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Quantitative microbial risk assessment for assessing risks to recreational users in Port Phillip Bay

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EPA acknowledges Aboriginal people as the first peoples and Traditional custodians of the land and water on which we live, work and depend.
We pay respect to Aboriginal Elders, past and present.

As Victoria's environmental regulator, we pay respect to how Country has been protected and cared for by Aboriginal people over many tens of thousands of years.

We acknowledge the unique spiritual and cultural significance of land, water and all that is in the environment to Traditional Owners, and recognise their continuing connection to, and aspirations for Country.

Foreword

Environment Protection Authority Victoria (EPA) has been conducting the Beach Report program for almost three decades. Beach Report provides recreational water quality advice for 36 beaches around Port Phillip Bay during the summer season when recreational use is highest. Advice for the public is provided based on daily forecasting and weekly sampling for water quality analysis. Information and reporting for this program is available on EPA's website (epa.vic.gov.au/summerwater).

Current criteria for assessing water quality are based on indicator levels recommended by the National Health and Medical Research Council's (NHMRC) *Guidelines for managing risks in Recreational Waters* (2008), which were also adopted in the State Environment Protection Policy (Waters) in 2018. These levels rely on the monitoring of enterococci as an indicator organism for faecal contamination, and make use of studies which may not be as relevant to the conditions of Port Phillip Bay. Indeed, the NHMRC Guidelines recommend that local health risks assessments be undertaken for water bodies such as Port Phillip Bay to ensure that the objectives for enterococci truly reflect the risks for recreational users.

Almost two million people visit Port Phillip Bay each year to enjoy water-based recreational activities. These activities are part of a healthy lifestyle for many Victorians. To assess the risks to these recreational users, EPA commissioned quantitative microbial risk assessments (QMRAs) of water quality during the 2017–18 swimming season for three popular beaches within Port Phillip Bay: Altona, Elwood and Frankston. QMRA is a holistic approach from source to receptor (in this case humans) that integrates all factors likely to affect microbial health risks to effectively provide a clear understanding of these risks.

To undertake the QMRA, a study of pathogens and faecal contamination in Port Phillip Bay was conducted and a risk analysis completed. The QMRA provided insights on the potential health outcomes for the recreational users of the Bay and quantified the risk of swimming at these three beaches. By quantifying health risk and understanding faecal sources, this approach can inform policy and Regulations (QMRA-based regulatory approach), guide investment in mitigation activities to reduce faecal contamination in the Bay and allow EPA to better communicate risk.

This report outlines the QMRA method, results and recommendations regarding risk characterisation in Port Phillip Bay, to support EPA's role in protecting Victorians from the effects of pollution and waste.

Executive summary

Unsafe levels of pathogens in coastal waters can result in illnesses in people and restrictions to water-based recreational activities due to beach closures, . Swimming and other recreational activities in pathogen-contaminated waters most frequently lead to gastroenteritis.

It is not possible to routinely measure all viruses, parasites and pathogenic bacteria in seawater. Therefore, representative faecal bacteria are used as 'indicators'. The presence of these bacteria indicates a potential contamination by faecal material from warm blooded animals (including humans). For marine waters, the indicator bacterium used most frequently is enterococci because it has been shown to have a dose-response relationship with gastrointestinal and respiratory illnesses in marine waters.

Testing for enterococci is relatively simple and low cost. However, their use as indicators of microbial water quality is limited as they cannot provide information on the different sources of faecal material or hazards in a catchment. They also conservatively assume that all sources of pathogens are from human origin. They may therefore under or overestimate the risk of exposure to pathogen concentrations and the potential impact on human health. To better understand these health risks from water-based recreation in Port Phillip Bay, a quantitative microbial risk assessment (QMRA) study was conducted at three locations (Altona, Elwood and Frankston) over the 2017-18 summer.

QMRA is a holistic approach from faecal source to receptor. It integrates all factors likely to affect health risks from microbial exposure. These factors include pathogen densities, routes of exposure, volumes ingested or inhaled, population exposed, infectious doses and probabilities of getting ill when infected. The QMRA approach relies on monitoring reference pathogen densities and applies dose-response models to assess the probability of infection or illness based on exposure and pathogen densities. This approach is a lot more costly than faecal indicator testing and therefore is not used routinely to establish site-specific criteria. However, this technique provides a lot of valuable site-specific information that can be applied in Victorian waters and offers an opportunity to estimate potential adverse health outcomes based on local conditions at a relatively lower cost than an extensive epidemiological study.

In order to gain the most value from this study, the QMRA was supported by a concurrent source tracking study, to determine the biological origin of the faecal contamination at the three locations. Knowing the source of the faecal contamination (for example from dogs, human, birds etc) allows a better understanding of the risks to human health.

Relatively high bacterial indicator densities were observed in Altona, Elwood and Frankston but pathogen concentrations were much lower and rarely found above the detection limit of the testing method. Overall, the densities of different bacterial indicators correlated with each other, but correlations between indicators and pathogens were less clear. The exception to this was *E. coli* and enterococci indicator organisms which did correlate with *Salmonella* pathogen concentrations. Other parameters such as water clarity and turbidity were also significantly correlated with bacterial indicators and *Salmonella* concentrations.

The results of this QMRA indicated that there was less than 1% chance of a person contracting a gastrointestinal illness during a single primary-contact recreational event at Altona, Elwood or Frankston. This is a much lower risk than predicted by the NHMRC *Guidelines for managing risks in recreational waters* which suggested there is a 10% risk. This shows that faecal indicator testing may not provide an accurate representation of potential health outcomes in the bay. It can be overly conservative as it does not take local conditions into consideration.

Indicator organism testing assumes that 100% of the faecal material is of human origin. However, this source tracking study showed that human faeces only contributed an average of 13% of the total faecal contamination and the main contributors to faecal contamination were of avian and canine origin. These carry comparatively lower risks to human health. This suggests that considering the origin of the contamination should be a primary factor in assessing risks of water-based recreation in Port Phillip Bay, since it can significantly impact the outcome of the risk assessment.

The QMRA was limited to three locations and represented only a snapshot in time of the risk of potential illness. Despite this limited scope, the results of this QMRA have proved very informative. It is recommended that the results be validated with further study at the same sites, as well as at other sites within the bay to ensure the results are reflective of the ongoing risks to recreational users. This validation study should include microbial source tracking to confirm the influence of the source of faecal contamination on the risks to recreational users.

The study highlighted the benefits of establishing site-specific objectives based on the identified sources of contamination. These site-specific objectives, developed using a tiered-risk assessment approach, would mean that beach grades determined based on site-specific objectives would more accurately reflect potential health outcomes and enable EPA to provide better targeted information to recreational water users.

Contents

List of tables and figures

Glossary of terms

Sanitary inspection	Survey of all potential sources of contamination and their likelihood to impact the water quality at a beach.
D-R	Refers to dose-response models that link a dose of ingested pathogen to a probability of getting infected or contracting an illness.
Recovery rate	Percentage of a known microorganism number recovered after inoculation of that known microorganism number in a water sample
Gastrointestinal	Related to the stomach and the intestines
Recreational contact (Primary, secondary and tertiary contact)	Includes primary (whole body) contact recreation, that is activities in which the whole body or the face and torso are frequently immersed or the face is frequently wet by spray and it is likely that some water will be swallowed or inhaled, or come into contact with ears, nasal passages, mucous membranes or cuts in the skin. Secondary (incidental) contact recreation activities are activities in which only the limbs are regularly wet and in which greater contact (including swallowing water) is unusual and include occasional and inadvertent immersion through slipping or being swept away into the water by a wave. Tertiary (aesthetic uses) contact

	recreation activities are activities in which there is normally no contact with water (for example angling from shore), or where water is incidental to the activity (such as sunbathing on a beach).
Pathogen	Causative agent of disease. Microbial pathogens are microscopic organisms. They include bacteria, viruses, protozoa and fungi.
Disease	Illness caused by a pathogen.
Indicator	Microorganism, usually bacteria, that indicates the potential presence of pathogens
E. coli	Bacteria used as a faecal indicator due to its presence in high numbers in the faeces of warm-blooded animals. *E. coli* is the recommended indicator for freshwater. In marine waters, it usually indicates recent faecal contamination
Enterococci	Bacteria used as a faecal indicator due to its presence in high numbers in the faeces of warm-blood animals. Enterococci is the recommended indicator for marine waters.
Enteric	Relating to, or occurring in, the intestines
Monte Carlo approach	A Monte Carlo simulation uses computational algorithms to provide a large number of iterations by repeated random sampling of several variables. This approach means that uncertainty is accounted for, providing a fuller picture of potential outcomes.
Bather shedding	Faecal matter shed by recreational users during water-based recreational activities.
16S microbial community profile	Microbial community profile based on the analysis of the 16S ribosomal RNA subunit gene

Acronyms and abbreviations

QMRA	Qualitative microbial risk assessment
qPCR	Quantitative polymerase chain reaction
SEPP (Waters)	State Environment Protection Policy (Waters)
MAC	Microbial assessment category
SIC	Sanitary inspection category
p(ill/inf)	Probability of illness given infection
GI	Gastrointestinal
ERS	Environment Reference Standard

Introduction

Faecal contamination is a leading cause of coastal water quality degradation and risks to human health (Lepesteur et al. 2006; Prüss 1998). Current guidelines to manage risks in recreational waters rely on the monitoring of indicator organisms to assess water quality because these organisms suggest the presence of pathogens which may cause disease in humans (Ashbolt et al. 2001).

Environment Protection Authority Victoria (EPA) forecasts water quality for recreational use by monitoring the densities of enterococci. This indicator organism generally indicates the presence of faecal contamination, thus the potential presence of pathogens and the risk of adverse health impacts from exposure to water contaminated by pathogens. Microbial assessment categories (MACs) have been derived from epidemiological studies that relate indicator organism densities to human illness rates. Such epidemiological studies are common for coastal waters with oceanic influence and known point-sources of pollution (such as raw or partially treated sewage) (Fewtrell & Kay 2015). However, Port Phillip Bay beaches are on a large embayment mainly impacted by non-point sources of pollution, such as urban stormwater discharges. Since the sources of contamination and environmental conditions are different, it is likely that actual risks of swimming in the bay are also different.

Beach grades reported by Beach Report on the EPA website are based on sanitary inspections as described in the SEPP (Waters) and MACs to feed into a risk matrix (NHMRC, 2008). The sanitary inspections are a stocktake of the potential sources of faecal contamination for a specific beach and their likelihood of impacting the water quality of that beach. This semi-quantitative approach is simple and low cost. However, it has limitations as it does not provide information regarding the different hazards and hazardous events throughout the catchment and assumes conservatively that all sources of pathogens are from human origin.

To better understand the health risks associated with water-based recreation in Port Phillip Bay, EPA commissioned QMRAs at three popular beaches in the bay, combined with microbial source tracking at each location over the summer season 2017-2018.

QMRA is a framework that uses quantitative scientific information and data, interprets them in the context of estimated health outcomes, supporting water management decisions and assisting in the prioritisation of remedial or further research efforts (WHO, 2016). QMRA involves the gathering of information about pathogen densities, the application of dose-response models to assess the probability of infection or illness based on exposure and pathogen densities. It therefore enables a better prediction of health risks than any assessment solely relying on faecal indicator monitoring (Ashbolt et al. 2010).

The results of the QMRA aimed to inform assessments of risk from microbial contamination for recreational uses as well as the human health risk assessment process. This report outlines the QMRA method, main results and recommendations regarding risk

characterisation in Port Phillip Bay, to support EPA's role in protecting Victorians from the effects of pollution and waste.

Objectives

The objectives of this project were:

- to ascertain whether indicators and pathogen densities could predict risks of illness at the three beach locations within the Bay
- to compare the probability of illness from water-based recreational activities at three beach locations within the Bay, as calculated using a QMRA approach, to the risk portrayed by SEPP (Waters) and the National Health and Medical Research Council (NHMRC) *Guidelines for Managing Risks in Recreational Waters* released in 2008
- to investigate what factor(s) could impact the level of risk in the Bay
- to provide an example of how a QMRA could be conducted and provide parameters and key assumptions for future application.

Methods

Site selection

Three beaches: Altona, Elwood and Frankston beaches, were selected for the study as these sites represented most beach types around Port Phillip Bay Melbourne (Figure 1). Site selection was a balance between many factors, including:

- frequent recreational use
- track-record of poor water quality
- variability of oceanic exchange rates between sites
- variability of pollution sources between sites.

Figure 1. Map of Port Phillip Bay beaches indicating the three sites used for the QMRA (red rectangles).

Sanitary inspections

Desktop surveys were conducted at the selected sites to identify:

- location of storm and sanitary sewers
- emergency release systems
- proximity to creeks or rivers
- existing enterococci data from Beach Report.

A field-based inspection was conducted for Elwood only, where water samples were collected from flowing stormwater drains and analysed for *E. coli* and enterococci.

The sanitary inspection form used to calculate the likelihood scores for each of the potential sources of faecal contamination identified during sanitary inspections at Altona, Elwood and Frankston is presented in Appendix A.

