



Near real-time notification of water quality impairments in recreational freshwaters using rapid online detection of β -D-glucuronidase activity as a surrogate for *Escherichia coli* monitoring

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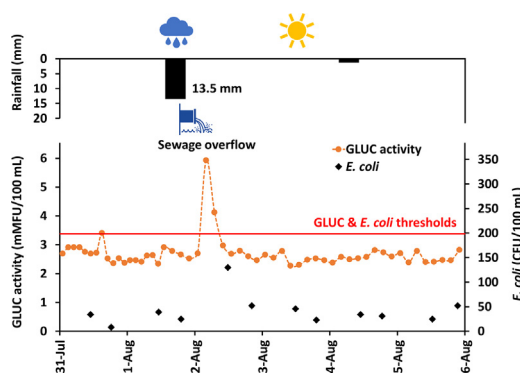
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HIGHLIGHTS

- Rapid online monitoring of β -D-glucuronidase (GLUC) activity in recreational waters
- Setting site-dependent GLUC activity thresholds based on regulatory *E. coli* ones
- Good threshold exceedance agreement with limited failures to act and false alarms
- Improved understanding of frequency, timing and amplitude of contamination events
- New insights for rapid recreational water quality assessment and decision-making

GRAPHICAL ABSTRACT



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ABSTRACT

Waterborne disease outbreaks associated with recreational waters continue to be reported around the world despite existing microbiological water quality monitoring frameworks. Most regulations resort to the use of culture-based enumeration of faecal indicator bacteria such as *Escherichia coli* to protect bathers from gastrointestinal illness risks. However, the long sample-to-result time of standard culture-based assays (minimum 18–24 h) and infrequent regulatory sampling (weekly or less) do not enable detection of episodic water quality impairments and associated public health risks. The objective of this study was to assess the suitability of an autonomous online technology measuring β -D-glucuronidase (GLUC) activity for near real-time monitoring of microbiological water quality in recreational waters and for the resulting beach management decisions. GLUC activity and *E. coli* concentrations were monitored at three freshwater sites in Quebec, Canada (sites Qc1-3) and one site in New Zealand (site NZ) between 2016 and 2018. We found site-dependent linear relationships between GLUC activity and *E. coli* concentrations and using confusion matrices, we developed site-specific GLUC activity beach action values (BAVs) matching the regulatory *E. coli* BAVs. Using the regulatory *E. coli* BAV as the gold

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standard, rates of false alarms (unnecessary beach advisories using GLUC activity BAV) and failures to act (failure to trigger advisories using GLUC activity) ranged between 0 and 32% and between 3 and 10%, respectively, which is comparable to the rates reported in other studies using qPCR-defined BAVs. However, a major benefit of the autonomous enzymatic technology is the real-time reporting of threshold exceedances, while temporal trends in GLUC activity can assist in understanding the underlying dynamics of faecal pollution and potential health risks. Our study is the first to describe the applicability of online near real-time monitoring of microbiological water quality as a tool for improved beach management and public health protection.

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1. Introduction

Fresh, estuarine and marine waters offer opportunities for recreational activities worldwide (World Health Organization, 2003). These water bodies are economically important, especially for tourism, and this is being recognised with increasing investments to rehabilitate riverbanks and urban watering places (Austin et al., 2007). However, recreational waters are frequently contaminated by faecal microorganisms thereby increasing the risks of gastrointestinal illness (GI) and associated costs for communities (DeFlorio-Barker et al., 2018). Waterborne pathogens are introduced into water bodies through point and diffuse pollution sources within urban, agricultural or mixed catchments, mainly through domestic wastewater discharges and run-off carrying faecal deposits from domestic and wild animals (Ferguson et al., 2003; Galfi et al., 2016).

To prevent exposure of bathers to waterborne pathogens, recreational water guidelines and standard methods have been proposed worldwide in association with defined microbiological water quality criteria (European Parliament and the Council, 2006; Health Canada, 2012; MfE and MoH, 2003; US Environmental Protection Agency, 2012). They recommend monitoring requirements and maximum acceptable concentrations of faecal indicator bacteria (FIB) in recreational waters to protect public health (Table 1). Based on epidemiological studies, *Escherichia coli* (*E. coli*) is the preferred FIB for assessing and monitoring freshwater microbial quality (Wade et al., 2003; World Health Organization, 2003).

