

## Pathogen performance testing of a natural swimming pool using a cocktail of microbiological surrogates and QMRA-derived management goals

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### ABSTRACT

In recent decades, natural swimming pools (NSPs) have gained popularity in Europe, especially in Germany and Austria. NSPs differ from swimming pools in that they utilize biological treatment processes based on wetland processes with no disinfection residual. However, data are missing on the specific log-reduction performance of NSPs to address enteric virus, bacteria, and parasitic protozoa removal considered necessary to meet the North American risk-based benchmark (<35 illnesses per 1,000 swimming events) set by the USEPA for voluntary swimming. In this study, we examined Canada's first NSP at Borden Park, Edmonton, Canada, to address the following three questions: (1) Given normal faecal shedding rates by bathers, what is the total log reduction (TLR) theoretically needed to meet the EPA benchmark? (2) what is the *in-situ* performance of the NSP based on spiking suitable microbial surrogates (MS2 coliphage, *Enterococcus faecalis*, and *Saccharomyces cerevisiae* [Baker's yeast])? and (3) how much time is required to reach acceptable bather risk levels under different representative volume-turnover rates? A reverse-quantitative microbial risk assessment (QMRA) revealed that of the four reference pathogens selected (*Norovirus*, *Campylobacter*, *Cryptosporidium*, and *Giardia*), only *Norovirus* was estimated to exceed the risk benchmark at the 50th, 75th, and 95th percentiles, while *Campylobacter* was the only other reference pathogen to exceed at the 95th percentile. Log-reduction values (LRVs) were similar to previous reports for bacterial indicators, and novel LRVs were estimated for the other two surrogates. A key finding was that more than 24 h treatment time would be necessary to provide acceptable bather protection following heavy bather use (378 bathers/day for *main pool* and 26 bathers/day for *children's pool*), due to the mixing dynamics of the treated water diluting out possible residual pool faecal contamination. The theoretical maximum number of people in the pool per day to be below USEPA's 35 gastro cases in 1,000 swimming events was 113, 47, and 8, at the 50th, 75th, and 95th percentiles. Further, the use of ultra-violet disinfection to the pool return flow had little effect on reducing the treatment time required.

**Key words:** biological treatment, natural swimming pool, *Norovirus*, QMRA, recreational water

### HIGHLIGHTS

- QMRA of the first natural swimming pool in Canada.
- Performance testing of the external biological treatment barriers and UV system at the natural pool using spiked surrogates for enteric pathogens (*Norovirus*, *Cryptosporidium*, and *Campylobacter*).
- Strategies for reducing enteric pathogen risks, which is driven by the number of infected swimmers, under EPA guidelines' threshold level for recreational water use.

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## INTRODUCTION

### Background

Recreational water is an economic, social, and cultural asset. Since the completion of the first natural swimming pool (NSP) in modern times in Austria, other countries have followed suit with some 20,000 NSPs constructed as of 2005 (Littlewood 2005). The natural approach to swimming pools is attractive to many, with NSPs mostly employing biological treatment in place of conventional physio-chemical processes and disinfection (Giampaoli *et al.* 2014; Schets *et al.* 2020; Petterson *et al.* 2021). Chlorine-based disinfection has its disadvantages, despite its general efficacy (i.e., disinfectant odour, disinfection by-products, and other aesthetic issues) (Manasfi *et al.* 2017). While there are concerns over a possible link between exposure to disinfection by-products and possible increased prevalence of bladder cancer (Costet *et al.* 2011), recent reviews infer co-factors (such as smoking) as a major part of the story (Cotruvo & Amato 2019). However, little is known regarding the efficacy of many of the natural processes used in NSPs for removing human pathogenic enteric viruses, bacteria, and protozoa that may pose a risk to human health, as most health programmes do not include routine monitoring of diseases associated with recreational water bodies (Neogi *et al.* 2014; Graciaa *et al.* 2018; Russo *et al.* 2020).

Though there is a limited understanding of the health risks associated with NSPs, numerous outbreaks have been documented from waterborne pathogens in recreational ponds and lakes (Blostein 1991; Paunio *et al.* 1999; Sinclair *et al.* 2009; Barna & Kádár 2012; Graciaa *et al.* 2018) and inadequately disinfected swimming pools (Sinclair *et al.* 2009; Barna & Kádár 2012). Similarities can reasonably be assumed between the health risks of ponds, lakes and swimming pools, and NSPs. For example, the chlorine-resistant parasitic protozoan oocysts of *Cryptosporidium* spp. are considered the leading cause of gastroenteritis in chlorinated swimming pools, resulting from faecal accidents by bathers (Suppes *et al.* 2017). Adequate removal of pathogenic viruses, bacteria, and protozoa is essential by the NSP system, given the absence of residual disinfectant and expected frequency of faecal shedding (Barna & Kádár 2012; Petterson *et al.* 2021), along with likely urination (Jmaiff Blackstock *et al.* 2017) and associated viral and urinary-tract pathogens (Henderson *et al.* 1998; Leonard *et al.* 2018). However, there is limited understanding of the microbial risks and general microbiological performance of NSPs. Currently, NSPs in North America are designated as general recreational water bodies, which have lower standards than pools/spas (though Borden NSP, which was investigated in the current study, is tested weekly) but could pose a higher theoretical risk of enteric infections.

### Performance surrogates to inform quantitative microbial risk assessment

The value of faecal indicator bacteria (FIB) as an index of gastrointestinal illnesses in bathers is well studied, particularly for sewage-impacted water bodies (Fewtrell & Kay 2015; Russo *et al.* 2020). However, there is a lack of data on non-enteric pathogens (e.g., *Pseudomonas aeruginosa*, *Legionella pneumophila*, and non-tuberculosis mycobacteria) in NSPs and recreational waters (WHO 2006). Timeliness for detecting FIB is of critical importance to manage pathogens that may be associated with faecal shedding in an enclosed pool system, as seen by outbreaks of cryptosporidiosis each summer in chlorinated swimming pools (Ryan *et al.* 2017). In a related field, performance-based targets for enteric viruses, bacteria, and protozoa along with opportunistic pathogen mitigation measures are emerging as part of best practice in managing pathogen risks with reuse waters (Schoen *et al.* 2017). In general, assessing the removal performance of waterborne pathogens through treatment barriers provides foundational data to better understand the potential health risks when various operational scenarios are modelled using a quantitative microbial risk assessment (QMRA) approach that allows for quantitative scientific data to be interpreted in the context of estimated health outcomes to support water safety management (WHO 2016).