Beach usage surveys

Twice per week during the 2017-2018 summer swimming season (November to March), the number of people and their activities were recorded over a 30-minute period. The days before and after major public holidays during this time (Christmas, New Year, Australia Day and Easter) were also surveyed. The following parameters were recorded:

- Proportion of primary, secondary (incidental) and tertiary contact (aesthetic) with the water. (Please refer to the glossary section for the full definition of primary, secondary and tertiary contact).
- Demographics, duration of activity, depth of wading and head immersion.
- Presence of birds and dogs on the beach and animals in contact or not with the water.

Relationships between rates of recreational use and parameters such as water temperature, air temperature, wind speed and cloud cover were assessed using Spearman Rank correlation analysis according to the method described in Spearman (2010).

Water quality monitoring

A total of 20 samples of water (60 L) were collected from each site twice per week during the 2017/2018 summer.

Water samples were analysed onsite for physical parameters using a Horiba U-52 (Horiba, Japan) for temperature, electrical conductivity, salinity, turbidity, dissolved oxygen and pH. Water clarity was assessed by the sampler as described in EPA protocol (EPA 2014).

Environmental parameters of cloud cover, wind speed and direction, air temperature, relative humidity, light intensity, were measured and recorded for each site. Detailed specifications of how each of these parameters are outlined in EPA (2014).

The samples were analysed for the faecal indicators and pathogens listed in appendices.

Reference pathogens were *Campylobacter, Salmonella, Cryptosporidium, Giardia,* enteroviruses, adenoviruses and noroviruses. Analytical methods used are listed in Appendix C.

On three occasions, 50 L samples were collected at each site and analysed using qPCR for noroviruses adenoviruses and enteroviruses (see methods in Appendix C).

Quality control: recovery rate testing

While published results can be used to estimate recovery rates and measurement uncertainties for each microorganism (Henry et al. 2016), it is far more accurate to obtain site-specific information about these aspects for QMRA modelling. Data from the recovery rate testing were used in the QMRA models to correct the measured concentrations for recovery rates and to take measurement errors into account.

On six occasions, water samples from Altona, Elwood and Frankston were spiked with known concentrations of each indicator and pathogen of interest. Each sample underwent the assay method as listed in Appendix C. The results of these recovery efficiency tests are shown in Table 1. On average, over 80% of *E. coli* and enterococci were recovered. The higher than 100% recovery with enterococci is likely due to the loss of specificity in the detection technique when spiking pure strains into marine waters. *Campylobacter* spp. and *Salmonella* spp. were recovered at an approximate rate of 50% and adenoviruses at

approximately 22%. These recovery rates were similar to, or slightly higher than, those reported in the literature for Victorian waters (Henry et al. 2015).

Table 1. Average recovery rates for each of the monitored organisms

Organism	Average Recovery (%)
E. coli	84
Enterococci	164
Campylobacter sp.	41
Salmonella sp.	58
Clostridium perfringens	38
Adenoviruses	22
Cryptosporidium sp.	52
Giardia sp.	41

Note: For *Giardia* and *Cryptosporidium,* each sample had its own recovery rate estimate.

For *Cryptosporidium* and *Giardia*, average recovery rates were 52% and 41% respectively, compared with 28-29% and 9-15% respectively reported by US EPA (2009).

QMRA model development

The model development followed the four steps of the risk assessment approach recommended by the World Health Organization (WHO 2016). That is, problem formulation, exposure assessment, health effect assessment and risk characterisation.

A literature review was conducted to define the parameters and assumptions for the QMRA model. Appendix C presents these model parameters and key assumptions.

Problem formulation – the model considered gastrointestinal illnesses (GI) and estimated risks for both primary and secondary contact exposure pathways for the general public. The model did not attempt to segregate the public into various groups such as children or those who have increased susceptibility to diseases. The QMRA considered seven reference pathogens: *Salmonella*, *Campylobacter*, *Cryptosporidium*, *Giardia*, adenoviruses, enteroviruses and noroviruses. The output of the model – that is the probability of illnesses per contact exposure – was modelled using a Monte Carlo approach to understand uncertainties and variabilities involved in QMRA modelling. The resulting probability distributions of risk were then compared to three broad thresholds combining the microbial assessment categories B and C from Table 13 in the SEPP (Waters) (gazette.vic.gov.au/gazette/Gazettes2018/GG2018S499.pdf into one category:

- Category 1: ≤ 1% additional GI (swim safely).
- Category 2: > 1% and ≤ 10% additional GI (swim at own risk).

- Category 3: >10% additional GI (do not swim).

MAC B and MAC C were combined to reflect the fact that beaches in Port Phillip Bay were not closed based on the result of one sample below 500 enterococci per 100 mL (short-term objective) which is the upper threshold of MAC C and corresponds to a probability of illness below 10% according to the NHMRC *Guidelines for Managing Risk in Recreational Waters* (2008).

Exposure assessment – the dose was a product of the exposure volume and the infectious pathogen density detected at each of the three beaches. The volumes of water ingested during primary and secondary contact recreation were estimated using distributions fitted to the datasets of Dufour et al. (2006) and Dorevitch et al. (2011). The primary contact exposure volume data was best estimated using an exponential distribution, producing a 50th percentile ingestion volume of 18.6 mL and a 95th percentile of 80.6 mL. The secondary contact ingestion volume was estimated using a log normal distribution, producing a 50th percentile ingestion volume of 2 mL and a 95th percentile of 17.1 mL.

Health Effect Assessment - dose-response (D-R) model and parameters used are listed in Appendix D. Other models used for sensitivity testing are also listed in Appendix D.

Appendix E provides the probabilities of illness given infection (p(ill|inf)) used in this study.

Risk characterisation – the QMRA modelling was conducted using Monte Carlo techniques to enable variations in doses, reflecting the variations observed in the literature for exposure volumes and pathogen concentrations in the water column. Exposure volumes for 100 people were generated, randomly drawn from either the primary or secondary ingestion volume distributions defined above (McBride et al. 2013). These 100 people were then exposed to 1000 different days of pathogen concentrations, randomly drawn from the datasets acquired from the three beaches used in this study. For the purpose of the QMRA, pathogen concentrations below the detection limit were assumed equal to half of the detection limit. The pathogen concentrations were then adjusted using the average recovery efficiency and the proportion of detected pathogens that are infectious, which was assumed to be 100% for the baseline QMRA. The dose was determined and used in dose-response models to calculate the probability of infection (or the probability of illness for *Salmonella*). The probability of infection was then used to determine the probability of illness using the p(ill|inf) distributions. This was repeated for each chosen pathogen and the aggregate probabilities of illness were calculated and used to determine statistical distributions for ingestion exposure during both primary and secondary contact. The resulting distributions were then compared to the broad thresholds indicated earlier. Further details on the key assumptions and methods used are available in Schang et al. (2020).

Two groups of QMRAs were run using this methodology:

- The baseline QMRA, which represents the best and likely most conservative estimate of risk.

- The second QMRA involves a series of sensitivity scenarios, where the sensitivity of some of the assumptions and uncertainties involved in the baseline QMRA were explored. This sensitivity testing is extremely important, as there are many uncertainties and assumptions involved in the QMRA process and only through a thorough understanding of these impacts may the outcomes of the baseline QMRA be truly appreciated. These sensitivity scenarios included:

    - testing the baseline QMRA by assuming that norovirus densities were equal to the maximum densities of enteroviruses or adenoviruses
    - testing the baseline QMRA using different dose-response models for *Campylobacter*, *Cryptosporidium*, adenovirus and norovirus (see Appendix D). For *Campylobacter*, two other dose-response models available in the literature were tested. For *Cryptosporidium*, the exponential model proposed by NRMMC (2006) was replaced with the model proposed by US EPA (2005). For adenoviruses, the Crabtree et al. (1997) model was replaced by the model developed by Teunis et al. (2016). For norovirus, the model proposed by Soller et al. (2017) was replaced by the models developed by Teunis et al. (2008) and Messner et al. (2014).

Source tracking

Source tracking was performed on 35 samples from the three beaches. Samples were selected to ensure that an even mix of the following conditions were present at each site:

1. high concentrations of indicators and pathogens
2. pathogen detection but low indicator concentrations
3. no pathogens detected but high indicator concentrations
4. low indicator concentrations and no pathogens detected.

As much as possible, samples were also chosen to represent an even mix of rainfall-influenced and dry-weather conditions.

Source tracking was performed using two methods. First, the HF183/BacR287 human *Bacteroides* marker set was used, following the US EPA standardised protocol, which detects human faecal pollution using a TaqMan® quantitative polymerase chain reaction (qPCR) assay. In brief, the samples were processed following the steps of method 1609, and 190 µL of final elute was then processed following the steps of Method 1696. All standards, method blanks, positive spikes, extraction blanks, internal amplification controls and extraction controls were performed as outlined in EPA Method 1696 (US EPA, 2019).

The second method used for source tracking was based on Henry et al. (2016) and McCarthy et al. (2017a). The SourceTracker model uses microbial fingerprints of source (gene sequence present in the sample) and sinks (gene sequences in known sources) to determine approximate contributions to each sample. In brief, samples were filtered on

0.22 µm filters, DNA was extracted and then sequenced using a variable region 3 and 4 of the 16s gene. Microbial community fingerprints were then compiled (Henry et al. 2016). These fingerprints were then used in the publicly available Source Tracker program and compared to an array of available sink fingerprints using a Bayesian approach. In this case, the following sink fingerprints were selected from our local Melbourne database including human sewage from the Eastern Treatment Plant and septic systems from around Melbourne, dog, chicken, waterfowl, seagull, horse and cattle. The output of the Source Tracker program is an estimate of the proportion of the beach sample community that is made up of each sink sample. The method is explained in detail in Henry et al. (2016).

Sensitivity and specificity tests were used to compare the results of the source tracking techniques to the pathogen data presented in this report. Sensitivity is defined here as the ability of the source tracking method to correctly identify the presence of a pathogen (calculated as the number of true positives divided by the sum of the true positives and false negatives), while specificity is the ability of the source tracking method to correctly identify the absence of a pathogen (calculated as the number of true negatives divided by the sum of the true negatives and false positives). Values of sensitivity and specificity close to 100% are desirable. Odds ratios were also calculated as the product of the true positives by the true negatives divided by the product of the false positives and false negatives.

Spearman correlation analyses (Zar 1999) were also undertaken to assess any relationships between the source tracking markers and the concentrations of indicator organisms or pathogens (MATLAB 2018).

Site-specific QMRAs

Soller et al. (2010) investigated the probability of illness after swimming at beaches that were contaminated by different sources of non-point and point sources of pollution. Their study demonstrated that for beaches that were all contaminated with 35 enterococci /100 mL, the risks would differ by almost three orders of magnitude depending on the source of the enterococci, for example human sewage or seagull faeces (Figure 2).

Additional QMRAs were run using the approach described by Soller et al. (2010) and the percent contributions from each site. The assumptions and inputs for the modelling were:

- All QMRA parameters were as per the baseline QMRA – that is dose response models, infectious percentage, etc. (See Appendix B, D and E).
- Pathogens used for this analysis were *Campylobacter*, *Salmonella*, *Giardia*, *Cryptosporidium*, and norovirus.
- For input into the QMRA, 1000 water quality scenarios were created. One thousand enterococci concentrations were generated for each site by fitting various distributions to each site dataset – it appeared that the log-normal was the best to

second best for all sites. As a result, a log normal distribution was created for each site, providing 1000 enterococci datapoints, representing 1000 different days.

- For each 1000 enterococci datapoints, the number of enterococci that belonged to each source was estimated by simply multiplying the average percentage from each source for the sources that were detected (that is sewage, dog, gull, horse and chickens). The resulting estimated enterococci concentrations from each of these sources in the water was represented as $C_{enterocciWATER}^{Y}$; where Y is the source of interest.

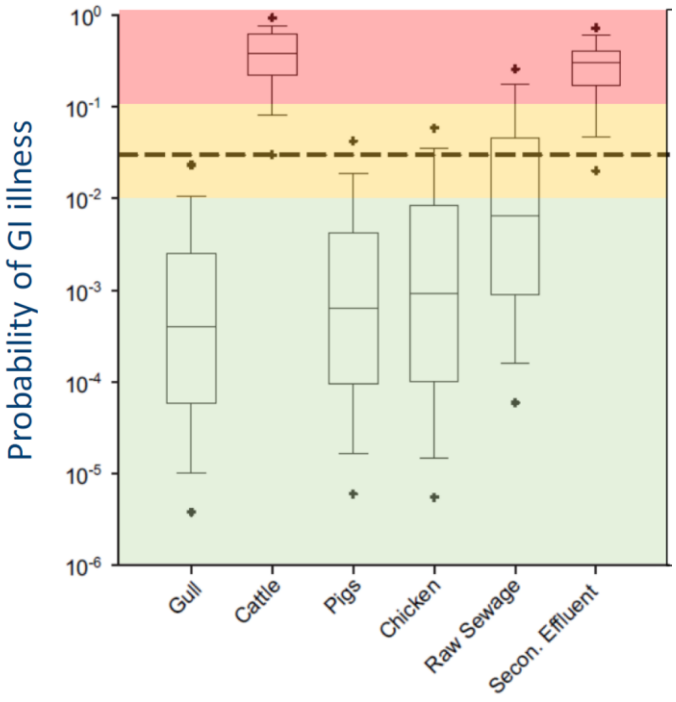


Figure 2. Probability of contracting a gastrointestinal illness by ingesting contaminated water containing 35 enterococci per 100 mL according to the source of faecal contamination.
(adapted from Soller et al. 2010)
The risk of 35 enterococci from secondary effluent is higher than the risk from 35 enterococci from raw sewage due to the inability of the secondary treatment to remove protozoa and viruses as efficiently as bacteria, meaning than 35 enterococci would represent more viruses and protozoa in secondary effluent than in raw sewage. The illness benchmark (dashed line) represents a geometric mean probability of illness of 0.03. Red shading indicates a GI illness risk of >10%, yellow of between 1% and 10% and green <1%.

