Data from the Maalouf et al. (2011) experiments is summarized in Table E-4. Focusing on the lowest dose and noting that the concentrations reported for the gills and mantle change little between 1 and 24 hours, we make the following assumptions:

- the log-transformed steady-state concentrations $\log_{10}c_g^*$ and $\log_{10}c_n^*$ lie within the measured ranges for these tissues after 24 hours as reported in Table E-4, and
- the log-transformed predicted concentrations $\log_{10} c_g$ and $\log_{10} c_n$ after 1 hour are equal to proportions ε_g and ε_n of the log-transformed steady-state concentrations.

Furthermore, in the interest of simplicity, we also assume that the elimination rate of virus particles from non-digestive tissue is the same as that in the gill tissue (i.e., $k_n = k_g$). The range of possible values for $\log_{10} c_g^*$ and ε_g yields a range of possible values for k_g and σ. Based on these ranges, we select $k_g = 0.8$ hr^{-1} and $\sigma = 6.4 \times 10^{-4}$ ($\log_{10} c_g^* = 2$ and $\varepsilon_g = 0.9$). Less information can be drawn for the non-digestive tissue, however the first assumption does give bounds for the ratio α/η.

Next, we note that when the log-transformed digestive tissue concentrations from both Maalouf et al. (2011) and Ventrone et al. (2013) are normalized by the log-transformed seawater concentrations, the

results from both sets of data seem to follow a single curve (see the Appendix of Harper and McBride 2015). Comparing this normalized ratio with our model, the curve should be of the form

$$y = a + b_1 e^{-r_1 t} + b_2 e^{-r_2 t}, \quad (27)$$

where $r_1 = k_b$ and $r_2 = \eta$. Fitting an equation of this form to the data using Matlab's Curve Fitting Toolbox yields $k_b = 7 \times 10^{-3}$ hr^{-1} and $\eta = 0.76$ hr^{-1}. Manually exploring the model results with the selected parameter values, we choose $\beta = 0.06$ hr^{-1} as providing a reasonable fit to the curve. The remaining parameters are estimated from the ratio bounds for α/η and Equation (E.5). The full set of parameter values is summarized in Table E-5. It should be noted the experiments on which these values are based were conducted in fairly cold temperatures, and there is likely to be a temperature dependence in at least some of the parameters, particularly the specific filtration rate λ.

Table E-3: Adopted values for oyster physiological parameters.

Parameter	Adopted Value	Suggested Range	Source
λ	0.1 L hr^{-1} g^{-1} (appropriate for warmer temperatures	0.01–0.1 L hr^{-1} g^{-1} ('g' refers to wet weight)	Based on a greenweight (including shell) of 67 g and proportion meatweight (wet) of 0.1352 for Pacific oysters (King & Lake, 2013), a clearance rate of approximately 0.01 – 0.1 L hr^{-1} cm^{-1} at 8 °C (Ren et al. 2000)* and a body length of 7 – 10 cm.
ω	0.59 hr^{-1}	0.40 – 0.66 hr^{-1}	Based on a time of 90 – 150 min for food to pass through the oyster digestive system (Galtsoff, 1964; Le Guyader et al. 2006).
ϕ_g	0.20	0.16 – 0.28	Based on relative masses of 12 – 21% (gills) and 4 – 7% (labial palps) for Pacific oysters (Honkoop et al. 2003).
ϕ_d	0.15	0.09 – 0.18	Based on typical dissections of approximately 5 – 10 g digestive tissue from 6 oysters (Greening, G., personal communication, 24 June 2013); meatweight as above.
ϕ_n	0.35		Estimated based on the remaining proportion of oyster meatweight, assuming the adductor muscle (not included) accounts for 20 – 40% (Maryland Sea Grant, 2013).

*Temperature selected to match bioaccumulation experiments (Maalouf et al. 2011; Ventrone et al. 2013)

Table E-4: Bioaccumulation data (Maalouf et al. 2011).

Strain	Seeded concentration ($\log_{10}$ RNA copies L^{-1})	Tissue compartment	Mean ± SD ($\log_{10}$ RNA copies g^{-1})	
			1 hr	24 hrs
GI.1	6.4 ± 0.3	gills	1.9 ± 0.8	2.0 ± 0.6
		mantle	2.0 ± 1.0	2.0 ± 0.7
		digestive tissue	3.6†	4.5 ± 0.9
	7.4 ± 0.3	digestive tissue	-	5.5 ± 0.8
	8.5 ± 0.3	gills	-	3.2 ± 1.0
		digestive tissue	-	6.4 ± 0.7

†Estimated from the percent of recovered virus concentrations in digestive tissues after 1 hr (mean of all seeded concentrations).

Table E-5: Adopted values for the virological parameters.[67]

Parameter	Adopted Value	Parameter	Adopted Value
α	1.5×10^{-3} hr^{-1}	k_b	7.0×10^{-3} hr^{-1}
β	0.06 hr^{-1}	k_f	0.11 hr^{-1}
σ	6.0×10^{-4}	k_g, k_n	0.80 hr^{-1}

E.10.2 Comparison with experimental data

Figures E-3 and E-4 show the model predicted concentrations for GI.1 (the data set on which the parameterization is based) compared with the measured concentrations according to Maalouf et al. (2011) and Ventrone et al. (2013). In Figures E-3 and E.4, the model predicted concentrations generally fall within the error bounds for the measured concentrations, however the model seems to overestimate the concentration in digestive tissues early in the bioaccumulation period (≤ 24 hours; Figure E-4). The corresponding depuration curve is shown in Figure E-5. Contamination in the gills and non-digestive tissue is reduced fairly rapidly, whilst contamination in the digestive tissues persists for much longer, as expected. According to the curve, the time to reduce virus concentrations in digestive tissues by 90% is around 13 days, whilst the time to remove 99% is around 27 days. These finding seem to be *reasonably* consistent with the depuration studies summarized in Table E-1 (i.e., contamination can persist in the order of 20 days).

The model predicted concentrations compared with the measured concentrations for GII.3 are shown in Figures E-6 and E-7.[68] Although the model is parameterized based on GI.1 data, the predicted concentrations for GII.3 generally lie within the error bounds for the measured concentrations. Once again, however, the model overestimates the concentration in digestive tissues between around 1 and 24 hours. A contributing factor to this overestimation could be the series expansion for ln(1+x) used to parameterize the model.[69] Only the first term in the series expansion is used, however the magnitude of the remainder terms is greater for small t. Further exploration of the parameter space may reduce this issue.

[67] Includes data from Maalouf et a1. 2010, 2011)

[68] Although the model is parameterised for GI.1, it also provides reasonable results for GII.3 given the data we have

[69] This issue should be examined further—there are only limited data available to work with.