Despite the existence of regulatory frameworks, waterborne disease outbreaks associated with recreational activities still occur in developed countries (Graciaa et al., 2018) and their frequency was shown recently to increase in natural recreational waters (Gharpure et al., 2019). This reflects the need for an appropriate and robust monitoring and management framework for natural recreational waters. Two issues are often raised for recreational water quality monitoring. Firstly, regulatory water sampling is often sporadic because of resource and analytical methods limitations and it is not adapted to the high temporal and spatial fluctuations in FIB concentrations in water (Boehm, 2007; Converse et al., 2012; Muirhead and Meenken, 2018). As a result, beaches can be often closed due to single-day contamination events, days after the actual health risk for bathers has passed. Conversely, beach access is maintained during contamination peaks that are missed by regulatory sampling, thereby exposing bathers to illness risks (Leecaster and Weisberg, 2001; Whitman and Nevers, 2004). Secondly, current microbiological methods recommended for the monitoring of recreational water quality of waters are culture-based, involving a minimal 18–24 h delay between sample and results, which makes same-day sampling and decision making impossible. Finally, as current culture-based methods quantify culturable organisms only, any FIB such as *E. coli* that has turned into a viable but non culturable (VBNC) and remains metabolically active will not be detected by standard culture-based enumeration methods (Servais et al., 2009). Thus, aged contamination containing more VBNC bacteria is less

Table 1
Comparison between current regulations on recreational water quality for freshwaters in Quebec, European Union, New Zealand and the United States of America. CFU, colony-forming unit, GM, geometric mean, MPN, most probable number, STV, statistical threshold value.

Regulation	Water quality grading system	<i>E. coli</i> thresholds (CFU or MPN per 100 mL)	Sampling strategy	Recommended standard method
Québec	A – Excellent B – Good C – Fair D – Polluted	≤20 ^a Between 21 and 100 Between 101 and 200 ≥201	At least 2 times (per bathing season) At least 3 times At least 5 times At least 5 times	mFC-BCIG agar (CEAEQ, 2016)
European Union ^b	Excellent Good Sufficient Insufficient	95th percentile ^c <500 95th percentile <1000 90th percentile <900 90th percentile >900	Min. 1 sample before and 3 during bathing season (<1 month between consecutive samples). If short-term pollution, one additional sample to be taken to confirm that the incident has ended	Chromocult Coliform Agar (ISO 9308-1) or miniaturized MPN method (ISO 9308-3)
New Zealand ^d	Acceptable Alert Action	<260 CFU/100 mL 1 sample > 260 CFU/100 mL 1 sample > 550 CFU/100 mL	Routine (usually weekly) sampling, (except for beaches graded Very Good and Very Poor), at least 20 samples from each site + additional sampling after exceedances	mTEC agar (USEPA, 2010) or Colilert Quanti-Tray/2000 (IDEXX, USA)
United States of America	Estimated Illness Rate = 36/1000 or 32/1000 for primary contact recreators	GM < 126 CFU/100 mL, STV < 410 CFU/100 mL Or < 235 CFU/100 mL GM < 100 CFU/100 mL, STV < 320 CFU/100 mL Or < 190 CFU/100 mL	At least 1 sample per week, evaluation of the GM and the STV over a period of 30 days	mTEC agar (USEPA, 2014) or any other equivalent method that measures culturable <i>E. coli</i> .

^a During each sampling occasion, samples are collected along longitudinal transects at two different depths, yielding in final two composite samples that are analysed and subsequently averaged for threshold-based decision making.

^b A long-term profile of the beach, aiming at characterizing the site and its upstream territory by identifying the potential sources of pollution must be made beforehand, and revised according to the rating obtained during routine monitoring (every 4, 3 or 2 years for a good, satisfactory or unsatisfactory quality, respectively). Each member state defines the dates of its bathing season and a monitoring schedule is established every year before the beginning of the bathing season.

^c The percentiles are calculated using data collected over four successive bathing seasons.

^d Surveillance criteria are used for routine weekly monitoring for recreational sites and are different from the long-term Suitability for Recreation grading (SFRG) criteria based on a Sanitary Inspection Category (SIC) and 95th percentiles used for Microbiological Assessment Criteria (MAC) (MfE/MoH, 2003).

likely to be detected, despite the fact that they represent a potential risk for health (Boehm et al., 2018; Oliver, 2010).