- Concentrations of enterococci, *Campylobacter*, *Salmonella*, *Giardia*, *Cryptosporidium* and norovirus were estimated based on pathogen concentrations in sewage and in the faeces of dog, gull, horse and chickens reported in the literature (always reported in ranges). These concentrations were referred to as C_x^Y – that is the concentration of parameter X (enterococci, *Campylobacter*, etc.) in each source Y.

- For each of the 1000 simulated water quality days, the proportion of each source ($Prop^Y$) that was contained in the sample was estimated using: $Prop^Y = C_{enterocciWATER}{}^Y / C_{enterococci}{}^Y$.
- To estimate the concentration of each pathogen from each source, the proportion of each source was multiplied by the concentration of the pathogen in that source: $C_{X\ WATER}{}^Y = Prop^Y \times C_x{}^Y$.
- Prevalence and infective ratios were then applied to estimate the number of infective pathogens from each source in each water quality day.
  The model was then run 1000-10000 times with these concentrations of infective pathogens from each source to provide site-specific QMRAs (Cheun et al. 2019).

Results

Sanitary inspections

The desktop based sanitary survey confirmed that all three sites were in the highest risk category revealing multiple sources of faecal contamination at each site from bather shedding (Table 2).

Table 2. Summary of likelihood scores for each of the sources identified during sanitary inspections at Altona, Elwood and Frankston.

Source	Likelihood scores		
	Altona	Elwood	Frankston
Bathers	0.15	0.6	0.2
Toilet facilities	1	1	1
STP outfall within 2 km	0.2	-	-
STP bypasses or overflows	-	-	-
Sewage overflows within 1 km	0.2	-	0.2
Sewage chokes and leakages within 1 km	0.2	0.2	1
Onsite sewage disposal systems within 1 km	-	-	-
Wastewater reuse within 100 m	-	-	-
River discharge within 1 km	-	2	
Stormwater	1.3	0.6	2
Lagoon discharge within 500 m	-	-	-
Boats within 100 m	0.2	0.2	0.1
Animals present on site	1	0.2	0.6
TOTAL SCORE	4.25	4.8	5.1
Sanitary inspection category	HIGH	HIGH	HIGH

Notes: STP = sewage treatment plant

Beach usage surveys

Beach usage monitoring revealed that most people attending the beaches were tertiary users (that is no evidence of swimming or wading). Most people were simply walking, sunbathing or eating on the beach and a smaller fraction were secondary contact users

(boats, etc.). Only 5-15% of the surveyed persons entered the water for a primary contact exposure event (Figure 3).

Usage patterns varied considerably over the 12 weeks of monitoring (Figure 4), with usage peaks observed to coincide with sharp increases in air temperature. A significant positive relationship was observed between average air temperature during the survey period and the number of primary and secondary contact exposures (Figure 5). These surveys were used to estimate approximately 1.9 million primary and secondary contact exposure events in Port Phillip Bay beaches each year, a figure similar to EPA Victoria's beach usage estimate of 1.4 million per year.

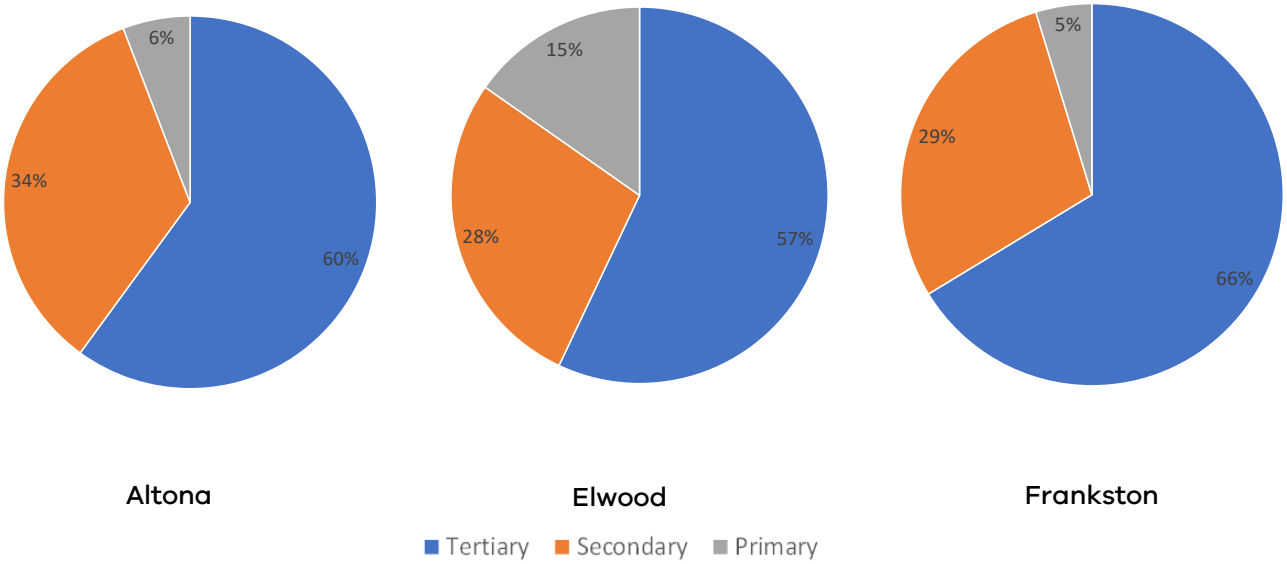


Figure 3. Average proportion (%) of primary, secondary and tertiary users observed at Altona, Elwood and Frankston

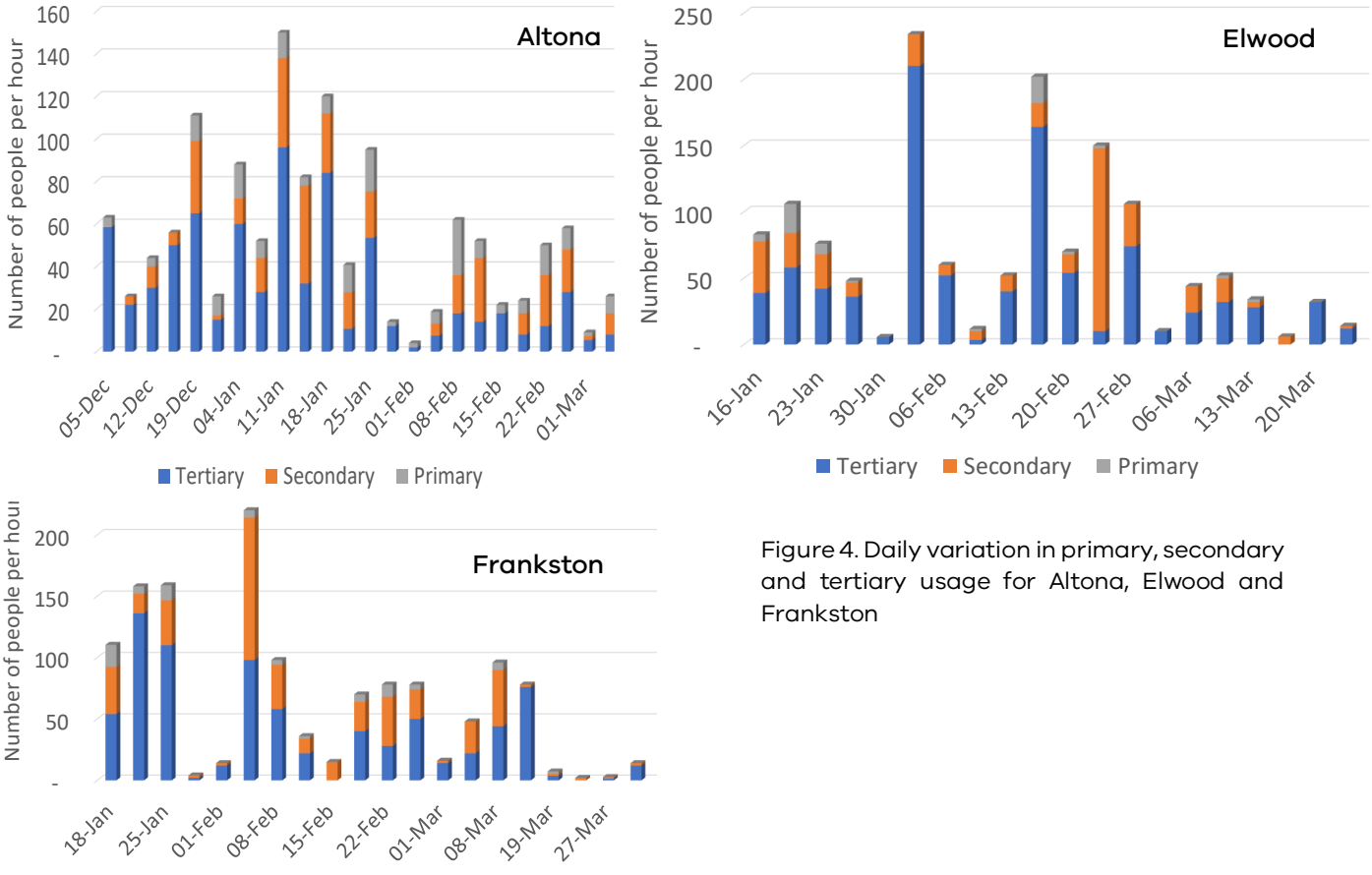


Figure 4. Daily variation in primary, secondary and tertiary usage for Altona, Elwood and Frankston

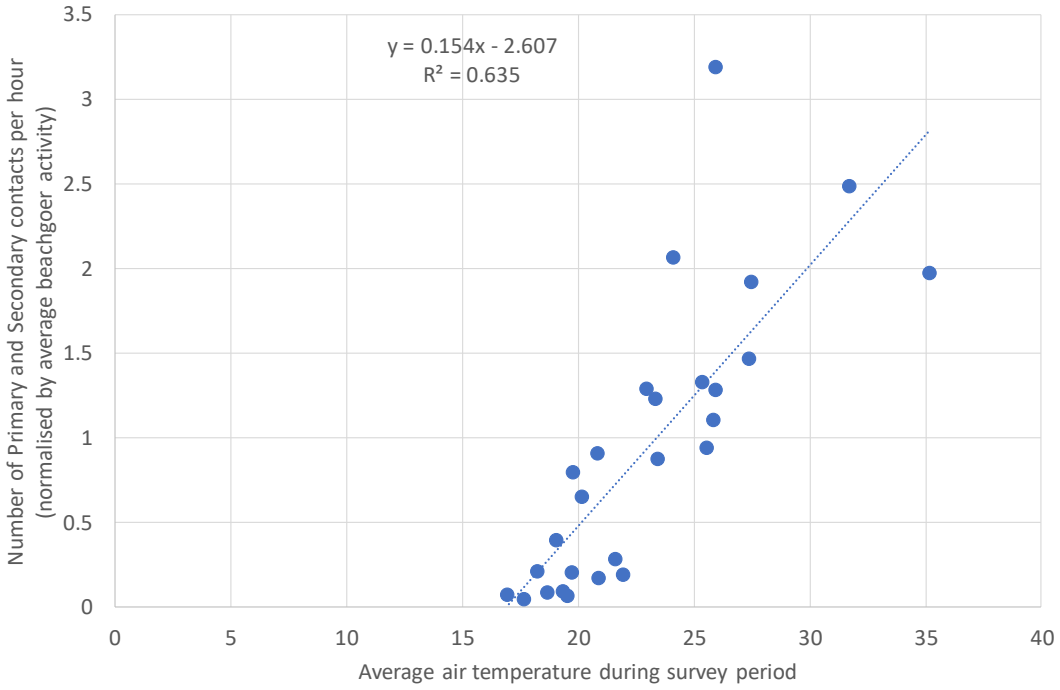


Figure 5. Spearman Correlation between air temperature and primary/secondary contact usage (number of contacts).