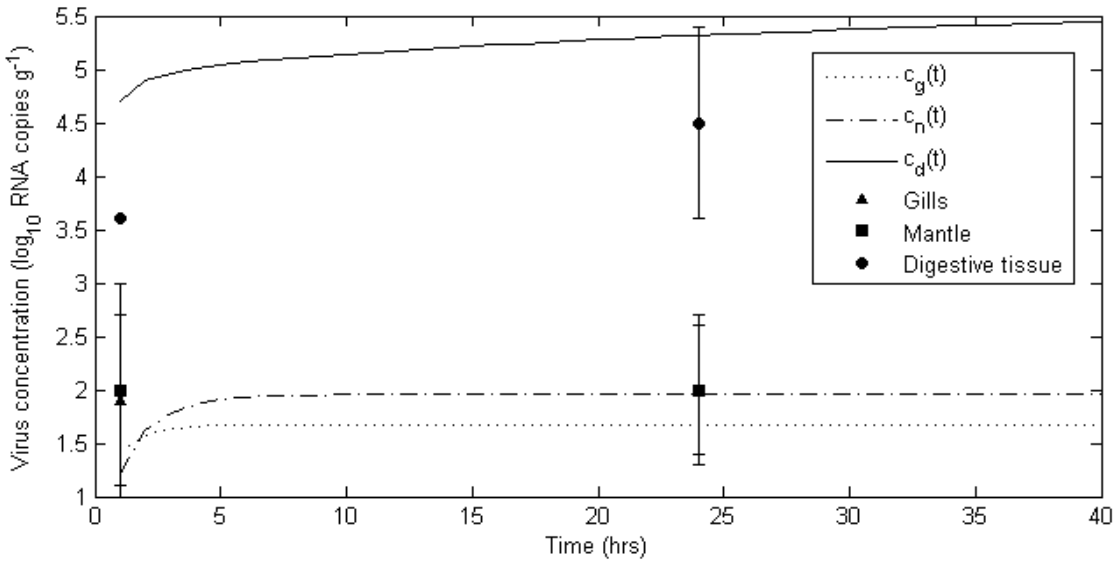


Figure E-3: Model predicted concentrations for gill, digestive and non-digestive tissues compared with measured concentrations for GI.1 (lowest seeded concentration) reported by Maalouf et al (2011). Measured concentrations for the gills and mantle tissues overlap at 24 hours. Parameters as in Tables E-3 through E-5.

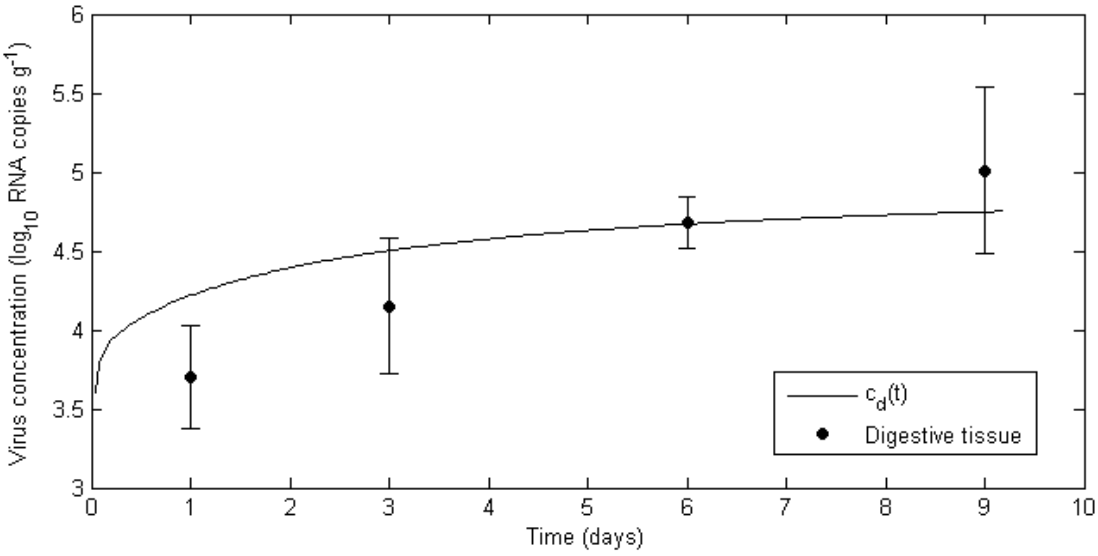


Figure E-4: Model predicted concentrations for digestive tissues compared with measured concentrations for GI.1 reported by Ventrone et al. (2013). Seeded concentration 5.0 log_{10} RNA copies L^{-1}. Parameters as in Tables E-3 through E-5.

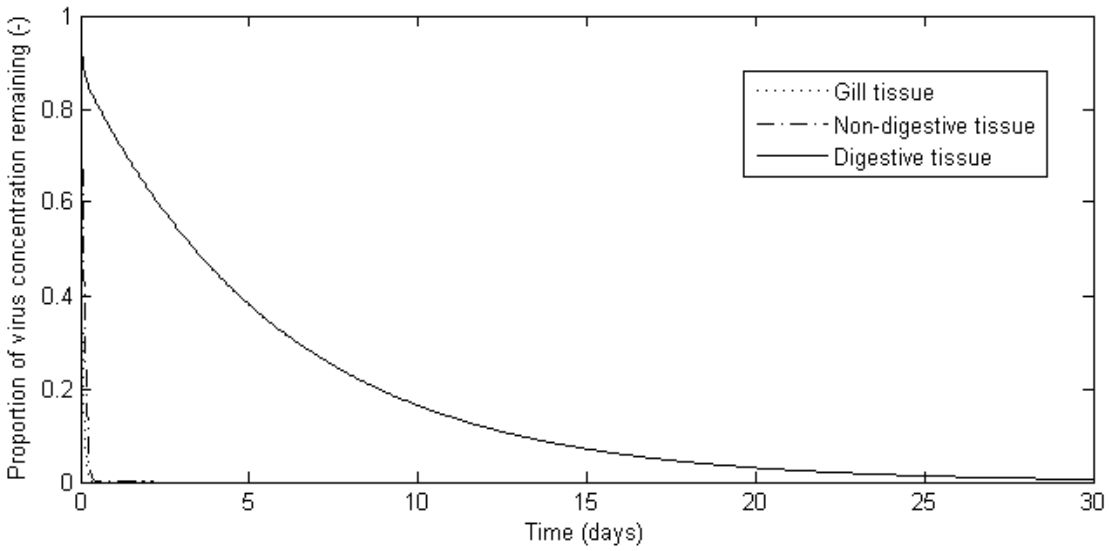


Figure E-5: Model predicted proportion of virus concentration remaining over time in gill, non-digestive and digestive tissues. Initial contamination reflects the final concentrations obtained in Figure E-4. Parameters as in Tables E-3 through E-5.

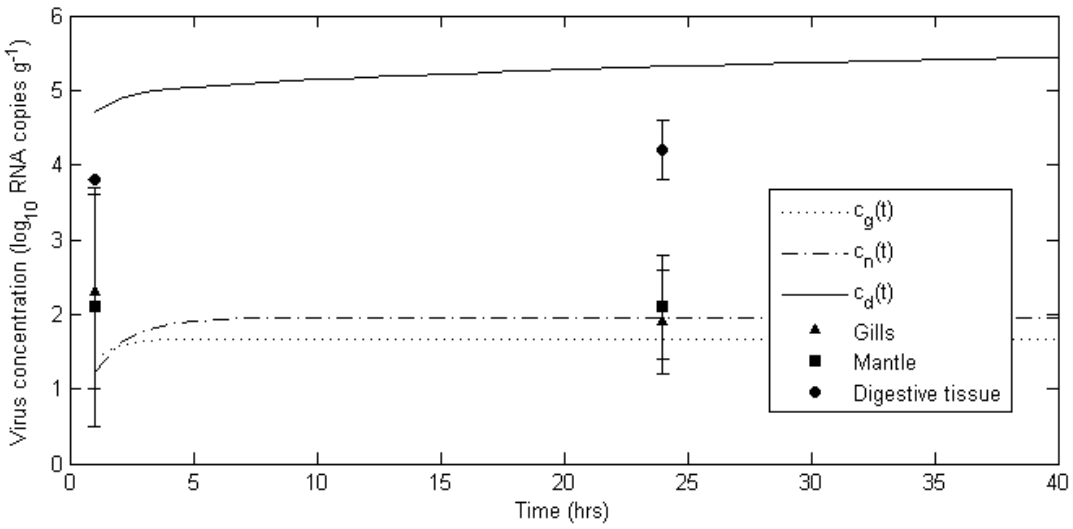


Figure E-6: Model predicted concentrations for gill, non-digestive and digestive tissues compared with measured concentrations for GII.3 reported by Maalouf et al. (2011). Seeded concentration 6.1 $\log_{10}$ RNA copies L^{-1}. Parameters as in Tables E-3 through E-5.