Automated culture-based technologies have been developed to reduce sample-to-result time, such as TECTA (Bramburger et al., 2015), ALERT (Angelescu et al., 2018), or Colifast ALARM™ (Tryland et al., 2015) systems. Through patented technologies, results are typically generated within 1 to 18 h depending on the initial concentration of culturable bacteria in the sample. Quantitative polymerase chain reaction (qPCR) has been successfully implemented to shorten the sample-to-result time in bathing waters and it can generate quantitative results within <4 h (Cao et al., 2018; Dorevitch et al., 2017; Mendes Silva and Domingues, 2015). A qPCR assay has been developed and standardized for the detection of *Enterococci* (ENT) in recreational waters (US Environmental Protection Agency, 2012). Also, automatization of qPCR assays was made possible for the enumeration of ENT and harmful algal species as reported by Yamahara et al. (2015). Despite the possibility to provide same-day results, qPCR assays usually require extensive laboratory equipment and technical expertise and they are prone to PCR inhibition due to matrix-related interfering compounds (Shanks et al., 2016; Griffith and Weisberg, 2011).

Given the costs and logistics associated with microbiological analyses, predictive modelling offers a rapid alternative method. Models are developed for real-time prediction of FIB concentrations using catchment-related information, historical FIB data and local hydrometeorology (Garneau and Vanrolleghem, 2017; Nevers and Whitman, 2005, 2009). However, the choice of the nowcast model is essential given that predictive models can be site-specific and display variable performances (e.g. Thoe et al., 2015; Brooks et al., 2016).

More recently, rapid enzyme-based assays developed in the late 1990s (Farnleitner et al., 2001; Fiksdal et al., 1994; George et al., 2000) were coupled to autonomous technologies to achieve near real-time monitoring of β -D-glucuronidase (GLUC) activity (Ryzinska-Paier et al., 2014; Koschelnik et al., 2015). They were tested and implemented for online monitoring of microbiological water quality of surface waters in rural (Stadler et al., 2016, 2019; Ender et al., 2017; Stott et al., 2019) and urban (Burnet et al., 2019a, 2019b) settings, but not yet in the context of recreational water quality.

The main objective of this study was to investigate the usefulness of rapid online monitoring of GLUC activity for timely and safe recreational water quality assessment and management. We first sought to determine GLUC activity thresholds that best matched equivalent *E. coli*-based regulatory beach action values (BAVs) at recreational sites in Quebec and New Zealand. We then tested for beach management decision agreements between both methods (enzymatic vs culture-based assays) by considering i) the 24-h sample-to-result time inherent to culture assays and ii) the possibility of an agreement due to chance only for such dichotomous decisions. Finally, we assessed the added value of online GLUC activity monitoring for near real-time assessment of recreational water quality based on optimized BAVs. The new perspectives offered by online enzymatic monitoring in terms of recreational water quality assessment as support to rapid informed decision making are further discussed.

2. Material and methods

1) Sampling strategy

Three sites in Quebec, Canada (Qc1, Qc2 and Qc3) and one site in the city of Hamilton, New Zealand (NZ) were sampled. Among the Qc sites, 2 are located in the Greater Montreal Area (Qc2 and Qc3) and 1 near Quebec City (Qc1) (Table 2). In Quebec, the sampling framework for beach water quality monitoring is described in the “Environnement Plage” program, but the latter concerns public beaches only and it is implemented on a voluntary basis. Also, a minimal sampling frequency is recommended although it can be increased (MELCC, 2019). At site Qc1, based on the prescribed “Environnement Plage” sampling plan,

two composite samples were collected daily (in the morning) at two different depths (0.3 and 1.2 m), each composed of 5 grab samples spatially distributed along a 100-m longitudinal transect. Two additional grab samples per day were collected near the transects at Qc1. The “Environnement Plage” program was not implemented by local authorities at Qc2 and Qc3. At site Qc2, single grab samples were taken routinely twice a day (around 10 AM and 11 PM). In addition, high-frequency sampling was carried out by the municipality following rainfall events (between 9 and 39 mm total rainfall over a 24 h period) that triggered local combined sewer overflows. At site Qc3, three grab samples were collected weekly from the intake of a drinking water treatment plant abstracting surface water from an urban river downstream from a wastewater treatment plant (WWTP) discharge. The Qc3 site is representative of the water body used for secondary contact recreation as the river is used for fishing and kayaking in summer. Bathing in this river is not regulated but local authorities were surveying a potential beach located downstream from the intake at the time of the study. All samples were analysed by the respective municipal laboratory within 24 h. At the NZ site, culturable *E. coli* were measured on grab samples collected manually or using an automated sampler under dry weather conditions and following storm events.

At Qc2, Qc3 and NZ sites, automated online monitoring of β -D-glucuronidase (GLUC) activity was carried out in situ using a ColiMinder® instrument (VWM Solutions, Austria) as described in Section 3. At Qc1, composite samples (as described above) were measured in the laboratory using the same technology. At Qc2 and NZ, automated samplers (Teledyne ISCO, USA) were programmed to match GLUC activity measurement frequency and deployed on-site to collect 1-L grab samples during selected GLUC activity peak events for parallel enumeration of culturable *E. coli*. All samples were analysed within 24 h of collection for the 4 sites.