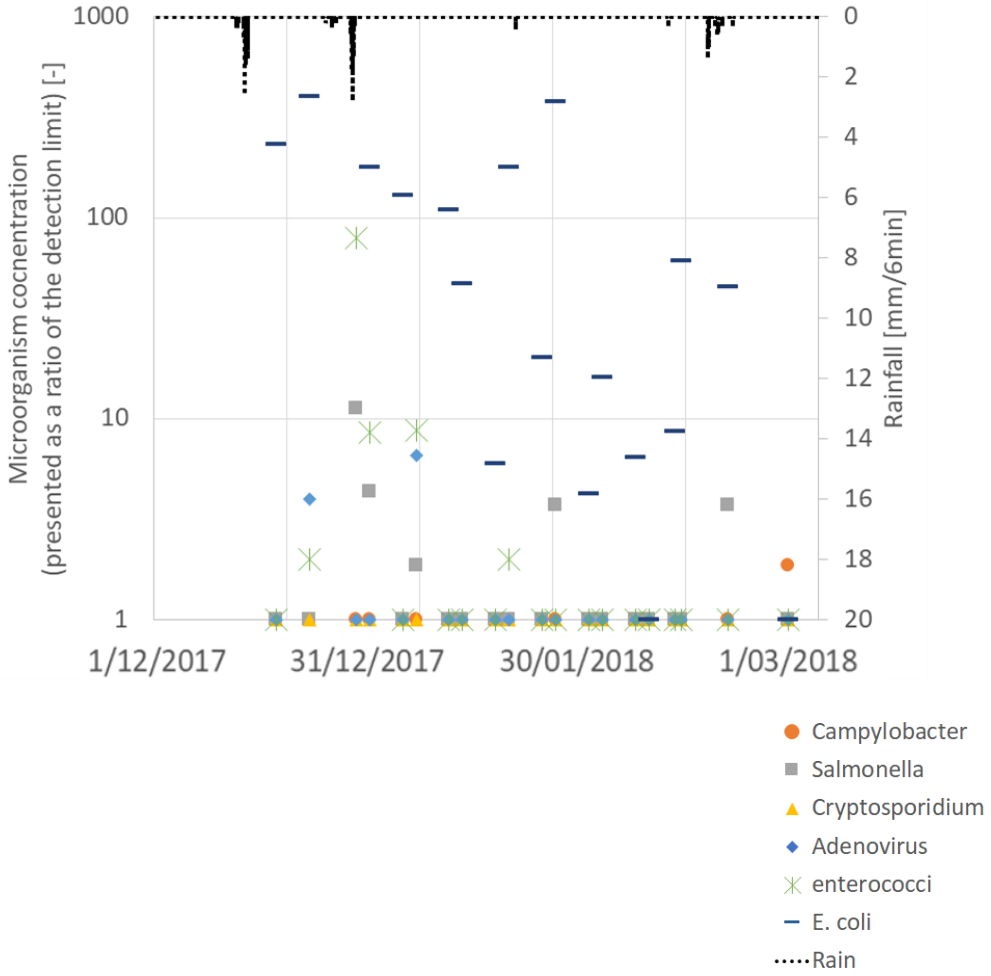
Water quality monitoring

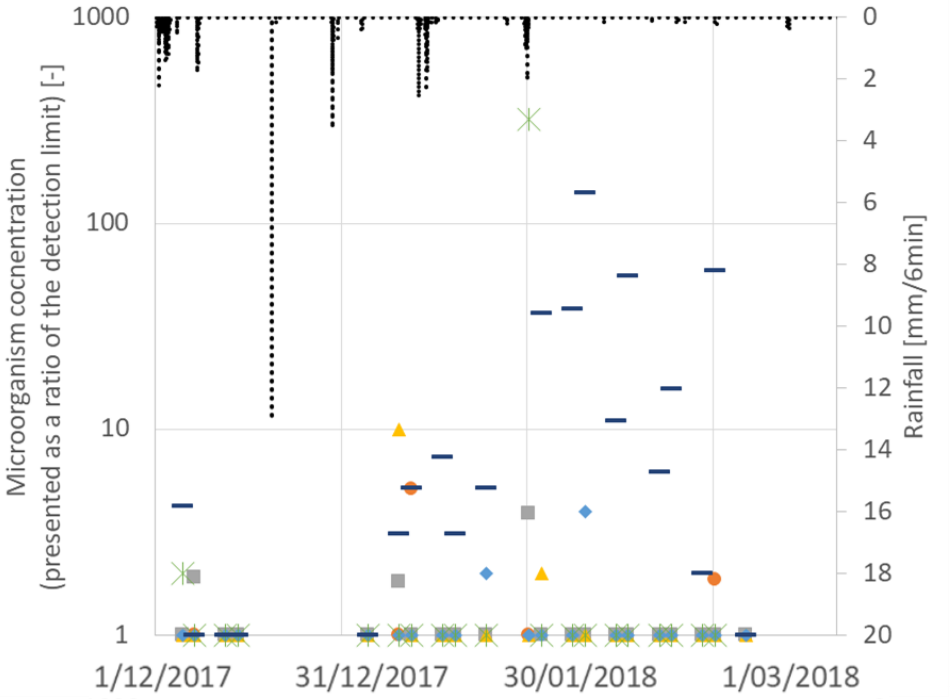
The detection rate of the indicator organisms was much higher than that of the reference pathogens (Figure 6). Because of the high uncertainties regarding the calculation of 95th percentiles using a small number of data points for each site, data were pooled across the sites, resulting in a median enterococci concentration of <10 MPN/100 mL and a 95th percentile of 366 MPN 100 mL. This 95th percentile, in the context of the NHMRC (2008) recreational guidelines, suggests that these sites are in the microbial assessment category C, with an estimated GI illness risk of 5-10% corresponding to category 2 (swim at your own risk).

Despite high enterococci measurements in the past, only 10% of all samples exhibited pathogen densities above the detection limit (Figure 6). *Salmonella* was the notable exception, with 27% of results above the detection limit. The average recovery rates of our recovery experiments were used to correct measured or assumed densities. Statistical analyses showed that there was insufficient evidence to conclude that the three sites behaved differently with regard to their pathogen concentrations (one-way ANOVA; p>0.05; Zar 1999).

While enterococci were detected in less than 50% of samples, *E. coli* was detected in around 80% of beach samples (median = 62 MPN/100mL; 95th percentile = 7028 MPN/100mL). *C. perfringens* was detected in more than 50% of samples, owing to its ability to survive environmental conditions that makes it a good tracer of residual pollution trends.

Campylobacter was only detected on five occasions, once on Altona and twice in Elwood and Frankston. *Salmonella* was detected almost three times as often (14 samples of the 61) across all three locations (Figure 6). *Giardia* and enteroviruses were never detected. *Cryptosporidium* was only detected twice in Elwood. Adenoviruses were detected twice in Altona and Elwood (Figure 6). Noroviruses were only measured on three occasions, but they were never detected (<1.3 copies/L).





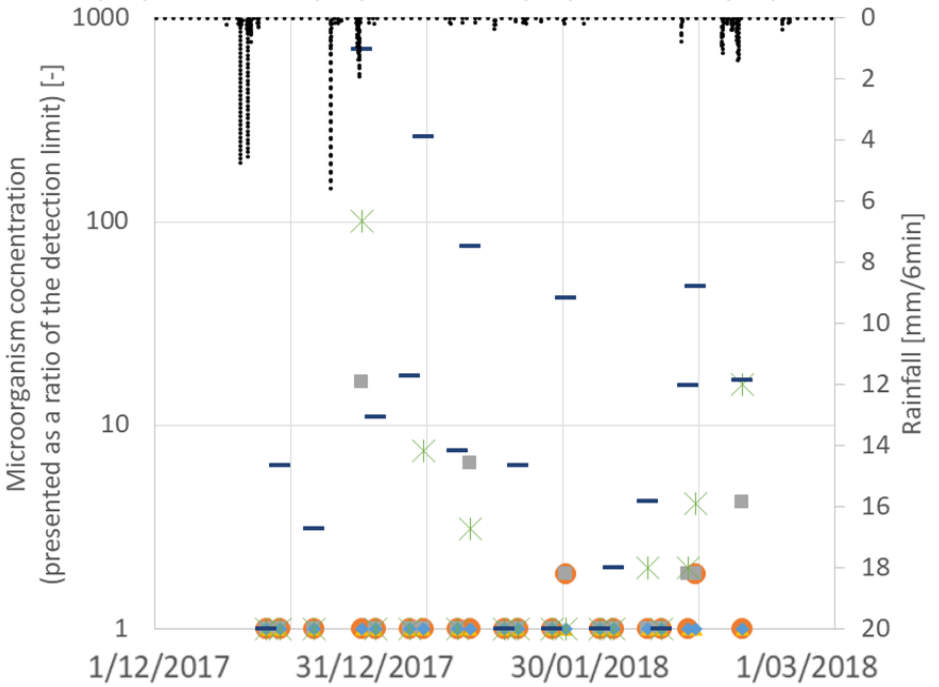


Figure 6. Enterococci concentration, rainfall intensities [mm/6 minutes] and pathogen levels for Altona (A), Elwood (B) and Frankston (C), December 2017 to April 2018.

Enterococci, *E. coli*, *Salmonella* and *Campylobacter* were detected at all locations. Adenovirus was detected at Altona and Elwood and *Cryptosporidium* only at Elwood on two occasions.

Pathogen concentrations have been normalised by their detection limit to allow direct comparison and to avoid differences in detection for determining their presence.

Overall, the level of pathogen detection was comparable with those in pathogen surveys reported in similar systems in other countries that is, fed primarily with non-point sources of pollution, or in embayments that receive a combination of disinfected wastewater effluent and stormwater runoff (Soller et al. 2015).

E. coli and enterococci were the only two indicators that significantly correlated with calculated probabilities of gastrointestinal illnesses due to a primary contact event (with the latter having a slightly higher correlation; p=0.024 and p=0.002, respectively) but these correlations were merely monotonic (Figure 7). No reliable objectives (i.e. set enterococci densities corresponding to specific probabilities of illness) could be derived from this correlation. However, detection limit issues were found to confound our analyses and may explain the absence of stronger correlation between risks and indicators.

Water clarity was the most correlated parameter tested, with significant correlations found between probabilities of illness during primary contact events and water clarity for all sites combined as well as for each site individually. This relationship is to be expected since higher densities and lower water quality coincide with rainfall events that drive pathogen and sediment mobilisation.

Both water clarity and turbidity were significantly correlated with *Salmonella* concentrations ($p<0.001$), but not with any other pathogens. Cloud cover was significantly positively correlated with most indicators except *C. perfringens*. Significant positive correlations were also observed between cloud cover and *Salmonella* (p=0.024) and adenoviruses (p=0.005). These results validate the current EPA Victoria forecast model which uses cloud cover as an input in its prediction. Antecedent rainfall totals prior to sampling were significantly positively correlated with most indicator organisms (except *C. perfringens*) and *Salmonella* concentrations, but not with any other pathogen.

However, the incidence of rainfall did not necessarily increase the estimated risk. Sometimes, the highest risk was observed during periods without any rainfall. The absence of a correlation between cumulative rainfall in the previous 24 hours or 72 hours prior to sampling and the estimated risk does not automatically imply no causative effect as this might be the result of high number of non-detects and limited number of samples collected.

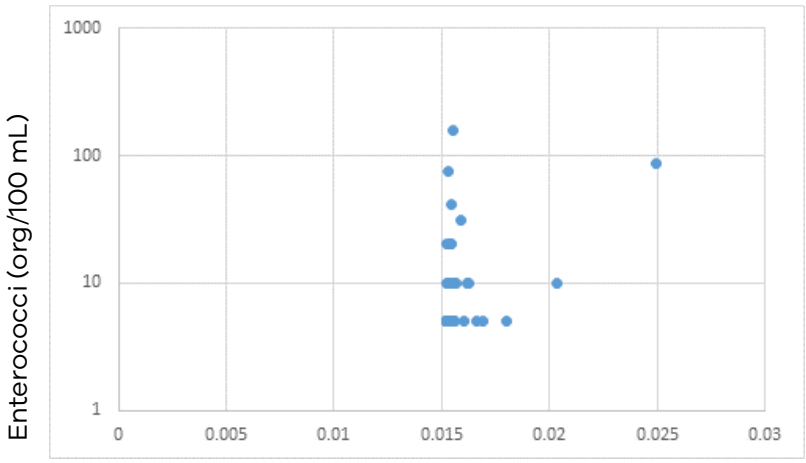


Figure 7. Monotonic correlation between enterococci densities and probability of GI illness (Spearman rank correlation)

E. coli and enterococci densities correlated positively with total rainfall in the 24 hours prior to sampling ($p<0.05$). Rainfall in the 72 hours prior to sampling correlated positively with enterococci only as *E. coli* dies off quicker in marine waters. The only pathogen to significantly correlate with antecedent rainfall totals was *Salmonella* concentrations ($p<0.001$) as shown in Figure 6 where hyetographs (rainfall time series) and pollutographs (pathogen time series) for each of the monitored pathogens are shown for the three beach sites. These graphs show that *Salmonella* increases in concentration after rainfall most of the time.

Baseline QMRA

Primary contact recreation

The baseline QMRA predicted that, of the 100,000 modelled exposures for primary contact recreation, 336 illnesses occurred, which equates to a mean probability of illness (p(ill)) of 0.33% (Table 3). Most of this probability was derived from the norovirus dose-response model, which is highly uncertain and seemed highly conservative considering the sampling undertaken. Adenoviruses contributed the next highest proportion of total risks. Bacteria and protozoa were the smallest contributors, with a p(ill) at or below 0.01%. The total predicted probability of illness from the baseline QMRA only exceeded 1.07% in 5% of the simulations (i.e. the 95th percentile p(ill) was 1.07%) (Schang et al. 2019).