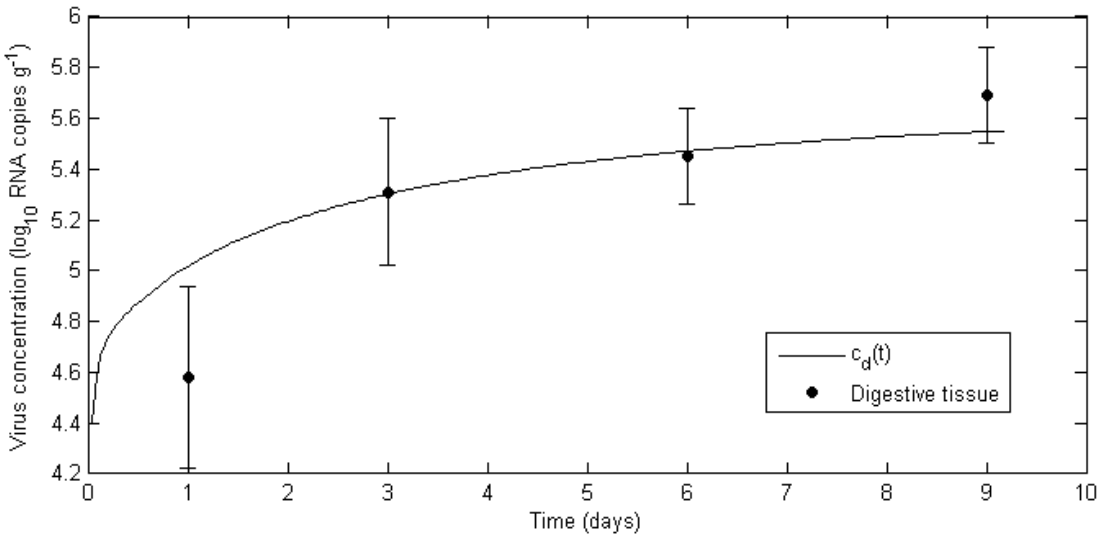


Figure E-7: Model predicted concentrations for digestive tissues compared with measured concentrations for GII.3 reported by Ventrone et al. (2013). Seeded concentration 5.8 log10 RNA copies L-1. Parameters as in Tables E-3 through E-5.

E.11 Numerical solutions

Even though we have presented some analytical solutions, for time-varying seawater concentrations we must resort to a numerical scheme to obtain approximate (but accurate) solutions.

We can consider using finite difference techniques to solve Equations (E.1)–(E.4) in which, for the left-hand-sides of these equations, we make the approximation $dc/dt \approx (c_{m+1} - c_m)/\Delta t$, where m denotes the time level (i.e., $t_m = m\Delta t$) after the initial time ($t = 0$).[70] The remaining issue is how to approximate the equations' right-hand-sides. In considering that, note that two potentially difficult issues arise in solving these equations: (i) sharp concentration fronts (when contamination first arrives at the oyster site) and (ii) with likely parameter settings Equations (E.1)–(E.4) are 'stiff'—having a mixture of fast and slow processes. So, approximating these right-hand-side terms at the prior time level (m) using explicit methods (Euler, Runge-Kutta), can cause some instabilities and front-smearing. However, it seems that these problems can generally be overcome using fully implicit solutions (Chapra & Canale 1988, Section 26.1; Press et al. 1992, Section 16.6).

At this stage, to keep things general, we use a time-weighted approximation to the right-hand-sides of the equations using

$$c(t) \approx w c_{m+1} + \bar{w} c_m : \quad t_m \le t \le t_{m+1} \tag{28}$$

where $0 \le w \le 1$ and $\bar{w} = w - 1$. Also, we simplify the equations by defining

$$\xi_1 = \frac{\sigma\lambda}{\phi_g}; \quad \xi_2 = (1-\sigma)\frac{\lambda}{\phi_d}; \quad \xi_3 = \frac{\alpha\phi_d}{\phi_n} \tag{29}$$

[70] It is usual to use n as the time index, but that would clash with our prior usage of n.

so that we obtain the direct time-weighted finite difference equations

$$c_{g,m+1} = \frac{c_{g,m} + \left[\xi_1\left(wc_{s,m+1} + \overline{w}c_{s,m}\right) - \overline{w}k_g c_{g,m}\right]\Delta t}{1 + wk_g\Delta t} \tag{30}$$

$$c_{f,m+1} = \frac{c_{f,m} + \left[\xi_2\left(wc_{s,m+1} + \overline{w}c_{s,m}\right) - \overline{w}\eta c_{f,m}\right]\Delta t}{1 + w\eta\Delta t} \tag{31}$$

$$c_{b,m+1} = \frac{c_{b,m} + \left[\beta\left(wc_{f,m+1} + \overline{w}c_{f,m}\right) - \overline{w}k_b c_{b,m}\right]\Delta t}{1 + wk_b\Delta t} \tag{32}$$

$$c_{n,m+1} = \frac{c_{n,m} + \left[\xi_3\left(wc_{f,m+1} + \overline{w}c_{f,m}\right) - \overline{w}k_n c_{n,m}\right]\Delta t}{1 + wk_n\Delta t} \tag{33}$$

Often such implicit approaches will require iterations (i.e., they are not 'direct'), because the right-hand-sides of the resulting simultaneous finite difference equations will contain values of unknowns that cannot be directly calculated. However examination of Equations (E.1)–(E.4) [or, equivalently, Equations (E.30)–(E.33)] shows that, if calculated in the order presented, all terms on the equations' right-hand-sides will be known: hence, a direct solution can be obtained without iteration.[71]

Note that for $w = 0$ we obtain the direct Euler backward difference equations

$$c_{g,m+1} = c_{g,m} + (\xi_1 c_{s,m} - k_g c_{g,m})\Delta t \tag{34}$$

$$c_{f,m+1} = c_{f,m} + (\xi_2 c_{s,m} - \eta c_{f,m})\Delta t \tag{35}$$

$$c_{b,m+1} = c_{b,m} + (\beta c_{f,m} - k_b c_{b,m})\Delta t \tag{36}$$

$$c_{n,m+1} = c_{n,m} + (\xi_3 c_{f,m} - k_n c_{n,m})\Delta t \tag{37}$$

while for $w = 1$ we obtain the direct fully implicit equations

$$c_{g,m+1} = \frac{c_{g,m} + \xi_1 c_{s,m+1}\Delta t}{\mu_g}: \quad \mu_g = 1 + wk_g\Delta t \tag{38}$$

$$c_{f,m+1} = \frac{c_{f,m} + \xi_2 c_{s,m+1}\Delta t}{\mu_f}: \quad \mu_f = 1 + w\eta\Delta t \tag{39}$$

$$c_{b,m+1} = \frac{c_{b,m} + \beta c_{f,m+1}\Delta t}{\mu_b}: \quad \mu_b = 1 + wk_b\Delta t \tag{40}$$

[71] This is an important time-saver for QMRA calculations.

$$c_{n,m+1} = \frac{c_{n,m} + \xi_3 c_{f,m+1} \Delta t}{\mu_n} : \quad \mu_n = 1 + w k_n \Delta t \tag{41}$$

E.11.1 Indicative results for general cases: parameter sensitivity

The numerical model has been used to obtain results for constant microbial concentration in the water (Figure E-8) and a 'top hat' concentration profile (Figure E-9). Parameter sensitivity is addressed using a 'Tornado Plot' from Monte Carlo simulations for a particular parameter constellation, as shown in Figure E-10.[72] Those results indicate that, for that constellation at least, λ (specific filtration rate) and β (binding rate from the 'free' phase in digestive tissue) are the most influential parameters.