Performance surrogates are used to assess the efficacy of a treatment process to remove the three classes of waterborne pathogens: viruses, bacteria, and protozoa (WHO 2016; Zimmerman *et al.* 2016). Given the potential presence of pathogenic viruses (Jiang 2006; Sinclair *et al.* 2009), bacteria (Caskey *et al.* 2018; Leoni *et al.* 2018), and parasitic protozoa (Pintar *et al.* 2010) in recreational waters, it is important to understand the reduction/inactivation kinetics of all three groups and potentially, those of helminth ova and fungal spores (Rudko *et al.* 2017). While performance testing using surrogates (indigenous or spiked-in) has become a more common practice to validate pathogen reductions associated with water reuse, its application to bathing waters is limited. Commonly used treatment surrogates spiked into waters include, MS2 coliphage for enteric (non-enveloped) viruses, *Escherichia coli* or *Enterococcus faecalis* for enteric bacteria, and spores of *Clostridium perfringens* or fluorescent microspheres to address parasitic protozoan oo/cysts (Shapiro *et al.* 2010; Zimmerman *et al.* 2016).



**Table 1** | Best estimate of log<sub>10</sub> reduction credits for treatment barriers within the NSP system; taken from [Petterson et al. \(2021\)](#)

Treatment barrier	Best estimate of elimination capacity (log <sub>10</sub> reduction) mean with plausible ranges applied in Monte Carlo simulation		
	Viruses	Bacteria	Protozoa
NF	1 (0.5, 2.5)	2 (1,3)	1.5 (0.2, 3)
ZF	0	0	0
Hydrobotanical filter/submerged substrate filter (HBF/SF)	0.5 (0, 2)	1 (0, 2)	1 (0.2, 2.5)
UV (25–40 mJ/cm <sup>2</sup> )	2.6	5	3

as the surrogate to represent enterococci. As an alternative to using microspheres as surrogates for parasitic protozoan oocysts, and to address loss in viability, we utilized *S. cerevisiae* as a relatively under-explored surrogate ([Chung 2012](#)).

### Reverse QMRA to estimate critical pathogen densities and required TLR

To address possible viral, bacterial, and parasitic protozoan enteric pathogen classes, we utilized four reference pathogens: human noroviruses, *C. jejuni*, and *C. hominis*/*G. intestinalis* oocysts, respectively. The benchmark risk target of 35 cases of gastroenteritis per 1,000 swimming events was used in accordance with [U.S. Environmental Protection Agency \(2012b\)](#) recreational water criteria. Using the WHO-described approach for a reverse QMRA ([Petterson & Ashbolt 2016](#)), we estimated the critical doses necessary to meet the benchmark risk for each class of enteric pathogen for potentially exposed children and adults when bathing in the children's pool and main pool, respectively ([Figure 1](#)).

Key assumptions for the determination of reference pathogen concentrations are outlined in [Table 2](#), including the number of bathers each day, the quantity of faeces shed, and estimated concentration of reference pathogens within faeces. Key assumptions for determining bather exposure to reference pathogens are outlined in [Table 3](#), including amount ingested, dose-response models, probability of illness, and the resulting critical doses for each organism.

### Efficacy of Borden Park NSP treatment barriers

#### Tracer pre-study using *E. coli* spike

Prior to the day of system performance testing using the three surrogates, a tracer study was performed using *E. coli* to gain a baseline understanding of flow times through the system and approximate LRVs at each treatment step. The protocol for the enumeration of *E. coli* is outlined below.

#### Preparation of microbiological performance surrogates

Three surrogates were selected to represent likely conservative removal/reduction of enteric viruses, bacteria, and parasitic protozoa, based on various proposed criteria ([Rice et al. 1996](#); [Nieminski et al. 2000](#); [Busta et al. 2003](#); [Sinclair et al. 2012](#)), being MS2 coliphage (ATCC 15597-B1), *E. faecalis* (ATCC 29212), and *S. cerevisiae* (Fleischmann, Germany), respectively.

**Table 2** | Key assumptions in estimating reference pathogen concentrations ([Petterson et al. 2021](#))

Bather assumptions				
Bathers per day	Adult pool–Max: 378/day; Nominal: 252/day Kiddy pool–Max: 26/day; Nominal: 17/day			
Faeces excreted	0.06–6 g/bather			
Pathogen concentrations				
Reference pathogen	Norovirus	Campylobacter	Cryptosporidium	Giardia
Organisms per gram of faeces (infected bathers): Median (range) ( <a href="#">Bambic et al. 2011</a> )	10 <sup>8</sup> (10 <sup>4</sup> –10 <sup>10</sup> ) genome copies	10 <sup>7</sup> (10 <sup>1</sup> –10 <sup>8</sup> ) CFU	10 <sup>5</sup> (10 <sup>3</sup> –10 <sup>6</sup> ) oocysts	10 <sup>5</sup> cysts
Point prevalence (%)	0.80	0.39	0.11	0.39
Organisms shed per infected bather: Median (5th percentile, 95th percentile)	6.4E07 (2.3E05, 4.6E08)	8.5E05 (5.4E02, 7.6E07)	1.2E05 (5.7E03, 1.3E06)	3.4E04 (3.0E04, 5.7E05)

**Table 3** | Key assumptions for reference pathogen dose-responses and critical doses to meet benchmark risk (Pettersen *et al.* 2021)

<b>Bather exposure</b>				
Exposure (accidental ingestion): Adults (children)	16 (37) mL (Dufour <i>et al.</i> 2006)			
<b>Reference pathogen dose-responses</b>				
	<b>Norovirus</b>	<b>Campylobacter</b>	<b>Cryptosporidium</b>	<b>Giardia</b>
Dose-response model: Exact Beta-Poisson parameters for infection	$\alpha = 0.063; \beta = 0.032$ (Messner <i>et al.</i> 2014)	$\alpha = 0.024; \beta = 0.011$ (Teunis <i>et al.</i> 2005)	$\alpha = 0.115; \beta = 0.176$ (Teunis <i>et al.</i> 2005)	Exponential model $k = 0.02$ (Rendtorff 1954)
Probability of illness given infection	0.7 (Teunis <i>et al.</i> 2008)	0.2 (Black <i>et al.</i> 1988)	0.7 (USEPA 2006)	0.7 (USEPA 2006)
Critical dose (number of organisms): Adults (children)	4.9 (2.1)	18.4 (7.9)	8.4 (3.6)	160 (69)

One day prior to assessing the performance of the treatment barriers, overnight cultures of *E. faecalis* (ATCC 29212) and MS2 bacteriophage (ATCC 15597-B1) were grown as follows. *E. faecalis* was inoculated in tryptic soy broth (TSB) and incubated overnight at 37 °C and estimated as most probable numbers (MPNs) and qPCR cell equivalents as described below. MS2 was grown by infecting *E. coli* (ATCC 15597) during its exponential phase (about 18 h at 37 °C) as described in Method 1601 (USEPA 2001), then filtered through a 0.22 µm membrane to recover the coliphages. *S. cerevisiae*, also known as Baker's yeast (Fleischmann, Germany), was purchased as a 'moist cake' from a local grocer in Edmonton, Alberta, and directly suspended by vigorous vortexing in 1% phosphate-buffer saline (PBS).