This baseline QMRA suggests that the beaches were:

- in category 1 (swim safely) with a predicted probability of illness of ≤1% per primary contact recreational exposure about 94% of the time
- in category 2 (swim at your own risk) with a probability of illness between 1% and 10% only 6% of the time, and
- never in category 3 (do not swim) (see Figure 8).

Table 3. Mean and 95th percentile of the probability of contracting a gastrointestinal illness (%) due to specific

	Primary Contact		Secondary Contact	
	Mean(%)	95th Percentile (%)	Mean (%)	95th Percentile (%)
Campylobacter	0.01	0.03	<0.01	0.01
Salmonella	<0.01	0.02	<0.01	<0.01
Giardia	<0.01	0.01	<0.01	<0.01
Cryptosporidium	0.01	0.03	<0.01	<0.01
adenoviruses	0.07	0.23	0.01	0.03
enteroviruses	<0.01	0.01	<0.01	<0.01
noroviruses	0.23	0.82	0.04	0.13
Total (all pathogens)	0.33	1.07	0.05	0.18

Secondary contact recreation

For secondary recreation, the modelled 100,000 exposures resulted in 48 predicted illnesses, which equates to a mean probability of illness of 0.05%. As with the primary contact QMRA, most of this probability was derived from the norovirus dose-response model which appears conservative. The predicted probability of illness exceeded 0.18% in 5% of the simulations (that is the 95th percentile was 0.18%). Most of the time (99.7%), the total probability of gastrointestinal illness for a single secondary contact exposure was <1%. A single secondary contact exposure would rarely (0.3% of the time) result in a probability of illness of between 1 and 10% (Figure 8).

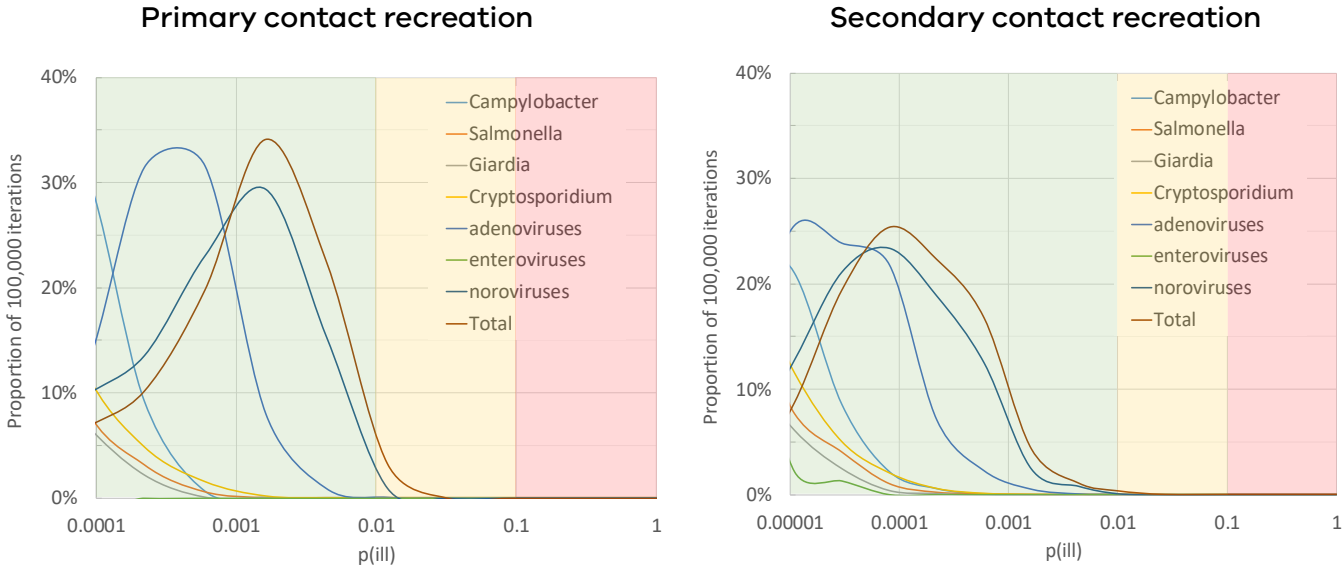


Figure 8. Baseline QMRA – probability density distributions for p(ill) for each pathogen and total p(ill).

Note: Green areas indicate that p(ill) is less than 1% (Swim safely), yellow areas indicate that p(ill) is between 1% and 10% (Swim at your own risk) and red areas indicate that p(ill) is greater than 10% (Do not swim).

Sensitivity analyses

Pathogen concentrations

Three samples were collected and analysed for noroviruses by qPCR and all three were below the detection limit (<1.3 copies per L). As enteroviruses may be used as a surrogate for the presence of noroviruses (Soller et al. 2015), the baseline QMRA was run again assuming that the norovirus densities were equal to the maximum densities of enteroviruses or adenoviruses, noting the absence of detection in the limited sampling undertaken. The results of the baseline QMRA were more conservative than those of this sensitivity testing (Table 4). Soller et al. (2017) suggested that the dose-response model used for the QMRA baseline was also conservative compared with epidemiological datasets (US EPS 2009).

Table 4. Sensitivity testing QMRA for primary contact exposure, where norovirus densities were assumed to equal to the maximum adenovirus densities - Probabilities of contracting a gastrointestinal illness.

	Baseline QMRA		d(NoV) ≈ d(adenoviruses)	
	Mean	95th Percentile	Mean	95th Percentile
noroviruses	0.23%	0.82%	0.04%	0.14%
Total (all pathogens)	0.33%	1.07%	0.14%	0.40%

Dose-response models

The baseline QMRA was run again using different dose-response models for *Campylobacter*, *Cryptosporidium*, adenovirus and norovirus (Table 5).

No significant change was observed when using the Medema et al. (1996) dose response model for *Campylobacter*. Both the mean and 95th percentile probability of illnesses increased when using the dose response model proposed by Teunis et al. (2005) because that model includes data on children, while the other models were essentially developed for the general population. The results of the Teunis et al. model still indicates relatively good microbial assessment categories with these beaches rated 80% of the time in Category 1 (swim safely), 20% of the time in category 2 (swim at your own risk), and 0% of the time in category 3 (do not swim). Similarly, the results of the Medema et al. (1996) model indicates that these beaches would be in category 1 (swim safely), 94 % of the time and 6% of the time in category 2 (swim at your own risk).

For *Cryptosporidium*, a small increase was observed for both the predicted mean and 95th percentile illness rates compared with the baseline QMRA when using the US EPA (2005) dose response model, but the MAC categorisation of the beaches did not change (Table 5).

For adenoviruses, the use of the Teunis dose-response model resulted in a decrease in the predicted enteric illness outcomes for primary contact recreation (Table 5).

For noroviruses, the full effects of the choice of dose-response model must be considered in the light of the lack of norovirus detection. The assumption that all noroviruses detected are infectious is also highly conservative. However, the MACs of the beaches frequently remain in the category 1 (swim safely) most of the time (Table 5). If the Messner

et al. (2016) dose response model was used instead of the 'cloud' dose response model devised by Soller et al. (2017), beaches would be 100% of time in category 1 (swim safely), 0% of time in category 2 (swim at your own risk), 0% in category 3 (do not swim).

Table 5. Results of sensitivity testing of the impact of the dose-response model selected on the enteric disease outcomes of primary contact recreation - percentage of time the beaches would be in various MACs

			Microbial assessment categories		
			Category 1 swim safely	Category 2 swim at your own risk	Category 3 do not swim
Baseline QMRA			94%	6%	0%
Alternative dose-response model	*Campylobacter*	Teunis et al. 2005	80%	20%	0%
		Medema et al. 1996	94%	6%	0%
	Cryptosporidium	US EPA 2005	94%	6%	0%
	Adenoviruses	Teunis et al. 2016	97%	3%	0%
	Noroviruses	Teunis et al. 2008	82%	18%	0%
		Messner et al. 2016	100%	0%	0%

To summarise, adjustment of the *Cryptosporidium* and adenovirus dose response relationships did not change the outcomes significantly or result in reduced probabilities of illnesses per contact event. One of the additional *Campylobacter* dose-response models resulted in an increased probability of illness, while the other resulted in a decrease. The same was found for the two-additional norovirus dose-response models tested. Nonetheless, regardless of the dose-response model chosen, the 95th percentile probability of enteric illness due to a single primary contract recreational event rarely exceeded 2.02% (95th percentile).

Source tracking

Bacteroides – *Bacteroides* HF183/BacR287 were detectable in 13 samples, ranging in concentrations from 6.2x10^{2} copies per 100 mL to 4.2x10^{7} copies per 100 mL (the latter was found at the Frankston site). Elwood had the highest number of detections (8/12 samples), followed by Frankston (5/12) and Altona (2/11). Further analysis of the data (using sensitivity and specificity tests) demonstrated that the detection of *Bacteroides* did not statistically correlate with the detection of pathogens.

SourceTracker – The proportion of the microbial communities within each beach water sample that was comprised of the faecal sources in our database was relatively low; ranging from less-than detection to around 0.8% (Table 6). While this may seem low, it must be recognised that the 16s microbial community within marine waters contains many other sources of bacterial communities other than faecal pollution.

On average, sewage and dog faeces were the highest contributors to faecal pollution at the beaches. High levels of dog faeces were found at Altona beach, suggesting that people allow their dogs to defecate or enter the water at or near this site. The total proportion of human sewage in the samples ranged from less-than-detection to 0.29%, with an average of 0.03% across all sites.

There was a statistically significant correlation between the concentrations of the *Bacteroides* HF183/BacR287 and the proportion of the microbial communities in the beach samples that were like human sewage (p=0.008). This correlation is reflected in the comparison shown in Table 7. Altona only had one sample that resembled human faeces, which is surprising given it is located near a wastewater treatment plant. It is important to note that two of the samples were positive for human adenoviruses at Altona and neither of these matched the sample that was positive for human sewage. Frankston seemed to be most influenced by human sewage, with five of its samples having detectable human sewage contributions, yet there were no human viruses (enteroviruses or adenoviruses) detected at this site. Four of these five samples had detectable levels of *Salmonella*.

Table 6. Mean proportions (in %) of microbial community that resemble each faecal source microbial fingerprint – Source Tracker model.

	Total faecal	Sewage	Dog	Chicken	Seagull	Horse	Waterfowl
Altona	0.08 [0.21]	<0.01 [0.09]	0.06 [0.21]	0.01 [0.07]	<DL [<DL]	<DL [<DL]	<DL [<DL]
Elwood	0.03 [0.14]	<0.01 [0.08]	<DL [0.03]	0.01 [0.06]	<DL [0.05]	<DL [0.02]	<DL [<DL]
Frankston	0.16 [0.79]	0.06 [0.29]	0.01 [0.07]	0.03 [0.24]	0.01 [0.08]	0.01 [0.07]	0.04 [0.26]

Notes: The values in square brackets are maximum values detected at each site. Total faecal is the addition of all proportions for all sources tested (chicken, waterfowl [wood duck, swamphen, etc.], seagull, bat, cat, cow, deer, dog, horse, possum, human sewage, sheep, wallaby, wombat).

There was a statistically significant relationship between enterococci concentrations and the total proportion of faecal microbial communities (p<0.001), perhaps confirming that enterococci are providing an estimate of the overall level of faecal contamination. Enterococci concentrations were significantly correlated with the proportion of the microbial community within the beach samples that were like human sewage communities (p=0.004). A correlation was suggested between enterococci and the proportion of the microbial community within the beach samples that were like waterfowl communities. This correlation would need to be confirmed by further sample collection and analyses as only three samples had detectable levels of waterfowl microbial communities.

Favourable comparisons were made between the source tracking methods and the likelihood estimations of human contamination from the desktop sanitary survey, with sites ranked in the same manner by both source tracking methods and the sanitary survey in terms of its likeliness to be human-contaminated (Table 7). Human sources identified during the sanitary survey included bather shedding (release), toilet facilities,

sewage treatment plant (STP) outfalls, STP bypasses, sewage overflows, sewage chokes, and boats.

Although human sources of faecal contamination exist at these sites, there is little doubt that they coexist with other sources which could influence the health risks for recreational users. The SourceTracker and sanitary surveys also identified several other animal sources of pollution that could be affecting these sites, as correlations were found between the estimated animal contributions from our source tracking methods and the presence of *Salmonella*.

Table 7. Comparisons between the sanitary survey likelihood scores and human source contributions as identified using *Bacteroides* and SourceTracker methods.