Calculations of steady-state analytical solutions for constant water virus concentration (c_s = 0.007 # L^{-1}) agree with the asymptote of the long-term numerical solutions [i.e., $c(t \to \infty)$ = 16.485 # L^{-1}], as indicated on Figure E-8.

E.12 Incorporation into QMRA procedures

The main difficulty in incorporating the mathematical model into the QMRA procedure is that the model is inherently time-based whereas the risk calculation is frequency-based. The Monte Carlo simulation also involves a large number of iterations, which is computationally prohibitive towards directly incorporating the model. A possible option has been identified that would allow the model to inform the QMRA procedure without having to significantly modify the existing calculation structure, as discussed below. This option relies on the fact that a time history of microbial concentrations in the water overlying the shellfish is already generated (as a preceding step to the Monte Carlo simulation). These time histories are usually determined using one or more pollutant transport models and are then converted to probability distributions, from which the microbial concentrations in seawater are drawn. Using the model to inform the QMRA calculation would then be a two-step process:

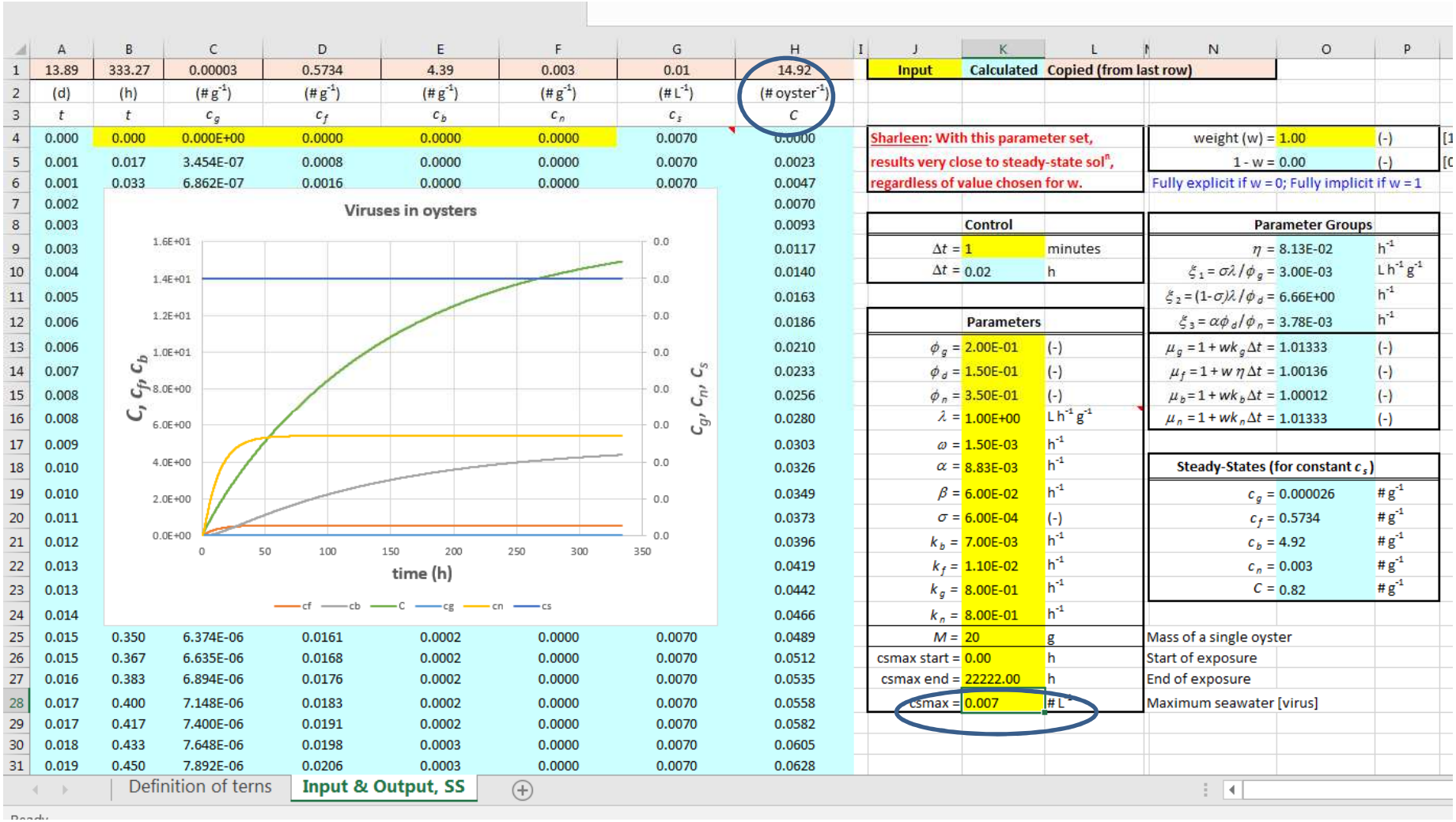
1. Use the time history of microbial concentrations in the overlying water to inform the shellfish model only, and from the results calculate a probability distribution for the microbial concentration in shellfish. This may involve either:
    a. running the model with the time history as an input and then converting the results to a probability distribution, or
    b. using the time history to characterise the site (e.g., frequent small contamination events vs infrequent large contamination events), and then selecting an appropriate shellfish distribution from a set of pre-defined model runs.
2. Use the probability distribution for the microbial concentration in shellfish flesh as an input into the Monte Carlo simulation.

In essence, this leaves the existing basic QMRA model structure unchanged, and is therefore much less cumbersome than trying to incorporate the model directly (note that there is little need to incorporate a time history into swimmers' exposures). Implementation of the process will be the subject of further work in 2014/15.

[72] Computed using @RISK (Palisade Corp. 2013).

See the Appendix of Harper and McBride (2015) for further details concerning:

- Deriving model equations from first principles.
- Performing mass balance checks.
- Fitting curves to measured digestive tissue concentrations and comparison with model equations.