### Spiking and sampling of treatment components

A spiking cocktail was created with MS2 bacteriophage, *E. faecalis*, and Baker's yeast surrogates. Prior to spiking, all pumps leading into the buffer tank, which fed the biological treatment units (Figure 1), were turned off to drain the tank. The spiking cocktail was added to the buffer tank part-way through filling it with fresh water to aid distribution of the organisms to final concentrations of 7.2, 5.6, and 7.8 log<sub>10</sub> per 100 mL of viable MS2, *E. faecalis*, and Baker's yeast, respectively. Once the tank was filled, a hose originating from an air compressor was submerged into the buffer tank to continue mixing.

Separate pumps leading to the NF and SSF/HBP were then turned on, and approximately 1 min later (based on earlier *E. coli* tracer studies of flow times), 500-mL samples were taken from the sample points prior to any treatment barriers to obtain baseline surrogate concentrations. Also based on the prior tracer test, after 12 min, 500-mL samples were taken at post-treatment sample points for surrogate enumeration. Samples were immediately stored in an ice-packed cooler prior to being transported back to the laboratory. This procedure of draining the basin, adding the surrogate cocktail, turning on the pumps to the NF and HBF/SF, and sampling was repeated two additional times over the 1 day to obtain triplicate samples and ensure steady-state surrogate concentrations throughout the system.

### Enumeration of surrogates

*E. faecalis* and *E. coli* assays using Enterolert™ and Colilert™. Enterolert™ and Colilert™ were used according to the manufacturers' specifications (IDEXX Canada, ASTM Method #D6503-99) to estimate viable *E. faecalis* and *E. coli*, respectively (ASTM International). Samples were either undiluted or diluted with Milli-Q water to a final volume of 100 mL in sterile bottles (IDEXX, USA), Enterolert™/Colilert™ reagent dissolved, sealed in a Quanti-Tray 2000™, and then incubated for 24 h at 41 and 37 °C for *E. faecalis* and *E. coli*, respectively. After incubation, Quanti-Tray 2000™ fluorescent wells were counted to estimate the MPN of *E. faecalis* and *E. coli*.

*E. faecalis* by qPCR. *Enterococcus* qPCR was performed as per Method 1611 (USEPA 2012a), with the following modifications: the qPCR master mix used was PrimeTime Gene Expression Master Mix (Integrated DNA Technologies, Canada) and the reactions were run on an ABI 7500 Fast thermocycler with fast cycling conditions (95 °C 3' [95 °C 5'', 60 °C 30''] × 40 cycles). See Supplementary Table S1 for a list of primers/probe sequences used. In brief, bacteria from

100 mL samples were collected on 0.4 µm polycarbonate filters in a disposable funnel (FMFNL1050; Pall), and filters were rolled and placed into 2 mL O-ring tubes and frozen at –80 °C until being processed further. DNA was extracted in lysis buffer spiked with Salmon Sperm DNA using a Bead Mill 24 (Fisher Scientific, USA) at a speed of 3.1 m/s for 1 min. Sketa qPCR was first performed prior to *Enterococcus* qPCR to determine if there was any PCR inhibition in the samples. Samples were directly quantified (in triplicate), and results were expressed relative to a standard curve consisting of serial dilutions (5–50,000 target gene copies/reaction) of a standard culture of *E. faecalis* (ATCC 29212) and a no template control.

**MS2 bacteriophage by qPCR.** MS2 coliphages were concentrated using a modified polyethylene glycol (PEG-6000, Merck, Germany) precipitation method described by Hamza *et al.* (2009), and quantified human adenovirus (HAdV 40/41, provided by Prof. X. L. Pang, Provincial Laboratory for Public Health, Edmonton) was added as a process control (Hamza *et al.* 2009). A comparison of viruses detectable after filtration compared with the number of spiked HAdV particles showed a recovery rate between 50 and 75% (data not shown). Infectivity was estimated using a propidium monoazide (PMA) pre-treatment and optimized protocol described by Dinh Thanh *et al.* (2017). Total viral nucleic acids of samples pre-treated and not pre-treated with PMA were co-extracted from 200 µL of viral suspension using the DNeasy Blood and Tissue Kit™ (Qiagen, Germany) according to the manufacturer's instructions. Virus DNA and RNA were eluted in 100 µL of elution buffer and stored at –20 °C until assayed.

To quantify bacteriophage MS2, complementary DNA from its RNA genome was produced using the High Capacity cDNA Reverse Transcription Kit™ (Thermo-Fisher, USA) following the manufacturer's protocol. Cycling conditions and concentrations of primers and probes for MS2 and HAdV are described in Pecson *et al.* (2009) and Heim *et al.* (2003), respectively, and summarized along with protocols for the preparation of standards in Leifels *et al.* (2015).

***S. cerevisiae* (Baker's yeast) culture.** Baker's yeast samples were diluted as needed in sterile tap water and 20 µL was spread plated onto malt extract agar (MEA; Sigma-Aldrich, Millipore-70145 MSDS) with a pH of 5.5 to reduce bacterial growth and incubated for 48 h at 28 °C. Yeast colony forming units (CFU) were counted to estimate the concentration of yeast in triplicate.

### Simplified model to aid NSP management

Model equations following the law of dilution (Gage *et al.* 1926) were developed to provide operators with a practical tool to estimate TLR achieved and pool water turnovers needed under the following pool water circulation conditions: (1) pool (main and children's) turnover time, defined as the time required to circulate one pool volume-equivalent through the system; (2) system removal capacity, which is LRVs provided in a single pass through the treatment barriers; and (3) duration of operation. The equations were developed based on a simplified single pool model assuming that the cross contamination between the main and children's pools was negligible, which is satisfied in normal operation as shown by Monte Carlo simulation of the NSP system.