	Altona			Elwood			Frankston		
	Sanitary survey	*Bacteroides*	Source Tracker	Sanitary survey	*Bacteroides*	Source Tracker	Sanitary survey	*Bacteroides*	Source Tracker
Human sources	1.95	0.01%	5%	2.0	0.5%	12%	2.5	5%	21%

Site-specific QMRAs

The first six box plots obtained from Soller *et al*. (2010) were compared with the probability of illness estimated in this study (Figure 9). Soller *et al* (2010) assumed a constant faecal pollution level of 35 enterococci/100 mL, while the data used in this study's QMRA had a median faecal pollution level of <10 enterococci/100 mL and a 95th percentile of over 300 enterococci /100 mL. As such, the purpose of this is not to directly compare this study to that of Soller *et al*. (2010), as their QMRA model differed from this QMRA. However, observing these distributions (especially the median, 5th and 95th percentiles), the risk of swimming in Port Phillip Bay appeared to be most comparable to a site with a faecal contamination level of 35 enterococci /100mL which is primarily fed with non-point sources of gull, pig and chicken pollution and marginal amount of faecal contamination of human origin, and less comparable to a beach primarily sourced from cattle, raw sewage or secondary treated effluent. Using the baseline QMRA assumptions, the probability of illness was calculated for various sources identified in Altona, Elwood and Frankston (Figure 9). Results were similar to those reported by Soller et al. (2010), confirming that the risk in Port Phillip Bay is strongly dependant on the source of faecal contamination. The risk associated with the canine source, which was identified at all sites in Port Phillip Bay was also calculated (Figure 9).

The results of the site-specific QMRAs that were run using Soller et al. (2010) approach and the proportions of the sources of contamination specific to each site were:

- The 95th percentile probability of illness per recreational contact event at Altona was 0.4%
- The 95th percentile probability of illness per recreational contact event at Elwood was 0.98%
- The 95th percentile probability of illness per recreational contact event at Frankston was 1.43%

with an average across the three sites of 0.94%. This average probability of illness compared favourably with the probability the baseline QRMA obtained using the pathogen datasets developed during this study as the 95th percentile probability of illness per recreational event for all three sites obtained used this latter approach was 1.07%.

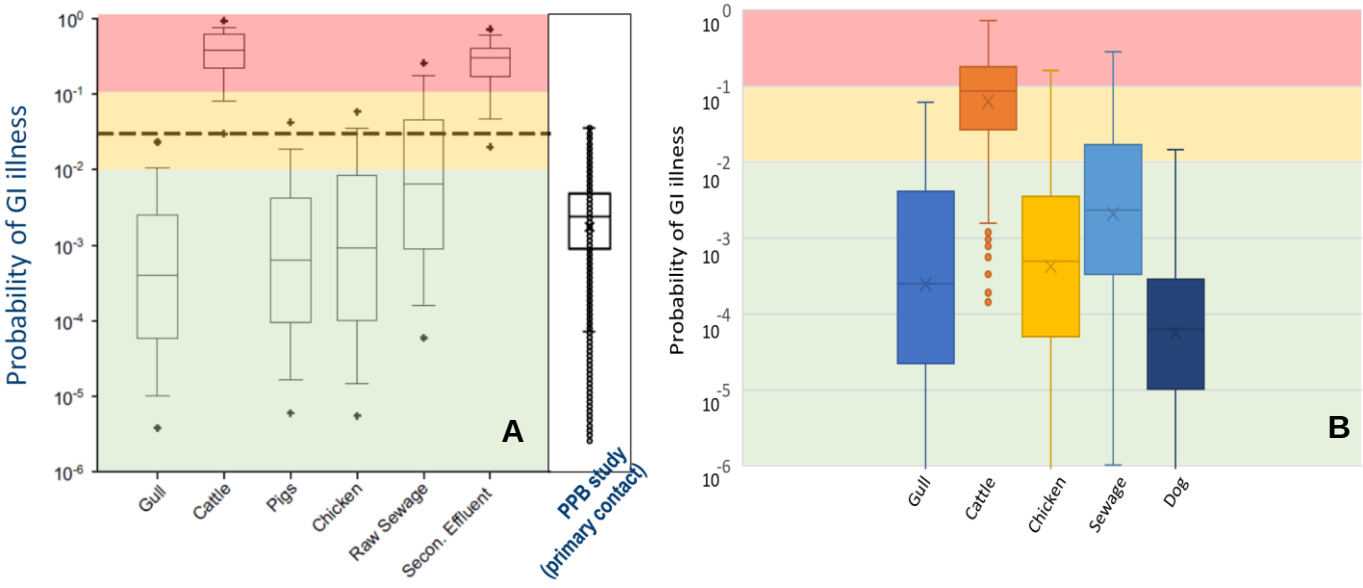


Figure 9. Probability of illness corresponding to constant faecal pollution levels of 35 enterococci/100 mL according to the source of contamination.

Probability of GI illness compared to Soller et al. (2010) calculations (A), probability of GI illness calculated for various sources using the same assumptions than those used for the QMRA (B).

Discussion

Can pathogen densities be predicted using indicators?

No, except for *Salmonella,* pathogen densities cannot be predicted using indicators**.**

No significant correlation was found between indicators and pathogens, except *Salmonella* which concentrations were significantly correlated with *E. coli* and enterococci densities ($p<0.05$). No reliable objectives (that is correlation linking set enterococci densities and specific probabilities of illness) could be derived from this correlation. However, detection limit issues confounded our analyses and may explain the absence of stronger correlation between risks and indicators. The absence of stronger correlation could also be explained by the diversity of sources of faecal contamination, with different ratios between indicators and pathogens according to the source.

The results of this study suggest that indicators cannot reliably predict the densities of specific pathogens in Port Phillip Bay. However the contribution of each pathogen to the overall illness rate varies according to numerous factors, including infectious dose, virulence, etc. As a result, a lack of correlations between indicators and specific pathogen densities will not automatically equate with a lack of correlation between indicators and illness rates.

What is the probability of recreational users contracting a gastrointestinal illness at Altona, Elwood and Frankston?

Based on this study, the probability of contracting a gastrointestinal illness at the three locations was low and much lower than would be estimated using SEPP(Waters) criteria.

The baseline QMRA predicted that the mean probability of illness for primary contact recreation was 0.33%.

For secondary recreation, the mean probability of illness was 0.05%.

These probabilities are much lower than the probabilities above 10% that would have been predicted by the NHMRC *Guidelines for managing risks in recreational waters*.

Can illness rates be predicted?

Some parameters can indicate a potential increase in probability of illness as the probability of illness tends to increase as the value of these parameters increases. While these trends can be used for the notification of recreationists through Beach Report, they cannot be used to accurately predict a probability of illness.

E. coli and enterococci were the only two indicators that significantly correlated with calculated probabilities of gastrointestinal illnesses due to a primary contact event. However, this relationship was merely a trend (that is faecal bacterial indicators were higher when risks increased) rather than a strong correlation from which bay-specific objectives could be derived.

Water clarity was the parameter that most correlated with the probabilities of illness during primary contact events. Both water clarity and turbidity were significantly correlated with *Salmonella* densities ($p<0.001$).

Significant positive correlations were also observed between cloud cover and *E. coli*, enterococci, *Salmonella* and adenoviruses densities. These results validate the current EPA Victoria forecast model which uses cloud cover as an input to its prediction.

Antecedent rainfall totals prior to sampling were significantly positively correlated with *E. coli*, enterococci and *Salmonella* concentrations, but not with any of the other pathogens. The highest risk was sometimes observed during periods without any rainfall. However, the absence of a correlation between cumulative rainfall in the previous 24 hours or 72 hours prior to sampling and the estimated risk does not automatically imply an absence of causative effect in view of the high number of non-detects and limited number of samples collected during this study.

Illness rates cannot be predicted in Port Phillip Bay using enterococci densities or environmental parameters. The correlations observed were merely trends that would not predict illness rates accurately.

This means that EPA's current monitoring program, which is based on best available scientific evidence, cannot precisely predict the risk of illness. Nonetheless, it provides semi-quantitative, conservative information about microbial water quality in the bay.

How do the results of this QMRA compare with NHMRC Guidelines 2008 and published epidemiological studies?

The results of this QMRA are similar to results found in QMRA and epidemiological studies conducted in areas contaminated by non-point sources of faecal contamination.

The NHMRC (2008) guidelines, which formed the basis for the revised SEPP (Waters) (2018), were derived following Kay *et al*. (2004) interpretation. The Kay et al. (2004) study was conducted in the UK, at a beach with oceanic influence and a human point-source of contamination. It linked excess gastrointestinal illness rates with 95th percentile enterococci concentrations, such that:

- ≤40 MPN/100 mL related to less than one illness incident per 100 exposures (<1%)
- 41-200 MPN/100 mL related to an illness incidence of between one in 100 and one in 20 exposures (1-5%).
- 201-500 MPN/100 mL related to an illness incidence of between one in 20 and one in 10 exposures (5-10%)
- >500 MPN/100 mL related to an illness incidence of greater than one in 10 exposures.

The three beaches used in this QMRA had a combined 95th percentile enterococci concentration of 366 MPN/100 mL. Kay *et al*. (2004) interpretation suggests that the probability of contracting a gastrointestinal illness would be between one in twenty (5%) and one in ten (10%) exposures at Altona, Elwood and Frankston. This QMRA predicted low illness rates of over an order of magnitude lower compared with the probability of illness predicted by the interpretation of Kay et al. (2004). The uncertainties involved in the QMRA process need to be considered in this interpretation, especially since the probability of illness predicted by the QMRA is heavily influenced by the pathogen densities detected in the water, most of which were below their detection limit. It is reasonable to assume that the mean probability of illness would be lower than 0.33% if the measurements were repeated with lower detection limits.

The human-sourced enterococci levels found in Port Phillip Bay were compared with the NHMRC (2008) MACs. Using the source tracking results, the percentage of faecal contamination of human origin was estimated to average 13% across the three sites. Multiplying each of our enterococci data points by the proportion of human sources found at our sites, the 95th percentile enterococci concentration derived from human sewage would be 48 MPN/100 mL. Comparing this with the NHMRC (2008) MACs would suggest that the probability of GI illnesses at the Port Phillip beaches in this study would be just above 1%, which is consistent with the results of the baseline primary contact QMRA (95th percentile of 1.07%). It is important to note that solely attributing the human health risk from primary contact exposure to the 13% contribution from human source should be interpreted with caution because it relies on many assumptions (for example it ignores risks from non-point sources, assumes the risk is driven by faecal contamination from human origin). This approach also has limitations as estimating proportions of enterococci that are derived from human faecal contamination may also be extremely difficult.

A meta-analysis of the available marine epidemiological recreational studies conducted by McCarthy et al. (2017b) found that the probability of illness vs. indicator relationships were different depending on the beach type (oceanic vs. non-oceanic) and source type (point vs. non-point source of faecal contamination). The difference between the probability of illnesses estimated using the NHMRC 2008 guidelines and what was predicted in this QMRA may therefore be due to differences in the beach types and pollutant sources at the beaches studied (embayment-type beaches primarily fed by non-point source of stormwater pollution) compared with that used to derive guidelines (oceanic beaches fed by sewage point sources) (Cheun et al. 2019).

Comparisons were made with relevant epidemiological studies to further understand whether our QMRA predictions were similar to those that measured illness rates of swimmers in systems similar to Port Phillip Bay (Table 8). Only four studies reported results for beaches comparable to those used in this study. The maximum excess probability of gastrointestinal illness found in these four epidemiological studies was 1.85%. These excess GI rates are still higher than the probability of illness found in our current study (0.33%). However, the levels of faecal contamination reported were also generally higher than those from this study (expressed as median enterococci densities). Of interest is the study by Colford et al. (2007) conducted in a bay primarily fed by non-point sources of pollution. That study found a mean excess enteric illness rate of 1.1%, yet the levels of faecal contamination were significantly higher than those found in this study (median enterococci 29 MPN/100 mL vs. 7 MPN/100 mL).

Table 8. Comparison of enterococci and excess GI in this study with international literature

	Enterococci (MPN/100 ml)	Excess GI rates [epi/QMRA]
This study	7 [<10 – 4,205]	QMRA: 0.33%
Colford et al. 2007	29 [<10 – 60,000]	Epi: 1.10%^{S}
Sinigalliano et al. 2010	19 [- – 3,320]	Epi: 1.85%^{NS}
Colford et al. 2012	10-300 [2 - 41,000]	Epi: 1.30%
US EPA 2009 [epi] and Soller et al. 2015 [QMRA]	7.9 [<1 – 2,81]	Epi: 0.53^{NS} QMRA: 0.20%

Notes: Epi=Epidemiological studies. Enterococci values are reported as medians, and values in square brackets are the ranges. ND: non detected
Excess illness rates for GI are either derived from the epidemiological study or, in the case of this study and the study by Soller et al. (2015), these were derived from a QMRA. The Soller et al (2015) QMRA study was paired with an epidemiological study conducted by US EPA (US EPA 2009) at the same site.
^{S} signifies that a significant difference was observed between swimmers and non-swimmers, while
^{NS} signifies no significant difference was observed.

Can the origin of the contamination impact the level of risk in the bay?

Faecal contamination of human origin contributed an average of 13% of the total faecal contamination at Altona, Elwood and Frankston. The main contributions to faecal contamination were of avian and canine origins, which have comparably lower risks to human health and could explain the low probability of illness estimated by the QMRA.

This suggests that the origin of contamination impacts the probability of illness and should be a primary factor in assessing risks of water-based recreation in Port Phillip Bay.