	A	B	C	D	E	F	G	H
1	13.89	333.27	0.00003	0.5734	4.39	0.003	0.01	14.92
2	(d)	(h)	(# g^{-1})	(# g^{-1})	(# g^{-1})	(# g^{-1})	(# L^{-1})	(# oyster^{-1})
3	t	t	c_g	c_f	c_b	c_n	c_s	C
4	0.000	0.000	0.000E+00	0.0000	0.0000	0.0000	0.0070	0.0000
5	0.001	0.017	3.454E-07	0.0008	0.0000	0.0000	0.0070	0.0023
6	0.001	0.033	6.862E-07	0.0016	0.0000	0.0000	0.0070	0.0047
7	0.002							0.0070
8	0.003							0.0093
9	0.003							0.0117
10	0.004							0.0140
11	0.005							0.0163
12	0.006							0.0186
13	0.006							0.0210
14	0.007							0.0233
15	0.008							0.0256
16	0.008							0.0280
17	0.009							0.0303
18	0.010							0.0326
19	0.010							0.0349
20	0.011							0.0373
21	0.012							0.0396
22	0.013							0.0419
23	0.013							0.0442
24	0.014							0.0466
25	0.015	0.350	6.374E-06	0.0161	0.0002	0.0000	0.0070	0.0489
26	0.015	0.367	6.635E-06	0.0168	0.0002	0.0000	0.0070	0.0512
27	0.016	0.383	6.894E-06	0.0176	0.0002	0.0000	0.0070	0.0535
28	0.017	0.400	7.148E-06	0.0183	0.0002	0.0000	0.0070	0.0558
29	0.017	0.417	7.400E-06	0.0191	0.0002	0.0000	0.0070	0.0582
30	0.018	0.433	7.648E-06	0.0198	0.0003	0.0000	0.0070	0.0605
31	0.019	0.450	7.892E-06	0.0206	0.0003	0.0000	0.0070	0.0628

Input | Calculated | Copied (from last row)

Sharleen: With this parameter set, results very close to steady-state sol^n, regardless of value chosen for w.

weight (w) =	1.00	(-)
1 - w =	0.00	(-)

Fully explicit if w = 0; Fully implicit if w = 1

Control		
Δt =	1	minutes
Δt =	0.02	h

Parameters		
ϕ_g =	2.00E-01	(-)
ϕ_d =	1.50E-01	(-)
ϕ_n =	3.50E-01	(-)
λ =	1.00E+00	L h^{-1} g^{-1}
ω =	1.50E-03	h^{-1}
α =	8.83E-03	h^{-1}
β =	6.00E-02	h^{-1}
σ =	6.00E-04	(-)
k_b =	7.00E-03	h^{-1}
k_f =	1.10E-02	h^{-1}
k_g =	8.00E-01	h^{-1}
k_n =	8.00E-01	h^{-1}
M =	20	g
csmax start =	0.00	h
csmax end =	22222.00	h
csmax =	0.007	# L^{-1}

Parameter Groups		
η =	8.13E-02	h^{-1}
$\xi_1 = \sigma\lambda/\phi_g$ =	3.00E-03	L h^{-1} g^{-1}
$\xi_2 = (1-\sigma)\lambda/\phi_d$ =	6.66E+00	h^{-1}
$\xi_3 = \alpha\phi_d/\phi_n$ =	3.78E-03	h^{-1}
$\mu_g = 1 + wk_g\Delta t$ =	1.01333	(-)
$\mu_f = 1 + w\eta\Delta t$ =	1.00136	(-)
$\mu_b = 1 + wk_b\Delta t$ =	1.00012	(-)
$\mu_n = 1 + wk_n\Delta t$ =	1.01333	(-)

Steady-States (for constant c_s)		
c_g =	0.000026	# g^{-1}
c_f =	0.5734	# g^{-1}
c_b =	4.92	# g^{-1}
c_n =	0.003	# g^{-1}
C =	0.82	# g^{-1}

Mass of a single oyster
Start of exposure
End of exposure
Maximum seawater [virus]

Definition of terns | Input & Output, SS

Figure E-8: Numerical model results for constant water Norovirus contamination.

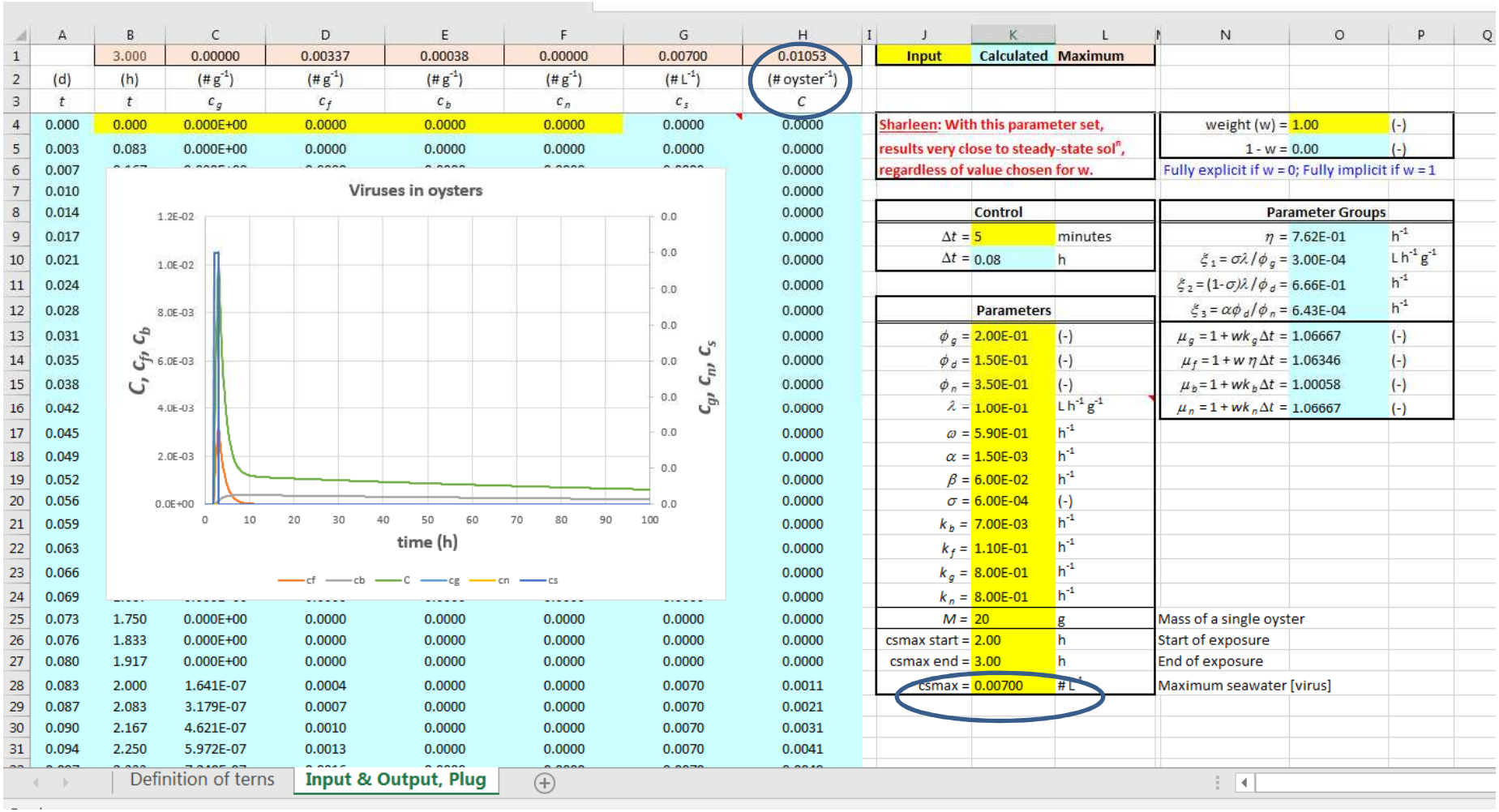


Figure E-9: Numerical model results for 'top-hat' water Norovirus contamination. Blue ellipses show key input (yellow cell) and output (orange cell).