2) Culture-based enumeration of *E. coli*

Enumeration of culturable *E. coli* was conducted with membrane filtration method using mFC-BCIG agar (method MA.700 - Ec.BCIG 1.0 (CEAEQ, 2016)) for Qc1 and Qc2 samples, and MI agar (USEPA method 1604 (US Environmental Protection Agency, 2002)) for Qc3 samples. For both methods, 100 mL of undiluted or diluted sample was filtered through a sterile 47 mm, 0.45 μ m pore size cellulose ester membrane filter (Millipore, Germany). The filters were then placed on 5 mL agar plates and incubated for 24 ± 2 h at 35 ± 0.5 °C for MI agar, and at 44.5 ± 0.5 °C for mFC-BCIG agar. Final concentrations were expressed in colony-forming units per 100 mL (CFU/100 mL). For the New Zealand (NZ) site, enumeration of *E. coli* was performed using Colilert Quanti-Tray/2000 (IDEXX, USA). Undiluted or diluted samples were processed following the manufacturer's instructions and incubated for 24 h at 35 ± 0.5 °C. Final concentrations were expressed in most probable number per 100 mL (MPN/100 mL) using the IDEXX MPN conversion table.

3) β -D-glucuronidase (GLUC) activity measurements

GLUC activity measurements were performed using ColiMinder® technology (VWM Solutions, Austria). For each measurement, the instrument collects a 6.5 mL-sample and mixes into it a proprietary fluorogenic substrate and buffer. To maximise subsequent emission of fluorescence, the incubation temperature is set at 44 ± 0.1 °C (Koschelnik et al., 2015). Optical measurement of the fluorescence emitted by the 4-methylumbelliferone (resulting from the cleavage of the 4-methylumbelliferyl- β -D-glucuronide (MUG) catalysed by GLUC) is carried out once the incubation temperature is stable. Under optimal excitation and emission wavelengths of 365 nm and 445 nm, respectively, the signal (measured in volts per second) is transformed into

Table 2
Sites characteristics, monitoring conditions and analytical methods.

Site name	Number of paired samples ^a		Site characteristics	Main faecal contamination sources	Monitoring periods	Monitoring strategy	Culture-based analytical methods
	Setting GLUC activity BAVs	Exceedance agreement analysis					
Qc1 ^b	54	21	Beach along the Saint Lawrence River (7000 to 15,000 m ³ /s).	Combined sewer overflow discharges, inputs of contaminants from agriculture and gulls (Garneau and Vanrolleghem, 2017)	June to September 2016	Daily sampling, arithmetic mean from 2 composite samples (MELCC, 2019)	Membrane filtration (MA, 700 - Ec.BCIG 1.0, (CEAEQ, 2016))
Qc2	155	41	Beach along the Saint Lawrence River (7000 to 15,000 m ³ /s).	Combined sewer overflows	July to September 2018	Daily sampling, 2 grab samples per day (between 10 AM and 11 PM) Event-based sampling ^c	Membrane filtration (MA, 700 - Ec.BCIG 1.0, (CEAEQ, 2016))
Qc3	65	35	Mille-Îles River (140 to 315 m ³ /s), drinking water intake, 250 m upstream from potential beach	Treated sewage effluents, sewage bypasses, combined sewer overflows.	May to September 2017 and May to September 2018	3 samples per week (between 9 and 11 AM)	Membrane filtration (MI agar, USEPA 1604, (USEPA, 2002))
NZ	220	37	Waikato River (150 to 450 m ³ /s), recreational activities (bathing, kayak, rowing), flow-controlled river (8 dams, 425km).	Mainly diffuse sources (livestock, runoff, cattle grazing, effluent spreading, stormwater and wildlife). 1 stormwater outlet immediately upstream.	December 2017 to April 2018 (over summer bathing season)	Baseflow and event samples	Defined Substrate Technology (Colilert Quanti-Tray 2000, (IDEXX, USA))

^a Paired samples for culture assays and GLUC activity measurements.

^b Two composite samples were taken each day following the "Environnement Plage" sampling program and the arithmetic mean was then. For the setting of GLUC activity BAV, a total of 20 averaged composite samples and 34 single grab samples were used. For exceedance agreement analysis, the remaining 21 averaged composite samples were used, but no single grab sample was used, since regulatory monitoring at Qc1 involves averaged composite samples (see Material and Methods Section 1).