Conclusions and recommendations

The QMRA conducted for primary and secondary contact recreational events using data collected from Altona, Elwood and Frankston beaches during the swimming season 2017-2018 showed that the probabilities of contracting an illness were very low compared to the rates of illness expected using the SEPP (Waters) (gazette.vic.gov.au/gazette/Gazettes2018/GG2018S499.pdf) and NHMRC interpretation (NHMRC 2008). Furthermore, the probabilities of contracting an illness were comparable to those found in the limited number of epidemiological studies with similar water body-types and pollution sources.

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

Water clarity was the parameter most correlated with probabilities of illnesses during primary contact events. Both water clarity and turbidity were significantly correlated with *Salmonella* densities. *E. coli*, enterococci, *Salmonella* and adenoviruses densities increased with the cloud cover, validating the current EPA Victoria forecast model which uses cloud cover as an input to its prediction. However, illness rates could not be predicted in Port Phillip Bay using these environmental parameters. The correlations observed were merely trends that would not predict illness rates accurately.

On average, 13% of the total faecal contamination originated from a human source, which is believed to drive the risk at Altona, Elwood and Frankston. The main contributions to faecal contamination were of avian and canine origins, which carry comparatively lower risks to human health. This study clearly established that current microbial water quality objectives from SEPP (Waters) may be conservative as they assume that all faecal contamination is from human origin. There is therefore a need to establish site-specific objectives based on the identified sources of contamination. These site-specific objectives, developed using a tiered-risk assessment approach, would mean that beach grades determined based on site-specific MACs and sanitary inspection categories would more accurately reflect site-specific risks and potential health outcomes for recreational users. Information about the source of contamination would enable EPA to provide better targeted information to recreational water users. This would likely result in EPA issuing fewer unnecessary closure notices. It would provide better advice to the almost two million people visiting Port Phillip Bay each year to enjoy water-based recreational activities. As the beaches remain open, visitors will continue to pursue activities, which are part of a healthy lifestyle and contribute to the economy of the region.

This QMRA presents an alternative approach to assessing risks in Australian recreational waters. The costs of such studies are high, but lower than traditional methods for more

extensive studies (for example epidemiological studies). Like epidemiological studies, QMRAs are limited to only providing an understanding of the situation at a certain point in time and at specific locations. Hence, it is recommended that the study be repeated at the same locations to validate the results, as well as expanded to more sites to better capture the potential spatio-temporal variations of pathogens in the bay, and to provide a strong scientific basis for the framework to develop site-specific objectives.

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Appendices

Appendix A. Sanitary inspection form

(Adapted from OEH NSW 2011)

SITE DESCRIPTION			
Site name and reference number (if any):			
Inspection date:			
Inspector's name:			
Type	Ocean ☐	Estuarine ☐	Freshwater ☐ Other ☐
Sandy Beach	☐ Yes	☐ No	
Size swimming area	Length (m)	Width (m)	Area (m^2)
Site location	Latitude	Longitude	
Site description			
Level of flushing:	High (coastal) ☐	Medium (estuarine) ☐	Low (lagoon) ☐
Elevated indicators	☐ After light rain (5 mm in 24hrs	☐ After moderate rain (10 mm in 24hrs)	
	☐ After heavy rain (20 mm in 24hrs)	☐ After very heavy rain (50 mm in 24hrs)	

SITE USE		
Activities one site	☐ Swimming	☐ Surfing
	☐ Fishing	☐ Canoeing/kayaking
	☐ Boating	☐ Diving
	☐ Playing in wet sand	☐ Other
Demographics	☐ < 7 years ☐ > 60 years ☐ Teenagers ☐ Adults ☐ Tourists	
Number of users per day *(Indicate min and max if possible)*	Weekends	
	Weekdays (outside holidays)	
	Weekday (holidays)	
Percentage of primary contact		
Number of illnesses recorded		

POLLUTION SOURCES						
Catchment land uses (%)		Bushland		Rural		Urban

Pollution sources likely to impact recreational water quality and Determination of Sanitary Inspection Category

- *Select in column A any source that is applicable.*
- *Fill the relevant section in pages 4 – 8 to obtain a likelihood rating. Refer to Table 1 if required when filling the relevant sections. Table 1 provides a frequency rating according to the event frequency.*
- *Enter the numerical likelihood value obtained for each applicable source in column B (Refer to Table 2)*
- *Add all the values to determine the Sanitary Inspection Category based on the total score (see Table 3).*

Source	A	B	
Bathers			
Toilet facilities			
STP outfall within 2 km			
STP bypasses or overflows			
Sewage overflows within 1 km			
Sewage chokes and leakages within 1 km			**The sanitary inspection category for this site is:**
On-site sewage disposal systems within 1 km			
Wastewater reuse within 100 m			**_____**
River discharge within1 km			
Stormwater			
Lagoon discharge within 500 m			
Boats within 100 m			
Animals present on site			
TOTAL SCORE			

Table 1. Frequency rating					
Frequency	May occur only in exceptional circumstances (1 in 10 years)	Unlikely to occur but could occur once in 5 years	May occur at least once or twice par bathing season	Will probably occur at least 3-4 times per bathing season	Will occur on a regular basis (once a week)
Frequency rating	**Very low**	**Low**	**Moderate**	**High**	**Very high**

Table 2. Numerical likelihood scores		
Source likelihood rating	**Animals**	**Other sources**
Very low	**0.1**	**0.1**
Low	**0.1**	**0.2**
Moderate	**0.2**	**1**
High	**1**	**3**
Very high	**1**	**12**

Table 3. Sanitary inspection category	
Total score	**Sanitary inspection category**
0 - 0.19	**Very low**
0.2 - 0.99	**Low**
1 - 2.99	**Moderate**
3 - 11.99	**High**
≥12	**Very high**

Bather shedding

Max bather density (= Max number/area) _____ person/m^2

(High bather density ≥0.2; Low Bather density <0.2)

Likelihood of pollution from bathers				
Flushing	Toilets		No toilet	
	Bather density		Bather density	
	<0.2	≥0.2	<0.2	≥0.2
Low	**Low**	**Moderate**	**Low**	**Moderate**
Medium	**Very low**	**Low**	**Low**	**Moderate**
High	**Very low**	**Low**	**Low**	**Moderate**

Comments

Toilet facilities				
Distance from site:				
Number of toilets:				
Number of showers:				
Type of disposal Sewered □ Onsite system □				
	Service frequency:			
Facility conditions Poor □ Good □				
Discharges/odours recorded:				
Likelihood of pollution from toilet facilities				
Facility conditions	Distance >50m		Distance ≤ 50m	
	Low use	High use	Low use	High use
Poor	**Low**	**Moderate**	**Moderate**	**High**
Good	**Very low**	**Low**	**Low**	**Moderate**
Comments				

STP Outfall (within 2 km)

Name and authority responsible:

Distance from site (m):

Likelihood of pollution from STP outfall

Outfall type	Treatment level							
	None	Preliminary	Primary	Secondary	Secondary + disinfection	Tertiary	Tertiary + disinfection	Lagoon
Direct	**Very high**	**Very high**	**Very high**	**High**	**Moderate**	**Moderate**	**Low**	**High**
Short	**High**	**High**	**High**	**High**	**Moderate**	**Moderate**	**Low**	**High**
Long	**Low**	**Low**	**Low**	**Low**	**Very low**	**Very low**	**Very low**	**Low**

Comments

STP bypasses/overflows (within 1 km)

Name and authority responsible:

Distance from site:

Average volume discharges per event (L):

Dilution	□ High □ Low
Min treatment level	□ None □ Primary □ Secondary □ Tertiary/lagoon
Disinfection	□ Never □ Sometimes □ Always

Likelihood of pollution from STP bypasses/overflows
(Refer to Table 1 for rating)

Very low	Low	Moderate	High	Very high

Comments

Sewage overflows (within 1 km)

Name	Address	Frequency/10 years	Volume

Dilution	☐ High ☐ Low

Likelihood of pollution from sewage overflows
(Refer to Table 1 for frequency rating)

Dilution	Frequency rating				
	Very low	Low	Moderate	High	Very High
High	**Very low**	**Very low**	**Low**	**Moderate**	**High**
Low	**Very low**	**Low**	**Moderate**	**High**	**Very high**

Comments

Onsite sewage disposal systems

Distance from site (nearest system):

Number of systems (excluding toilets):

Discharges/odours recorded:

Likely of pollution from onsite sewage disposal systems

Condition	Distance >50m		Distance ≤ 50 m	
	≤50 systems	> 50 systems	≤ 50 systems	> 50 systems
Good	**Very low**	**Very low**	**Low**	**Low**
Poor	**Low**	**Low**	**Low**	**Moderate**

Comments

Wastewater reuse (within 1 km) Location:				
Distance from site:				
Average volume discharged per event (L):				
Treatment before reuse	☐ No ☐ Yes, provide details:			
Likelihood of pollution from wastewater reuse *(Refer to Table 1 for frequency rating)*				
Very low	**Low**	**Moderate**	**High**	**Very high**
Comments				

River discharge (within 1 km) River name:				
Distance from site:				
Pollution sources in the river:	☐ Urban stormwater ☐ Agriculture run-off ☐ Intensive livestock production systems ☐ Leachate from onsite wastewater systems ☐ Wastewater discharge ☐ Other:			
Likelihood of pollution from river discharge *(Refer to Table 1 for frequency rating)*				
Very low	**Low**	**Moderate**	**High**	**Very high**
Comments:				

Stormwater				
Number of drains at the site:				
	Drain 1	Drain 2	Drain 3	Drain 4
Location				
Responsible authority				
Distance to site (m)				
Discharge area (see below)				
Direct discharge (<50 m)				
Main land use (see below)				

Likelihood of pollution from stormwater			
Land use	Discharge Area		
	Dune	Beach, offshore or direct > 50 m	Direct < 50 m
High density urban	**Low**	**Moderate**	**High**
Low density urban	**Very low**	**Low**	**Moderate**
Rural – grazing	**Very low**	**Low**	**Moderate**
Rural - cropping	**Very low**	**Low**	**Low**
Bushland/reserve	**Very low**	**Low**	**Low**
Comments			

Lagoons Name:				
Distance to site (m):				
Surface area (m^2):				
Catchment area (km^2)				
Sources of pollution to lagoon:	☐ Urban ☐ Other:	☐ Agriculture run-off	☐ Stormwater	
Time open to ocean (current %):				
Entrance managed or modified:	☐ No ☐ Yes, provide details:			
Likelihood of pollution from lagoons *(Refer to Table 1 for Frequency Rating)*				
Very low	**Low**	**Moderate**	**High**	**Very high**
Comments				

Boats			
Description of boating facilities:	☐ Marina ☐ Harbour ☐ Anchorage ☐ Boat ramp jetty ☐ Ferry berth ☐ Permanent moorings ☐ Temporary moorings		
Distance of nearest boat (m):			
Number of boats:			
Pump-out facilities provided	⍰ No ⍰ Yes, provide details:		
Complaints of boat discharge:			
Holding tanks required	☐ No ☐ Yes		
Onshore toilets provided	☐ No ☐ Yes		
Likelihood of pollution from boats			
Waste management	<20 boats	20-50 boats	50-100 boats
Good (holding tanks required)	**Very low**	**Very low**	**Low**
Poor (holding tanks not required)	**Low**	**Moderate**	**Moderate**
Comments			

Animals		
Wildlife:		
	Waterfowl	Native Animals
Density (low, medium or high)		
Domestic animals:	☐ Dogs ☐ Horse ☐ Others, provide details	
Dog waste bags available	☐ Yes ☐ No	
Animals access water	☐ Yes ☐ No	
Area cleaned regularly	☐ Yes ☐ No	
Likelihood of pollution from animals *(Refer to Table 1 for frequency rating)*		
Very low	**Low**	**Moderate**	**High**	**Very high**
Comments		

Appendix B: QMRA parameters and key assumptions

Problem formulation

Potential illnesses	Primary health outcome: gastrointestinal illnesses (GI)
Target populations	General population
Sources of contamination	Non-point sources, storm-water sources Bather shedding Point source sewage discharges (overflows and cross-connections)
Hazard identification	See table below
Health outcome	Probability of illness per contact recreational event (5th and 95th percentiles to compare with the probabilities of illnesses in described in NHMRC 2008.

Organisms	Reference pathogens (quantifiable using cultured based techniques except for noroviruses)
Bacteria	*Campylobacter, Salmonella*
Protozoa	*Cryptosporidium, Giardia*
Virus	Enteroviruses, adenoviruses, noroviruses

Exposure assessment

Concentration of pathogens	Based on the direct measurement of reference pathogens in the recreational amenities (min of 20 samples)
Microbial indicators	*E. coli*, enterococci, *Clostridium perfringens*, fRNA phages.
Analytical methods	See table below
Viability and Infectivity	Baseline assumption: 100% of organisms are infective See Appendix D for additional model runs using the following PERT# distribution (assuming contamination with a mixture of gulls, chicken and human sewage sources)
Exposure volumes	See table below
Duration of exposure	1 hour

Organisms	Method	Volume collected
Bacteria	Culture-based techniques	*Salmonella*: 2.5 L *Campylobacter*: 2.5 L Bacterial indicators: 500 mL
Protozoa	US EPA 1623 (+ viability step)	25 L
Viruses	Culture-based techniques	25 L
Norovirus	PCR method	50 L

- *Collection and analysis of replicate samples to understand measurement uncertainties.*
- *Spiking experiments to understand recovery rates and correct measured concentrations.*

	Primary contact	Secondary contact
Ingestion and inhalation	Exponential distribution 50th percentile: 18.6 mL 95th percentile: 80.6 mL (Dufour et al. 2006)	Log normal distribution 50th percentile: 2 ml 95th percentile: 17.1 mL (Dorevitch et al. 2011)

Health effects assessment	
Dose-response models	See Appendix DError! Reference source not found. As the endpoints of models are usually infection (*Salmonella* excepted), PERTs for the Probability of illness/infection are provided in
Health outcome	Appendix E.