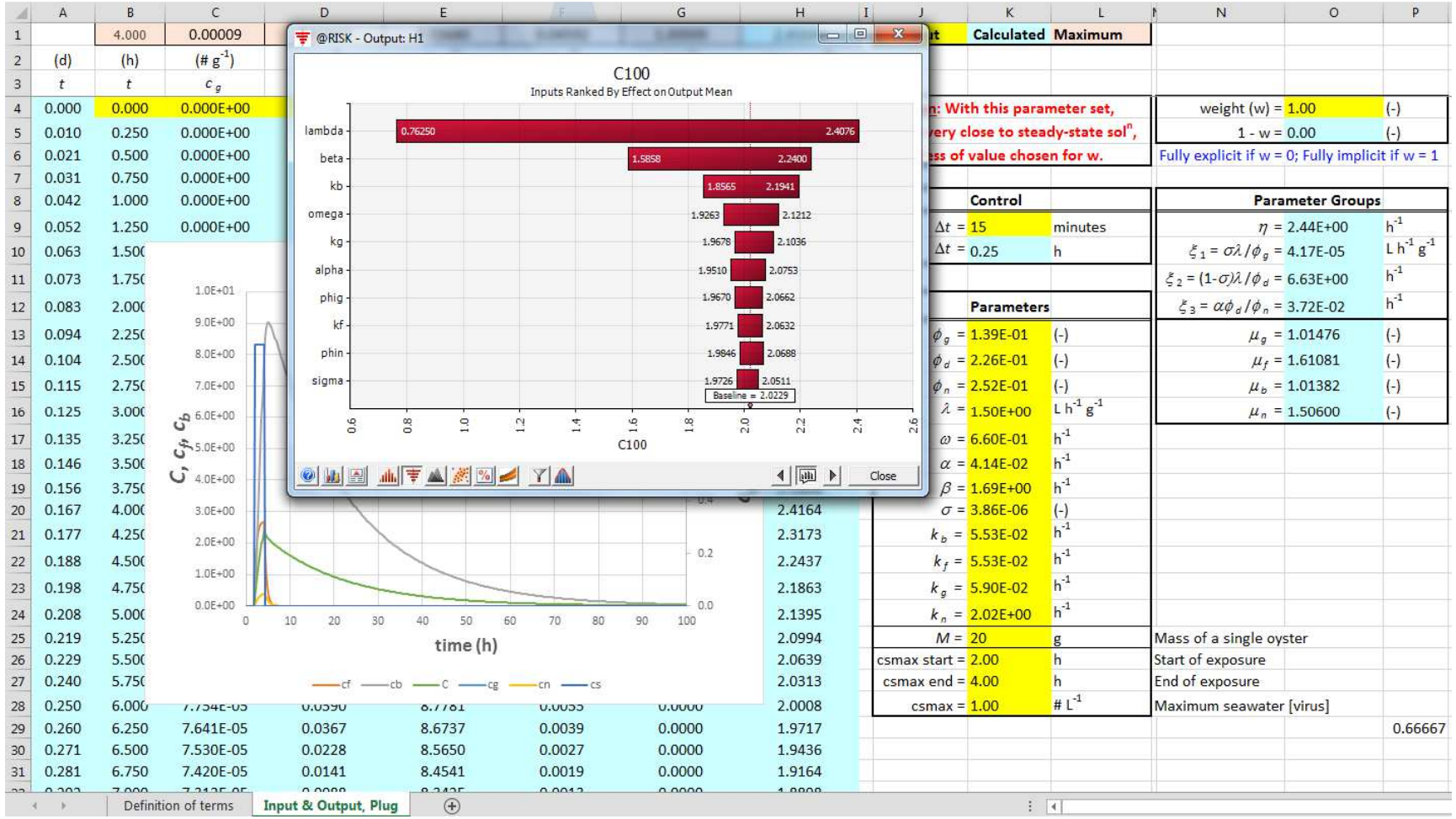


Figure E-10: Parameter sensitivity, obtained by running the fully implicit numerical model for 5000 iterations using @RISK.

Appendix F Models for choice of indicator(s) for brackish waters

Decision rules for this choice can be informed by appropriate models, described herein.

Approach

We set up a model for the time and longitudinal variation of *E. coli* and enterococci down an idealised estuary, to examine the point at which the advantage of one microbe outweighs the other in terms of indicated illness risk.

A one-dimensional physical estuary model

Figure F.1 displays a general estuary setup from an up-river origin (where the seaward flowrate is constant) to the mouth (where flow direction reverses between ebb and flood tides).

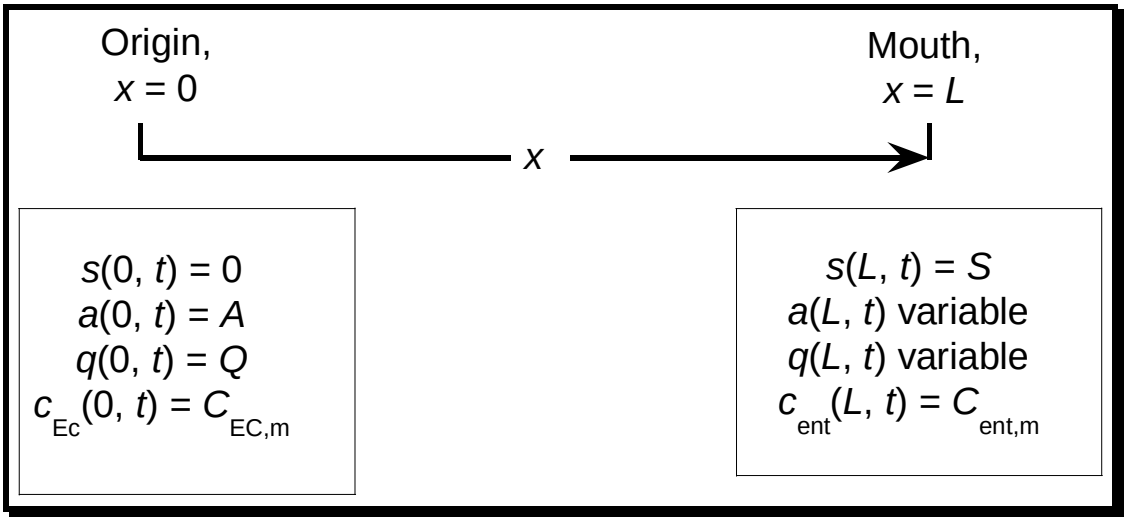


Figure F-1: Stylised estuary where, s is salinity, a is cross-section area, q is flowrate, c_{Ec} is *E. coli* concentration and c_{ent} is enterococci concentration. Upper case letters (S, A, Q, $C_{Ec,m}$ and $C_{ent,m}$) denote constant values.

In this setup, x is the distance along an estuary from a point on the inflowing river. The 'origin' is at $x = 0$ and the mouth is at $x = L$. We define salinity as a function of distance along the estuary and time (t), i.e., $s(x, t)$. There is never any salinity at the origin [i.e., $s(0, t) = 0$]. The (constant) coastal salinity on the flood tide (at the mouth) is S.[73] The cross-section area (a) and flow (q) at the origin are constant, i.e., $a(0, t) = A$ and $q(0, t) = Q$, but vary downstream.

Faecal indicator transition

E. coli concentration is denoted as $c_{Ec}(x, t)$.[74] *E. coli* is used as the faecal indictor at the origin and for some as-yet undefined point along x, between $x = 0$ and $x = L$. We denote this point as $x = x_t$, where the 't' subscript denotes 'transition'. Beyond x_t, the indicator-of-choice transitions to enterococci, with concentration denoted as $c_{ent}(x, t)$].

[73] This ignores return (to the estuary) of any part of the volume discharged on the previous ebb tide.