Assuming complete real-time mixing of water, when a small amount of water, that is,  $1/n$  of the total pool volume, where  $n$  is a large number, flows to the treatment barriers and then returns to the pool after treatment, the portion that is not circulated to the treatment barriers in the pool becomes  $(1 - (1/n))$ . As the system continues operating, the portion of water that is not circulated to the treatment barriers is then  $(1 - (1/n))(1 - (1/n)) (1 - (1/n)) \dots$ . Specifically, this process continues for  $n$  times for one turnover. And the water portion that is not circulated through treatment barriers is  $(1 - (1/n))^n$ , which becomes  $e^{-1}$  when  $n$  goes to infinity, meaning the assumed small amount of water is infinitely small. This is true for flowing water since we could think water goes through the treatment barriers bit by bit. Therefore, the circulation efficiency, that is, the portion of water that will flow through the treatment barriers during one turnover period, is  $1 - e^{-1}$ .

Generally, the portion of water that is not circulated to the treatment barriers in any duration of operation is

$$\left(1 - \frac{1}{n}\right)^{m n} \rightarrow e^{-m} \quad \text{if } n \rightarrow \text{infinity}$$

where  $m$  is the number of turnovers for the period of operation, which could be any positive real number. Accordingly, the portion that is circulated through the treatment barriers is  $1 - e^{-m}$ . Assuming a 100% system removal capacity, this would also be the treatment efficiency (TE), that is, the fraction of pathogen removal for any operation time.

Practically, the system removal capacity is always imperfect (<100%). In this case, we discuss the calculation of TE in a similar way to the calculation of circulation efficiency. Still assuming complete real-time mixing of water, when  $1/n$  ( $n$  is large) of the total pool volume flows to the treatment barriers that has a system removal capacity  $R$  (represented as a fraction, e.g.,  $1 - \log_{10}$  reduction = 0.9,  $2 - \log_{10}$  reduction = 0.99, etc.), and then returns to the pool after treatment, the portion of pathogens that is not filtered out or killed by the treatment barriers becomes  $(1 - (R/n))$ . As this process continues, the portion of total pathogen that is not filtered out or inactivated by the treatment barrier is

$$\left(1 - \frac{R}{n}\right)^{mn} \rightarrow e^{-mR} \quad \text{if } n \rightarrow \text{infinity}$$

It follows that

$$\text{TE} = (1 - e^{-mR}) \quad (1)$$

where TE is the treatment efficiency, and  $R$  is the system removal capacity, so TE can then be converted to a TLR value:

$$\text{TLR} = -\log(1 - \text{TE}) \quad (2)$$

where TLR is the total log reduction that has occurred in the pool as a whole, and TE is the total reduction as a fraction. By substituting TE in Equation (2) with Equation (1), we get the relationship between TLR and  $m$ :

$$\text{TLR} = mR \log(e) \quad \text{or} \quad m = \frac{\text{TLR}}{R \log(e)} \quad (3)$$

where  $e$  is Euler's number (i.e., 2.718...). The simplified model developed in Equation (3) was then used as an aid to the NSP operators to plot turnover periods needed against TLR required at various levels of system removal capacities.

## RESULTS AND DISCUSSION

### Reverse QMRA to estimate required TLR needed for safe operation of the Borden NSP

The theoretical concentrations of human *Norovirus*, *Campylobacter*, *Cryptosporidium*, and *Giardia* in bathing waters at 'normal' bather shedding are provided in Table 4, along with the TLR needed for each reference pathogen in the main and children's pools to meet the risk benchmark of 35 illnesses per 1,000 bathers. Notably, *Norovirus* was the determining reference pathogen for TLR. No reduction was needed for *Campylobacter*, *Cryptosporidium*, or *Giardia* at the 50th and 75th percentiles, and of these three, only *Campylobacter* required reduction at the 95th percentile. The risk-determining viral TLR value of 3.1 (corresponding to children's risk in adult pool at peak bather scenario) was used as a conservative value to derive risk management options described below since *Norovirus* had the highest required log reduction and the lowest anticipated log reduction from the NSP system. Given USEPA single-sample action levels for recreational waters are based on 75th percentiles (USEPA 2012b), we also utilized the 75th percentile for day-to-day management options.

It should be noted that the values displayed in Table 4 do not include overt faecal accidents. Faecal shedding conditions under nominal and peak bather numbers were used to gain an understanding of reference pathogen concentrations under day-to-day operation. While *Campylobacter*, *Giardia*, and *Cryptosporidium* played a risk-limiting role (compared with noroviruses), *Cryptosporidium* has previously been estimated to be at approximately 20,000 oocysts per litre during faecal accidents in pools (Gregory 2002) and may play a greater role in estimating the time required to treat NSP waters under such conditions.

### Assessment of Borden NSP treatment efficacy

The estimated  $\log_{10}$  reductions observed across the treatment barriers at Borden Park NSP are given in Table 5 and were similar to previous estimates (Table 1), with the notable exception of the Hydrobotanical filter and submerged filter (HBF/SF). See Supplementary Table S2 for estimated surrogate concentrations at each sampling point. Although measured concentrations of surrogates from samples taken after the HBF/SF process reached a steady state after approximately 30 min (based on *E. coli* pre-tests, data not given), HBF/SF delivered higher than expected LRVs for all three surrogates, as compared

**Table 4** | Summary quantiles of Monte Carlo sample of estimated pathogen concentration and required pathogen removal to achieve safe water quality<sup>a</sup> under routine bathing conditions