Risk characterisation	
Number of scenarios	To understand the uncertainties and variabilities inherent to QMRA assumptions, Monte Carlo simulations were run. They enabled the determination of the effect of the variations of parameters (measurement uncertainties, recovery rates, ingestion/inhalation volumes, proportion of viable/infective pathogens, probability of illness/infection, etc.) and exposed 100 people recreating on 1000 separate occasions. For each run, the total probability of illness is the sum of the probabilities of illnesses for all reference pathogens: $Pill = 1 - \prod_a (1-Pill_a)$; where *Pill* is the total probability of illness and $Pill_a$ is the individual probability of illness for pathogen *a* (Schoen & Ashbolt, 2010).
Output	Total probabilities of illness or frequency distributions of total probability of illness for various scenarios, compared to risks predicted by NHMRC 2008.

Appendix C: Summary of the microbial methods used to analyse the samples collected from the three beaches chosen for this QMRA.

Microorganism [unit][a]	Method	Volume analysed [L]
Faecal coliform *E. coli* [MPN (100 mL)$^{-1}$]	Colilert (IDEXX Laboratories) TECTA-CCA (TECTA)	0.01
Enterococci [MPN (100 mL)$^{-1}$]	Enterolert (IDEXX Laboratories) TECTA-ECA (TECTA)	0.01
Enterococci [CCE (100 mL)$^{-1}$]	US EPA method 1609 (USEPA, 2013)	0.1
Clostridium perfringens [org (100 mL)$^{-1}$]	AS/NZS 4276.17.1:2000 (Australian Standards, 2000)	0.1
Campylobacter [MPN.L^{-1}] Monash University for sampling and recovery testing	Samples were split into 11 subsamples and *Campylobacter* species were detected in each subsample following the modified AS 4276.19:2001 method described in Henry et al. (2015). The four highest volume filtered were also platted on blood-free charcoal agar and any positive colony was tested using secondary confirmation on horse blood agar and chemical testing as described in AS 4276.19:2001 (Australian Standards, 2001). Results were used to calculate the MPN according to Garthright and Blodgett (2003). Positive isolates were stored at -80°C until DNA extraction and full-genome sequencing analysis.	2.83L 0.51L*
Campylobacter [MPN. L^{-1}] ALS for recovery testing	Samples were split into 11 subsamples and *Campylobacter* species were detected in each subsample by AS 4276.19:2001. Results were used to calculate the MPN according to Garthright & Blodgett (2003). Confirmation media: blood free campy media and food chromogenic *Campylobacter* media	0.51*
Enteric viruses: adenovirus and enterovirus [MPN L^{-1}]	Samples were concentrated using the HF80S hollow fibre ultrafilter (Fresenius Medical). The retentate was collected and viruses adsorbed to the ultrafilter were eluted by recirculating buffer containing surfactant. Polyethylene glycol was added to the combined retentate and eluent and mixed at 4°C overnight. The sample was subsequently centrifuged at high speed and the pellet resuspended in cell culture media. The final re-suspended concentrate was used to inoculate 10 flasks of A549 cells, and the culture of adenovirus or enterovirus was confirmed in each flask by PCR after 28 days incubation (Allard et al. 1990; Zoll et al. 1992). The results were used to calculate the MPN according to Thomas's formula (A.P.H., A.W.W. & W.E, 1992).	25

Adenovirus recovery	Sample was spiked with known concentration of recombinant human adenovirus type 5 encoding green fluorescent protein from *Aequorea coerulescens* (AdGFP). The spiked sample was concentrated using HF80S hollow fibre ultrafilter (Fresenius Medical). The retentate was collected and viruses adsorbed to the ultrafilter were eluted by recirculating buffer containing surfactant. Polyethylene glycol was added to the combined retentate and eluent and mixed at 4°C overnight. The sample was subsequently centrifuged at high speed and the pellet resuspended in cell culture media. The TCID50 assay is used for the quantification of AdGFP in these sample concentrates. The final re-suspended concentrate is used to inoculate GH329 cells in 96-well cell culture plates. The plates are incubated at 37°C ± 1 in 5% carbon dioxide. Each well is monitored under FITC fluorescence and the TCID50 is calculated as described by Karber (1931).	
Protozoa (*Giardia* and *Cryptosporidium*) [cysts/oocysts L^{-1}]	United States Environmental Protection Agency method 1623 (USEPA, 2005) with sample spiking and recovery efficiency calculation as outlined in this standard. The described method is based upon US EPA Method 1623 (USEPA, 2005). The method involves the concentration of samples by collection/filtration, sample elution and concentration by centrifugation, the separation of the (oo)cysts from the debris by immunomagnetic separation (IMS) and the staining and microscopic examination of the purified (oo)cysts.	Up to 25L[c]
Infectivity *Cryptosporidium*	This method involves sample concentration by either filtering the water through an Envirochek® HV capsule (QWI-MIC.MP546) or centrifugation (QWI-MIC.MP548) in the laboratory. Retained particles are subject to immunomagnetic separation (IMS) for oocyst recovery and further concentration. Dissociation of captured oocysts from the magnetic beads is achieved by adding acidified HBSS solution and mixing by vortexing. After dissociation, the IMS beads are retained in the tube by the magnet and the supernatant containing the oocysts is removed. The recovered oocysts are washed with cell culture inoculation media to remove the IMS buffers and inoculated onto HCT-8 cells for infection. Developmental stages of Cryptosporidium (infectious foci) are detected by fluorescent labelled antibodies (Ab) [SporoGlo™ (Waterborne™ Inc.)] for immunofluorescent microscopy.	
***Salmonella* [MPN L^{-1}] Monash University for sampling and recovery testing**	Samples were split into 11 subsamples. Then following AS 4276.14:1995 (Australian Standards, 1995). Enrichment media: buffered peptone water Selective enrichment media: RVS Selective agar: XLD and CHROMagar Positive isolates were stored at -80°C until DNA extraction and full-genome sequencing analysis	2.83L 0.51*
***Salmonella* [MPN L^{-1}] ALS for recovery testing**	Samples were split into 11 subsamples. Then following AS 4276.14:1995 (Australian Standards, 1995). Enrichment media buffered peptone water- Selective enrichment media-RVS and MKTTN Selective agar-XLD and chromogenic Salmonella media- Biochemical analysis-using VITEK instrument and serology	0.51*

<table>
<tr>
<td>Norovirus G1 and G2, Hepatitis A Virus, Rotavirus, adenoviruses F and G, enteroviruses</td>
<td>Internal methods from ALS Environmental, as summarised below. PCR testing for three virus concentrates is complete. One hundred and fifty litres of water was concentrated at the beach using a Rexeed ultrafilter. They were then eluted with 1 L of beef extract solution, and then further concentrated using PEG. 1 mL concentrates that represented 50 L were stored at -80°C. This was then vortexed for 1 minute after thawing. Transfer 160 µl into 1.5 mL tubes. Centrifuge samples at 1,500 X g (~4,000 rpm) for 10 minutes. After centrifugation, transfer 140 µl of the supernatant (without disturbing the pellet) to a 2 mL conical screw cap tube without skirted base. Add 555 µl 1X PBS, 5ul RNA internal amplification control and 560 µl AVL to the 140 µl sample for a total of 1260µl. Carefully mix sample by pipette. Load samples in QIAcube (kit used- Qiamp Viral RNA kit). Final elution performed in 50 ul. For PCR – triplicate samples set up for all targets. IAC were used and passed for all three samples. The process controls included a kit positive PCR controls, NTC for PCR blank IAC as PCR/extraction control, PHE Virus Pellet as extraction positive and spiked PCR performed for all targets (plasmid added to eluted RNA and PCR performed).
Six standards run for each target (as triplicates; copy number from 100000- 1 per ul). Results reported as copies per litre.
<table>
<tr><th>Virus type</th><th>Reference</th></tr>
<tr><td>Norovirus G1 and G2</td><td>La Rosa et al., 2009</td></tr>
<tr><td>Hepatitis A virus</td><td>Costafreda et al., 2006</td></tr>
<tr><td>Rotavirus</td><td>Jothikumar and Hill, 2009</td></tr>
<tr><td>Adenovirus F and G</td><td>Xu et al., 2000</td></tr>
<tr><td>Enterovirus</td><td>Donaldson et al., 2002</td></tr>
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<td>50L</td>
</tr>
<tr>
<td>Source tracking</td>
<td colspan="2">Source tracking was performed using two methods. First, the HF183/BacR287 human Bacteroides marker set was used, following the US EPA standardised protocol (for details, please see Method 1696; US EPA, 2019). In brief, this detects human faecal pollution using a TaqMan® quantitative polymerase chain reaction (qPCR) assay. In brief, the samples were processed following the steps of Method 1609 and 190 µL of final elute was then processed following the steps of Method 1696. All standards, method blanks, positive spikes, extraction blanks, internal amplification controls and extraction controls were performed as per that outlined in EPA Method 1696 (US EPA, 2019). The second method used to source track was based on Henry et al. (2016). In brief, samples were filtered on 0.22 µm filters, DNA was extracted and then sequenced using a variable region 3 and 4 of the 16s gene. Microbial community fingerprints were then compiled as per that explained in Henry et al. (2016). These fingerprints were then used in the publicly available SourceTracker program and compared to an array of available sink fingerprints (the EPHM lab has over 1000 community profiles which can be used for comparison). In this case, the following sinks were selected from our local Melbournian database: human sewage from Eastern Treatment Plant and septic systems from around Melbourne, dog, possum, waterfowl, seagull, cat, cow, rabbit, etc. The output of the SourceTracker program is an estimate of the proportion of the beach sample community that is made up of each sink sample. The method is further explained in Henry et al. (2016) and McCarthy et al. (2017).</td>
</tr>
</table>

[q]Most probable number (MPN) [d]Only conducted when positive detection of *Giardia*/*Crypto*. * total volume of sample processed during recovery testing for *Campylobacter* and *Salmonella* testing. [&]only three samples were analysed for qPCR of these pathogens and these were done at the end of the swimming season (March-April 2018).

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Appendix D: Dose responses models and their parameters used for baseline QMRA and for sensitivity testing.

	D-R model type	D-R model parameters
Campylobacter		
Baseline	Beta-Poisson (Schmidt et al. 2013)	$\alpha = 0.1453$ $\beta = 8.007$
Sensitivity	Approx. Beta Poisson (Medema et al. 1996)	$\alpha = 0.145$ $\beta = 7.59$
Sensitivity	Beta-Poisson (Teunis et al. 2005)	$\alpha = 0.024$ $\beta = 0.011$
Salmonella		
Baseline	Hypergeometric – p(ill) (Teunis et al. 2010)	$\alpha = 8.53 \times 10^{-3}$ $\beta = 3.14$ $\eta = 8.23$ $\rho = 69$
Giardia		
Baseline	Exponential (Rose et al. 1991)	r = 0.0199
Cryptosporidium		
Baseline	Exponential (NRMMC, 2006)	r = 0.059
Sensitivity	Exponential (US EPA, 2005)	r = 0.09
Adenoviruses		
Baseline	Exponential – *p(inf|ingestion)* (Crabtree et al. 1997)	r = 0.4172
Sensitivity	Beta Poisson – *p(inf|ingestion)* (Teunis et al. 2016)	$\alpha = 5.11$ $\beta = 2.80$
Enteroviruses		
Baseline	Exponential	R = 0.0127
Noroviruses (assumed to be present at cultured MPN adenoviruses densities and NoV qPCR densities)		
Baseline	Fractional and Beta Poisson Cloud (Soller et al. 2017)	P = 0.72 $\mu = 1106$; $\alpha = 0.04$ $\beta = 0.055$
Sensitivity	Beta Poisson (Teunis et al. 2008)	$\alpha = 0.04$ $\beta = 0.055$
Sensitivity	Fractional Poisson (Messner et al. 2014)	P = 0.72 u = 1106

Note: All outcomes are a probability of illness, except where noted (that is *Salmonella* had probability of illness outcomes).

Appendix E: Probabilities of illness given infection used for baseline QMRA and for sensitivity testing.

	p(ill/inf)	
	Baseline	**Sensitivity test**
Campylobacter	PERT (0.1, 0.28,0.6)	-
Salmonella	N/A[1]	-
Giardia	PERT (0.2,0.45,0.7)	-
Cryptosporidium	PERT (0.2,0.5,0.7)	-
adenoviruses	PERT (0.25, 0.5,0.75)	Ingestion = $1-(1+{}^{Dose}/_{6.53})^{-0.41}$
noroviruses	60%	-

Note: [1]included in D-R model above