[74] 'c' denotes 'concentration'.

Assumptions

1. Inactivation[75] follows first-order kinetics.[76]
2. There are no point sources in the estuary.
3. Dispersion processes are ignored.
4. The estuary is completely marginal for compliance. Using the median faecal indicator requirements for *E. coli* and enterococci in the 1992 provisional guidelines (DoH 1992), for the inflowing river water the *E. coli* concentration is constant (126 per 100 mL), regardless of tide, i.e., $c_{Ec}(0, t) = C_{Ec,m}\ \forall t$. The inflowing enterococci concentration at the mouth on the flood tide is constant $c_{ent}(L, t) = C_{ent,m} = 33$ per 100 mL. The concentration on the ebb tide at the mouth is not specified, it can be calculated by the model.[77]

Inactivation coefficients

Denote these coefficients as k_{Ec} and k_{ent}, for *E. coli* and enterococci, respectively. We know that:[78]

a) The enterococci coefficient is independent of salinity but dependent on time, written as $k_{ent}(t)$.
b) The *E. coli* coefficient is dependent of time and salinity, written as $k_{Ec}(x, t)$.
c) For freshwater at any given time, the *E. coli* value is less than the enterococci value, i.e., $k_{Ec}(x=0, t) < k_{ent}(t)$.
d) The *E. coli* coefficient for coastal water is a constant multiple of the enterococci value at the same time. That is

$$k_{Ec}(x=L) \approx \eta k_e \tag{42}$$

where $\eta \approx 2$ (pers. comm., Dr Rob Davies-Colley, NIWA, Hamilton).

Equations to be solved

We need to solve mass balance equations for water, salinity, *E. coli* concentration and enterococci concentration, after specifying the profiles of cross-section area and velocity, $a(x, t)$ and $u(x, t)$ [$= q(x, t)/a(x, t)$], and specifying arbitrary initial conditions [$a(x, 0)$, $q(x, 0)$, $s(x, 0)$, $c_{Ec}(x, 0)$ and $c_{ent}(x, 0)$].[79]

The equation for the mass balance for water ('continuity equation') is

$$\frac{\partial a}{\partial t} + \frac{\partial q}{\partial x} = 0: \quad a(0,t) = A; q(0,t) = Q \tag{43}$$

---

[75] Sunlight (UV) drives inactivation. Note that other processes can lead to concentration loss (grazing, flocculation, settling). These are ignored, because: (i) They are assumed to be of secondary importance, and (ii) These processes were not included in the experiments to be referred to (pers. comm., Dr Rob Davies-Colley, NIWA, Hamilton).

[76] First-order decay kinetics are described by the simple ordinary differential equation $dC(t)/dt = -kC(t)$, where C is a concentration, t is time, and k (>0) is the inactivation coefficient. This equation has the general solution $C(x) = C_0 e^{-kt}$, were C_0 is microbial concentration at the origin [i.e., $C_0 = C(x=0)$]. It is 'first-order' because the right-hand-side of the differential equation contains C to the first power only, i.e., $C = C^1$. Note that in a steady-state system, t can be taken as a time-of-travel, in which case $t(x) = x/U$, where U is water speed.

[77] '$\forall$' denotes 'for all vales of'. We use the 1992 (Provisional) guidelines because they are based on medians, whereas the current guidelines (MfE/MoH 2003) are based on 95%iles. The 1992 medians require that $C_{Ec,m} \leq 126$ per 100 mL and $C_{ent,m} \leq 33$ per 100 mL.

[78] Davies-Colley et al. (1994), Sinton et al. (1994, 2002), Nelson (2018).

[79] These initial conditions have practically no influence on the results calculated after many tidal cycles: they merely provide a 'cold start'. So, they are taken as implied by each mass balance equation, not stated with those equations (avoiding clutter).

The equation for the mass balance for salinity is

$$\frac{\partial(as)}{\partial t}+\frac{\partial(qs)}{\partial x}=0:\quad s(0,t)=0;s(L,t)=S \text{ (flood tide only)} \tag{44}$$

The equation for the mass balance for microbes is

$$\frac{\partial(ac_{Ec})}{\partial t}+\frac{\partial(qc_{Ec})}{\partial x}=-ak_{Ec}c_{Ec}=-a\eta k_{ent}c_{Ec}:\quad c_{Ec}(0,t)=C_{Ec,m} \tag{45}$$

where the subscript on the last term ('m') denotes 'marginal for compliance'. For enterococci, we have

$$\frac{\partial(ac_{ent})}{\partial t}+\frac{\partial(qc_{ent})}{\partial x}=-Ak_{ent}c_{ent}:\quad c_{ent}(L,t)=C_{ent,m} \tag{46}$$

Solving these equations is 'do-able' but beyond the resources of this project. In any event it may not furnish more information than may be obtained from a rather simpler approach.

A simpler approach

Abandon these complicated models, including box models, and following O'Kane (1980), assume *quasi steady-state* at mid-tide in daylight, using time-of-travel as the one dependent variable. It is related to distance (x) and velocity (u) as

$$t = x / u \tag{47}$$

E. coli

Here we consider the ebb-tide, for which the indicator of interest is *E. coli*. We do so because the water flow is directed to the mouth, driven by the reducing sea level and river inflow.

In all that follows we transform equations to 'dimensionless form', considerably widening the applicability of results, because the number of parameters is then reduced by two, removing L and the initial *E. coli* concentration [$c_{Ec}(x = 0) = C_{Ec,m}$]. So, we define dimensionless distance (ξ) as

$$\xi=\frac{x}{L}:\quad 0\le\xi\le 1 \tag{48}$$

and define the dimensionless *E. coli* concentration as

$$\chi_{Ec}(\xi)=\frac{c_{Ec}(\xi)}{c_{Ec}(0)}:\quad 0<\chi_{Ec}(\xi)\le 1 \tag{49}$$

where, as before, $c_{Ec}(0) = C_{Ec,m}$.

We can now develop the equation to be solved for *E. coli*, i.e., a simplified version of equation (45). But before doing so we must account for the spatial behaviour pattern of k_{Ec}, given the preceding discussion of inactivation coefficients. So, if we assume that k_{Ec} varies linearly over x, we have

$$k_{Ec}(x)=k_{Ec}(0)+[k_{Ec}(L)-k_{Ec}(0)]\left(\frac{x}{L}\right):\quad 0\le x\le L \tag{50}$$

Using equations (42) and (48) we obtain

$$k_{Ec}(x) = k_{Ec}(0) + [\eta k_{ent} - k_{Ec}(0)]\xi: \quad 0 \le \xi \le 1 \tag{51}$$

Note that the units of k in these equations are now per unit length, not per unit time.

We now have the ammunition to develop the simplified governing equation, in which case Equation (45) becomes

$$\frac{d[c_{Ec}(x)]}{dx} = -\{k_{Ec}(0) + [\eta k_{ent} - k_{Ec}(0)]\xi\} c_{Ec}(x): \quad c_{Ec}(0) = C_{Ec,m}; 0 \le x \le L \tag{52}$$

and so, dividing throughout by $C_{Ec}(0)$ and using x = ξL [from equation (48)], the dimensionless form of equation (52) is

$$\frac{d[\chi_{Ec}(\xi)]}{d\xi} = -(\alpha + \beta\xi)\chi_{Ec}(\xi): \quad \chi_{Ec}(0) = 1 \tag{53}$$

where the two dimensionless parameters are

$$\alpha = k_{Ec}(0)L, \quad \beta = \eta k_{ent} L - \alpha \tag{54}$$

The analytical solution to equation (53), with coefficients given by equation (54), is

$$\chi_{Ec}(\xi) = \exp\left[-\left(\alpha\xi + \frac{\beta^2\xi^2}{2}\right)\right] \tag{55}$$

satisfying the initial condition requirement, i.e., $\chi_{Ec}(0) = 1$.