Percentile	Estimated reference pathogen concentration (org.L <sup>-1</sup> )			Total log <sub>10</sub> reduction required to achieve safe water quality <sup>a</sup>					
				Adults			Children		
	50	75	95	50	75	95	50	75	95
Main pool									
<i>Norovirus</i>									
Nominal bathers	230	1,300	8,000	1.7	2.4	3.2	2.0	2.8	3.6
Peak bathers	620	2,500	11,000	2.1	2.7	3.3	2.5	3.1	3.7
<i>Campylobacter</i>									
Nominal bathers	0.067	6.0	65	0	0	0.56	0	0	0.92
Peak bathers	1.1	15	93	0	0	0.70	0	0.26	1.1
<i>Cryptosporidium</i>									
Nominal bathers	0	0	0.43	0	0	0	0	0	0
Peak bathers	0	0.038	0.63	0	0	0	0	0	0
<i>Giardia</i>									
Nominal bathers	0	0	4.11	0	0	0	0	0	0
Peak bathers	0	0.34	5.73	0	0	0	0	0	0
Children's pool									
<i>Norovirus</i>									
Nominal bathers	0	0	2,300				0	0	3.0
Peak bathers	0	0	6,100				0	0	3.4
<i>Campylobacter</i>									
Nominal bathers	0	0	0.27				0	0	0
Peak bathers	0	0	9.7				0	0	0.15
<i>Cryptosporidium</i>									
Nominal bathers	0	0	0				0	0	0
Peak bathers	0	0	0				0	0	0
<i>Giardia</i>									
Nominal bathers	0	0	0				0	0	0
Peak bathers	0	0	0				0	0	0

<sup>a</sup>Benchmark risk of 35 illnesses per 1,000 swimming events, relying on assumptions described in the text.

with estimates from Table 1. Given the nature of the HBF/SF, which is a small pond allowing water to come into contact with the submersed plants, it is possible that our spiking study was not carried out over a long enough period for any potential dilution effect to play a role.

### Application of QMRA and treatment efficacy assessment for operation

Figure 2 illustrates the time required to treat Borden NSP water under normal system operation, based on 50th, 75th, and 95th percentiles for risk mitigation. Initially surprising was the lack of impact when using UV disinfection to meet the risk benchmark (35 cases in 1,000 bathers) for *Norovirus* (Figure 3). In essence, the rate of diluting out any pathogens shed within the two pools was the main limiting factor, not treatment efficacy.

A key finding was that the current estimated pathogen loadings to the Borden Park NSP were too high for sufficient removal within a 24 h period (Figure 2) at the 50th, 75<sup>th</sup>, and 95th percentiles of risk to be below the USEPA's benchmark for recreational waters across all flow rates within the system's capacity. Given the USEPA action level is based on the 75th percentile (U.S. Environmental Protection Agency 2012b), and the impracticality of continuously operating pumps at maximum capacity, it is recommended that mitigation measures be taken to reduce the loading of enteric viruses (indexed by

**Table 5** | Mean surrogate removal LRV by barrier<sup>a</sup>

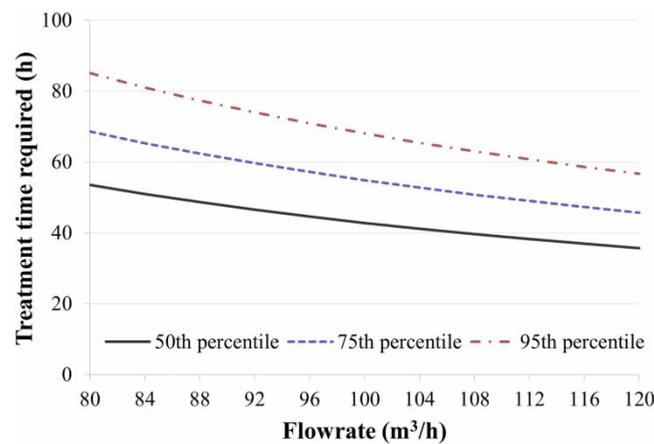
Barrier	MS2 (plaque count)	<i>E. faecalis</i> (culture assay)	<i>S. cerevisiae</i> (culture assay)
NF	1.49	1.05	3.99 <sup>b</sup>
HBF/SF	2.65 <sup>c</sup>	2.83 <sup>c</sup>	4.94 <sup>c</sup>
UV (Post NF)	2.50	4.03	2.91 <sup>b</sup>
UV (Post HBF/SF)	1.79	4.03 <sup>d</sup>	2.88

<sup>a</sup>LRVs were calculated from data in Supplementary Table S2, as the average of run 1 and run 2. Exceptions are as follows:

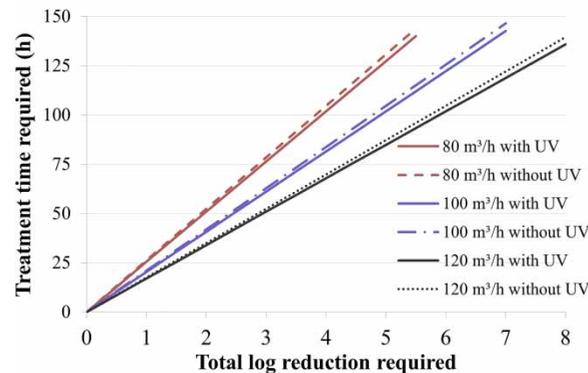
<sup>b</sup>Uncertain data from run 1 were ignored.

<sup>c</sup>As the difference between LRs from run 1 and run 2 is significant for HBF/SF, only run 2 numbers were used because run 2 allows longer time for spiking cocktail to flow through HBF/SF.

<sup>d</sup>Both LRs from run 1 and run 2 are uncertain. LR (Post NF) is used because the two UV systems are similar, and similar performance were shown in data for other two pathogen groups.

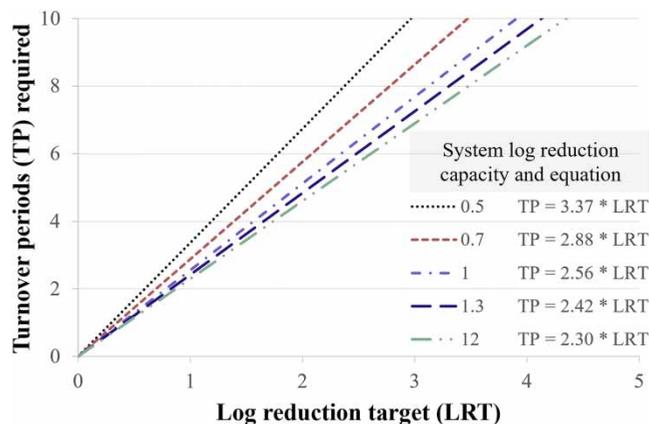


**Figure 2** | Estimated time required to treat Borden NSP waters to acceptable levels across possible flow rates under peak bather scenario, for 50th, 75th, and 90th percentiles of risk to be below the USEPA's benchmark of 35 illnesses in 1,000 swimming events (USEPA 2012b).



**Figure 3** | Estimated time needed to treat Borden NSP waters to acceptable levels across different TLR targets, at 80, 100, and 120 m<sup>3</sup>/h with and without the addition of UV, based on the criteria that 75th percentile of risk below the USEPA's benchmark for safety of 35 illnesses in 1,000 swimming events (USEPA 2012b).

noroviruses), either by reducing the number of bathers allowed in the pool each day and/or by taking stricter preventative measures, that is, ensuring bathers have not been ill for the preceding 2 weeks and are showering with soap prior to entering the pool (Ryan *et al.* 2017; Chalmers & Johnston 2018). If the NSP is used every day (or nearly every day), it is expected that the concentration of noroviruses, as well as the other reference pathogens, would rise over time, potentially increasing overall



**Figure 4** | Borden NSP water turnover periods needed for specified TLR targets, at various LR performance values (0.5, 0.7, 1.0, 1.3, and 12). Equations for each line are given, where 'y' represents the turnover periods needed to reach 'x', the TLR needed, depending on the LRV in a single pass through the system's treatment barriers, and included for each TLR to calculate total turnover periods required (Supplementary Figure S1).

risk to bathers. It should be noted that the Borden NSP, which was studied, undergoes marketing campaigns, weekly testing, and appropriate safety protocols to mitigate the risks of infected bathers entering the pool. See Supplementary Material for a detailed outline of such practices.

One of the more surprising results of this study was the low impact that the addition of UV had on decreasing the time required to treat the NSP water (see Figure 3). This lack of difference can be attributed to the recirculating hydraulic design of the pool system. Without the addition of residual chemical disinfectants, the system relies solely on the upstream treatment processes, where the water then flows back into the pools (Figure 1) and dilutes shed pathogens. This dilution effect leads to a diminishing return from additional LRVs, such as that from UV. With the current recirculating regime, any micro-organism reduction at the point source treatment in excess of 1.3 (Figure 4) has little impact under nominal faecal loadings (i.e., in the absence of an overt faecal accident). However, as the treatment needs increase (i.e., faecal accident resulting in excessive pathogen loading), the impact of the addition of UV increases slightly (see Figure 3).

### System operator model for practical use

The results presented in Figures 2 and 3 imply insufficient treatment within 1 day under current operational practices and assumptions (i.e., number of bathers per day and percentage of whom may be pathogen carriers). Hence, the QMRA model was rearranged to estimate the number of bathers per day to meet the USEPA benchmark risk. Based on all the same assumptions used in the reverse QMRA, it was determined that the theoretical maximum allowable bathers per day would be 113, 47, and 8, for the 50th, 75th, and 95th percentiles of risk, respectively. However, these numbers do not seem realistic at the 75th and 95th percentiles; hence, the number of pool turnover periods through treatment was investigated (Figure 4).

When the system was modelled for a low LRV, the number of turnover periods needed could be reduced more efficiently by increasing the system's LRV. However, this effect was diminished when the system operated with a higher LRV. For example, increasing LRV from 0.5 to 0.7, the turnover periods needed could be reduced by 15%, versus increasing LRV from 1.3 to 12 could reduce turnover periods by only 5% (Figure 4). Given the fact that additional LRVs in a single pass through the system (even with UV) did not meaningfully decrease the needed treatment time for the Borden Park NSP, it is suggested that mitigation measures focus on reducing the number of infected bathers and ensure participants are showering adequately prior to entering the pool.

## CONCLUSION

The results from this study utilized a multi-faceted approach to assess the treatment efficacy and potential waterborne pathogen risks associated with the Borden Park NSP. A 'cocktail' of spiked surrogates was used, representing human pathogenic viruses, bacteria, and protozoan oocysts to measure the performance efficacy of the treatment barriers under normal

operation. Additionally, a reverse QMRA was performed to determine under what operating scenarios the Borden NSP could meet the USEPA benchmark risk for recreational waters of 35 gastrointestinal cases per 1,000 swimming events. Overall, the individual treatment barriers performed as intended (Table 2); however, due to the constant dilution of treated waters entering the pool post-treatment, it was demonstrated that safe levels (based on the key reference pathogen, *Norovirus*) could not be attained at the 50th, 75th, or 95th percentile measurements of risk within 24 h. Further, our modelling found that treatment barrier log-reduction values in excess of ~1.3 (see Supplementary Figure S1) provided little improvement on the amount of time required to treat the NSP waters to meet the 35 illnesses per 1,000 bathers benchmark. To mitigate health risks, the following steps were recommended: (1) strict preventative measures be taken by ensuring bathers understand the necessity for showering with soap prior to entering the pool, (2) upon entry, bathers asked to not enter the pool if they have been ill within a 2-week period, and/or (3) the maximum number of bathers allowed per day be reduced to <45 to maintain an acceptable level of risk.

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## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