Enterococci

Here we consider the flood-tide, for which the indicator of interest is enterococci. We do so because the water flow is directed to the hills, driven by the increasing sea level, overcoming the river inflow. In that case we must replace the distance variable used for the *E. coli* model with *L*–*x*.

We now define dimensionless distance (θ) as

$$\theta = \frac{L - x}{L}: \quad 0 \le \theta \le 1 \tag{56}$$

and the dimensionless enterococci concentration as

$$\chi_{ent}(\theta) = \frac{c_{ent}(\theta)}{C_{ent,m}}: \quad 0 < \chi_{ent}(\theta) \le 1 \tag{57}$$

it being understood that θ is also bounded by 0 and 1. The dimensionless enterococci equation is simple first-order, viz. [80]

[80] The enterococci model is simpler than the *E. coli* model, because its inactivation coefficient (k_{ent}) is taken as constant at all points at half-flood tide in daylight.

$$\frac{d[\chi_{ent}(\theta)]}{d\theta} = -(k_{ent}\, L)\chi_{ent}(\theta): \quad 0 < \chi_{ent}(\theta) \le 1 \tag{58}$$

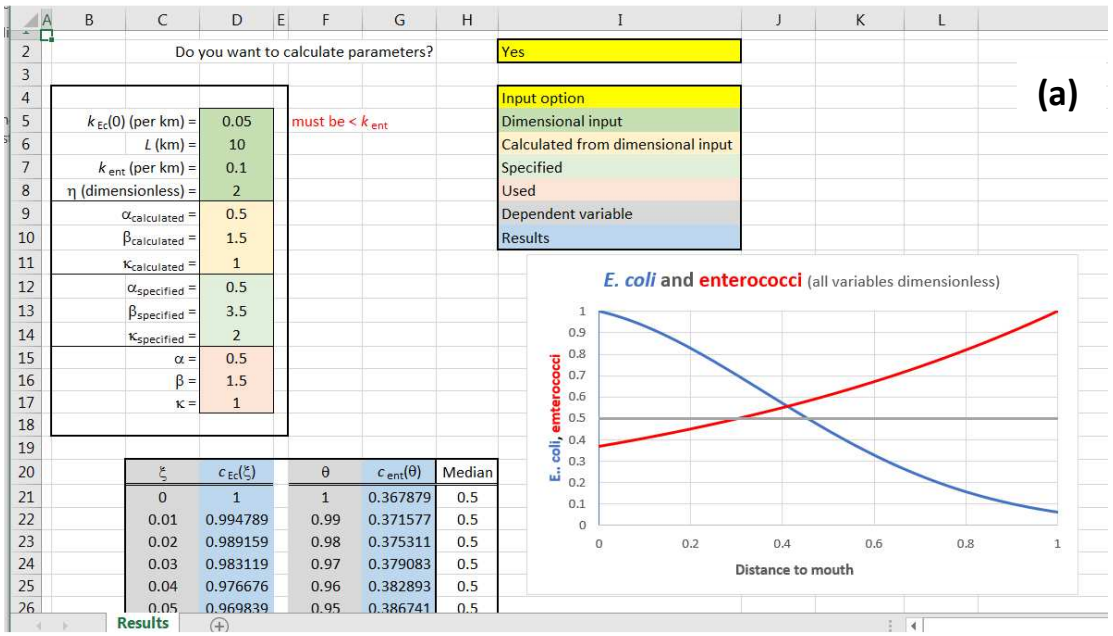
where k_{ent} has units of reciprocal length. The analytical solution is

$$\chi_{ent}(\theta) = e^{-\kappa_{ent}\,\theta}: \quad 0 < \chi_{ent} \le 1 \tag{59}$$

and where the single dimensionless parameter is

$$\kappa_{ent} = k_{ent} L \tag{60}$$

Results for a short residence time estuary are given in Figure F-1.



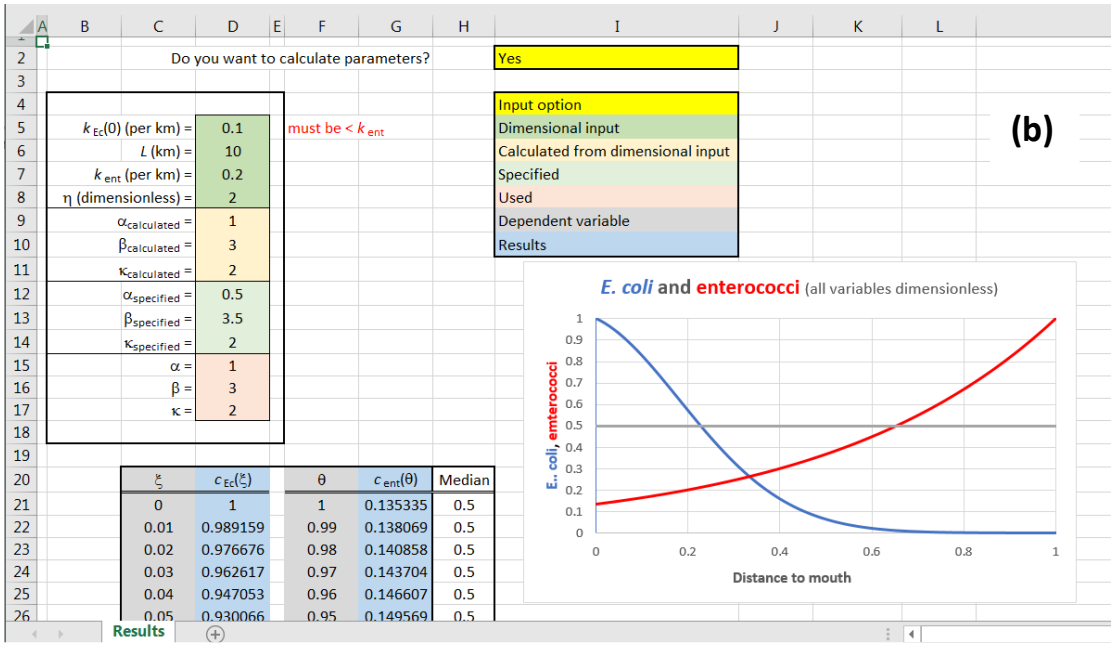


Figure F-1: Predicted half-tide dimensionless concentrations of *E. coli* (ebb tide) and enterococci (flood tide) for a short residence time 10 km river/estuary reach: (a) weak inactivation [$k_{Ec}(0)$ = 0.05 per km and k_{ent} = 0.1 per km]; (b) strong inactivation [$k_{Ec}(0)$ = 0.1 per km and k_{ent} = 0.2 per km].

Terms and units

a = channel cross-section area (L^2)

c = channel cross-section average microbe concentration (# L^{-3})[81]

k = first-order inactivation coefficient (L^{-1})

q = stream rate of water flow ($L^3\ T^{-1}$)

s = salinity (ppt)

t = time (T)

u = channel cross-section mean velocity is defined as $u = q/a$ ($L\ T^{-1}$)

x = distance along the channel (L).

[81] '#' denotes numbers of microbes; for substances like dissolved oxygen this symbol would be replaced by 'M', denoting mass.