## REFERENCES

- Adelman, M. J., Phelps, M., Hadacek, R. T., Slosser, O. R., Calvet, S., Oppenheimer, J. & Borhardt, J. H. 2016 Required C x T value for 5-log virus inactivation at full scale. *Journal - American Water Works Association* **108**, E18–E26.
- ASTM International. *Standard Test Method for Enterococci in Water Using Enterolert*.
- Bambic, D., McBride, G., Miller, W., Scott, R. & Wuertz, S. 2011 Quantification of pathogens and sources of microbial indicators for QMRA in recreational waters. WERF Research Report Series, IWA Publishing **10**. <https://doi.org/10.2166/9781843395430>.
- Barna, Z. & Kádár, M. 2012 The risk of contracting infectious diseases in public swimming pools. A review. *Annali Dell'Istituto Superiore di Sanità* **48** (4), 374–386.
- Black, R. E., Levine, M. M., Clements, M. L., Hughes, T. P. & Blaser, M. J. 1988 Experimental *Campylobacter jejuni* infection in humans. *The Journal of Infectious Diseases* **157**, 472–479.
- Blostein, J. 1991 Shigellosis from swimming in a park pond in Michigan. *Public Health Reports* **106** (3), 317–321.
- Busta, F. F., Suslow, T. V., Parish, M. E., Beuchat, L. R., Farber, J. N., Garrett, E. H. & Harris, L. J. 2003 The use of indicators and surrogate microorganisms for the evaluation of pathogens in fresh and fresh-cut produce. *Food Science & Technology* **2** (s1), 179–185.
- Caskey, S., Stirling, J., Moore, J. E. & Rendall, J. C. 2018 Occurrence of *Pseudomonas aeruginosa* in waters: implications for patients with cystic fibrosis (CF). *Letters in Applied Microbiology* **66** (6), 537–541.
- Chalmers, R. & Johnston, R. 2018 Understanding the public health risks of *Cryptosporidium* in swimming pools: a transmission pathway approach. *Perspectives in Public Health* **138** (5), 238–240.
- Chung, J. 2012 *Development of Fluorescently Labelled Cryptosporidium Oocyst Surrogates to Test the Efficacy of Sand Filtration Processes*. University of New South Wales, New South Wales.
- Costet, N., Villanueva, C. M., Jaakkola, J. J., Kogevinas, M., Cantor, K. P., King, W. D., Lynch, C. F., Nieuwenhuijsen, M. J. & Cordier, S. 2011 Water disinfection by-products and bladder cancer: is there a European specificity? A pooled and meta-analysis of European case-control studies. *Occupational and Environmental Medicine* **68** (5), 379–385.
- Cotruvo, J. A. & Amato, H. 2019 National trends of bladder cancer and trihalomethanes in drinking water: a review and multicountry ecological study. *Dose Response* **17** (1), 1559325818807781.
- Dinh Thanh, M., Agusti, G., Mader, A., Appel, B. & Codony, F. 2017 Improved sample treatment protocol for accurate detection of live *Salmonella* spp. in food samples by viability PCR. *PLoS One* **12** (12), e0189302.
- Dufour, A. P., Evans, O., Behymer, T. D. & Cantú, R. 2006 Water ingestion during swimming activities in a pool: a pilot study. *Journal of Water and Health* **4** (S2), 425–430.
- Fewtrell, L. & Kay, D. 2015 Recreational water and infection: a review of recent findings. *Current Environmental Health Reports* **2** (1), 85–94.
- Gage, S. D., Ferguson, H. F., Gillespie, C. G., Messer, R., Tisdale, E. S., Hinman Jr., J. J. & Green, H. W. 1926 Swimming pools and other public bathing places. *American Journal of Public Health* **16** (12), 1186–1201.
- Giampaoli, S., Garrec, N., Donze, G., Valeriani, F., Erdinger, L. & Spica, V. R. 2014 Regulations concerning natural swimming ponds in Europe: considerations on public health issues. *Journal of Water and Health* **12** (3), 564–572. <https://doi.org/10.2166/wh.2014.211>.

- Graciaa, D. S., Cope, J. R., Roberts, V. A., Cikesh, B. L., Kahler, A. M., Vigar, M., Hilborn, E. D., Wade, T. J., Backer, L. C., Montgomery, S. P., Evan Secor, W., Hill, V. R., Beach, M. J., Fullerton, K. E., Yoder, J. S. & Hlavsa, M. C. 2018 Outbreaks associated with untreated recreational water – United States, 2000–2014. *Morbidity & Mortality Weekly Report* **67** (25), 701–706.
- Gregory, R. 2002 Bench-marking pool water treatment for coping with *Cryptosporidium*. *Journal of Environmental Health Research* **1** (1), 11–18.
- Grunert, A., Frohnert, A., Selinka, H. C. & Szewzyk, R. 2018 A new approach to testing the efficacy of drinking water disinfectants. *International Journal of Hygiene and Environmental Health* **221** (8), 1124–1132.
- Hamza, I. A., Jurzik, L., Stang, A., Sure, K., Uberla, K. & Wilhelm, M. 2009 Detection of human viruses in rivers of a densely-populated area in Germany using a virus adsorption elution method optimized for PCR analyses. *Water Research* **43** (10), 2657–2668.
- Heim, A., Ebnert, C., Harste, G. & Pring-Akerblom, P. 2003 Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *Journal of Medical Virology* **70** (2), 228–239.
- Henderson, Y. C., Liu, T. J. & Clayman, G. L. 1998 A simple and sensitive method for detecting adenovirus in serum and urine. *Journal of Virological Methods* **71**, 51–56.
- Jiang, S. C. 2006 Human adenovirus in water: occurrence and health implications: a critical review. *Environmental Science & Technology* **40**, 7132–7140.
- Jmaiff Blackstock, L. K., Wang, W., Vemula, S., Jaeger, B. T. & Li, X.-F. 2017 Sweetened swimming pools and hot tubs. *Environmental Science & Technology Letters* **4** (4), 149–153.
- Lee, S., Hata, A., Yamashita, N. & Tanaka, H. 2017 Evaluation of virus reduction by ultrafiltration with coagulation-sedimentation in water reclamation. *Food and Environmental Virology* **9** (4), 453–463.
- Leifels, M., Jurzik, L., Wilhelm, M. & Hamza, I. A. 2015 Use of ethidium monoazide and propidium monoazide to determine viral infectivity upon inactivation by heat, UV-exposure and chlorine. *International Journal of Hygiene and Environmental Health* **218** (8), 686–693.
- Leonard, A. F. C., Zhang, L., Balfour, A. J., Garside, R., Hawkey, P. M., Murray, A. K., Ukoumunne, O. C. & Gaze, W. H. 2018 Exposure to and colonisation by antibiotic-resistant *E. coli* in UK coastal water users: environmental surveillance, exposure assessment, and epidemiological study (Beach Bum survey). *Environment International* **114**, 326–333.
- Leoni, E., Catalani, F., Marini, S. & Dallolio, L. 2018 Legionellosis associated with recreational waters: a systematic review of cases and outbreaks in swimming pools, spa pools, and similar environments. *International Journal of Environmental Research and Public Health* **15**, 8.
- Littlewood, M. 2005 *Natural Swimming Pools: An Inspirational Guide for Construction and Maintenance*. Schiffer Publishing, Pennsylvania.
- Manasfi, T., Coulomb, B. & Boudenne, J. 2017 Occurrence, origin, and toxicity of disinfection byproducts in chlorinated swimming pools: an overview. *International Journal of Hygiene and Environmental Health* **220** (5), 591–603.
- Messner, M. J., Berger, P. & Nappier, S. P. 2014 Fractional Poisson – a simple dose-response model for human norovirus. *Risk Analysis* **34** (10), 1820–1829.
- Neogi, S. B., Yamasaki, S., Alam, M. & Lara, R. J. 2014 The role of wetland microinvertebrates in spreading human diseases. *Wetlands Ecology and Management* **22** (5), 469–491.
- Nieminski, E. C., Bellamy, W. D. & Moss, L. R. 2000 Using surrogates to improve plant performance. *Journal – American Water Works Association* **92** (3), 67–78.
- Paunio, M., Pebody, R., Keskimäki, M., Kokki, M., Ruutu, P., Oinonen, S., Vuotari, V., Siitonen, A., Lahti, E. & Leinikki, P. 1999 Swimming-associated outbreak of *Escherichia coli* o157:H7. *Epidemiology and Infection* **122** (1), 1–5.
- Pecson, B. M., Martin, L. V. & Kohn, T. 2009 Quantitative PCR for determining the infectivity of bacteriophage MS2 upon inactivation by heat, UV-B radiation, and singlet oxygen: advantages and limitations of an enzymatic treatment to reduce false-positive results. *Applied and Environmental Microbiology* **75** (17), 5544–5554.
- Petterson, S. R. & Ashbolt, N. J. 2016 QMRA and water safety management: review of application in drinking water systems. *Journal of Water and Health* **14** (4), 571–589.
- Petterson, S. R., Li, Q. & Ashbolt, N. J. 2021 Screening level risk assessment (SLRA) of human health risks from faecal pathogens associated with a natural swimming pond (NSP). *Water Research* **18**, 116501. <https://doi.org/10.1016/j.watres.2020.116501>.
- Pintar, K. D., Fazil, A., Pollari, F., Charron, D. F., Waltner-Toews, D. & McEwen, S. A. 2010 A risk assessment model to evaluate the role of fecal contamination in recreational water on the incidence of cryptosporidiosis at the community level in Ontario. *Risk Analysis* **30** (1), 49–64.
- Rendtorff, R. C. 1954 The experimental transmission of human intestinal protozoan parasites II. *Giardia lamblia* cysts given in capsules. *American Journal of Hygiene* **50**, 209–220.
- Rice, E. W., Fox, K. R., Miltner, R. J., Lytle, D. A. & Johnson, C. H. 1996 Evaluating plant performance with endospores. *Journal – American Water Works Association* **88** (9), 122–130.
- Rudko, S. P., Ruecker, N. J., Ashbolt, N. J., Neumann, N. F. & Hanington, P. C. 2017 *Enterobius vermicularis* as a novel surrogate for the presence of helminth ova in tertiary wastewater treatment plants. *Applied and Environmental Microbiology* **83** (11), e00547-17.
- Russo, G. S., Eftim, S. E., Goldstone, A. E., Dufour, A. P., Nappier, S. P. & Wade, T. J. 2020 Evaluating health risks associated with exposure to ambient surface waters during recreational activities: a systematic review and meta-analysis. *Water Research* **176**, 115729.
- Ryan, U., Lawler, S. & Reid, S. 2017 Limiting swimming pool outbreaks of cryptosporidiosis – the roles of regulations, staff, patrons and research. *Journal of Water and Health* **15** (1), 1–16.

- Schets, F. M., van den Berg, H. H. J. L., Lynch, G., de Rijk, S., de Roda Husman, A. M. & Schijven, J. F. 2020 [Evaluation of water quality guidelines for public swimming ponds](#). *Environment International* **137**, 105516. <https://doi.org/10.1016/j.envint.2020.105516>.
- Schoen, M. E., Ashbolt, N. J., Jahne, M. A. & Garland, J. 2017 Risk-Based Enteric Pathogen Reduction Targets for Non-Potable and Direct Potable Use of Roof Runoff, Stormwater, and Greywater. *Microbial Risk Analysis* **5**, 32–43.
- Shapiro, K., Mazet, J. A., Schriewer, A., Wuertz, S., Fritz, H., Miller, W. A., Largier, J. & Conrad, P. A. 2010 [Detection of \*Toxoplasma gondii\* oocysts and surrogate microspheres in water using ultrafiltration and capsule filtration](#). *Water Research* **44** (3), 893–903.
- Sinclair, R. G., Jones, E. L. & Gerba, C. P. 2009 [Viruses in recreational water-borne disease outbreaks: a review](#). *Journal of Applied Microbiology* **107** (6), 1769–1780.
- Sinclair, R. G., Rose, J. B., Hashsham, S. A., Gerba, C. P. & Haas, C. N. 2012 [Criteria for selection of surrogates used to study the fate and control of pathogens in the environment](#). *Applied and Environmental Microbiology* **78** (6), 1969–1277.
- Suppes, L. M., Huang, C. H., Lee, W. N. & Brockman, K. J. 2017 [Sources of pharmaceuticals and personal care products in swimming pools](#). *Journal of Water and Health* **15** (5), 829–833.
- Teunis, P., Van Den Brandhof, W., Nauta, M., Wagenaar, J., Van Den Kerkhof, H. & Van Pelt, W. 2005 [A reconsideration of the \*Campylobacter\* dose–response relation](#). *Epidemiology and Infection* **133** (4), 583–592.
- Teunis, P. F., Moe, C. L., Liu, P., Miller, S. E., Lindesmith, L., Baric, R. S., Le Pendu, J. & Calderon, R. L. 2008 [Norwalk virus: how infectious is it?](#) *Journal of Medical Virology* **80** (8), 1468–1476.
- USEPA 2001 *Method 1601: Male-Specific (F+) and Somatic Coliphage in Water by Two-Step Enrichment Procedure*. Office of Water, United States Environmental Protection Agency, Washington, DC.
- USEPA 2006 *Economic Analysis for the Final Long Term 2 Enhanced Surface Water Treatment Rule*. U.S. Environmental Protection Agency, Washington, DC.
- USEPA 2012a *Method 1611: Enterococci in Water by TaqMan Quantitative Polymerase Chain Reaction (qPCR) Assay*.
- USEPA 2012b *Recreational Water Quality Criteria*. U.S. Environmental Protection Agency, Washington, DC.
- Wade, T. J., Calderon, R. L., Brenner, K. P., Sams, E., Beach, M., Haugland, R., Wymer, L. & Dufour, A. P. 2008 [High sensitivity of children to swimming-associated gastrointestinal illness: results using a rapid assay of recreational water quality](#). *Epidemiology* **19** (3), 375–383.
- WHO 2006 *Guidelines for Safe Recreational Water Environments – Volume 2: Swimming Pools and Similar Environments*. World Health Organization, Geneva.
- WHO 2016 *Quantitative Microbial Risk Assessment: Application for Water Safety Management*. World Health Organization, Geneva.
- Zimmerman, B. D., Korajkic, A., Brinkman, N. E., Grimm, A. C., Ashbolt, N. J. & Garland, J. L. 2016 [A spike cocktail approach to improve microbial performance monitoring for water reuse](#). *Water Environment Research* **88** (9), 824–837.

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