^c Triggered by rainfall-induced CSO discharges recorded in real-time by the city (between 9 and 39 mm 24-h cumulative rainfall episodes).

Modified Fishman Units per 100 mL (MFU/100 mL) according to the Sigma Quality Control Test Procedure (Sigma-Aldrich, 1998). A GLUC activity measurement takes about 25 min, including a 10-min cleaning step.

Validation (precision, robustness) and comparison of GLUC activity measurements with culture-and molecular-based enumeration of *E. coli* was recently performed by Burnet et al. (2019a). Measurement frequency was set at 2 or 3 h under baseline conditions (absence of rainfall) and was increased to hourly following rainfall episodes. Measurement results were generated by the instrument within 15 min after automated sample collection. GLUC activity results were transmitted in real-time on a secured web portal that could be accessed remotely. Instruments were also controlled remotely through a virtual private network (VPN) to adjust measurement frequency when required.

4) Data analysis

The overall methodological approach is illustrated in a flow chart (Fig. S1). A first part of the site-specific datasets was used to determine the GLUC activity threshold that best matched the equivalent *E. coli* regulatory threshold value. The remaining part of the datasets was used to perform exceedance agreement analyses using the optimized GLUC activity threshold. The number of samples from the site-specific datasets used for both analyses is summarized in Table 2.

4.1. Definition of GLUC activity beach action values (BAV) using confusion matrices

Routine and high-frequency (event-based) monitoring data (paired measurement of GLUC activity and *E. coli* concentrations) were pooled to explore the relationship between GLUC activity and concentrations of culturable *E. coli*. At Qc1, both composite and grab samples were pooled to maximise the size of the dataset. For different GLUC activity threshold values, beach management decisions (open, close) were

then compared with the regulatory *E. coli* threshold (Table S.1). False-positive closures (hereafter referred to as a "false alarm") were defined as GLUC activity-mediated beach closures not supported by *E. coli* results. False-negative closures (hereafter referred to as a "failure to act") were defined as GLUC activity-mediated beach opening while *E. coli* results recommended closure. The optimal GLUC activity threshold was determined for each study site by minimizing the rate of "failure to act" while trying to maintain the rate of "false alarm" as low as possible.

4.2. Beach management decision agreement

Using the remaining datasets, exceedance agreement analyses between GLUC activity and culturable *E. coli* were performed i) for Day 0 *E. coli* results and Day 0 GLUC activity results (same sample, but *E. coli* and GLUC activity results known on Day 1, and Day 0, respectively), and ii) for Day 1 GLUC activity results (sample taken on Day 1) and Day 0 *E. coli* results (sample taken on Day 0 but *E. coli* results known on Day 1). We used Day 0 *E. coli* result as the "gold standard" to assess the frequency of correct and incorrect beach management decisions given that it is used in current regulations worldwide (Table 1). For the exceedance agreement analysis, the definitions of "false alarm" and "failure to act" remained the same as for the confusion matrices (Section 4.1). Comparisons were performed on routine monitoring samples only (daily sample, usually collected in the morning) to reflect current beach monitoring and management practices, which are performed daily at best. For methods leading to dichotomous decisions (i.e. beach opening or closure) there is likely a level of agreement due to chance and reliability studies should therefore take this random agreement into account (Dorevitch et al., 2017). To account for chance due to random agreement, Cohen's kappa was calculated according to McHugh (2012). For the NZ site, only the BAV corresponding to the surveillance "action" threshold of 550 *E. coli*/100 mL was considered because only those *E. coli* threshold exceedances require immediate action to protect swimmers from exposure to potential waterborne pathogens. Above 550 *E. coli*/100 mL, there may be a significant risk of high levels of *Campylobacter* infection (MfE and MoH, 2003). The recommended "alert"

threshold of 260 *E. coli*/100 mL represents a substantial elevation in the probability of *Campylobacter* infection compared to the New Zealand background rate but does not involve recreational site closure.

3. Results and discussion

1) Relationships between GLUC activity and *E. coli* counts

Linear regressions were performed between \log_{10} -transformed on-line GLUC activity measurements and \log_{10} -transformed counts of culturable *E. coli*, both obtained under dry and wet weather conditions. Linear relationships and coefficients of correlation between GLUC activity and *E. coli* counts in paired samples were site-dependent (Fig. 1). The use of different culturable *E. coli* enumeration methods at Canadian and New Zealand sites may have influenced in part these correlations. Defined substrate technology has been shown to yield higher *E. coli* concentrations than membrane filtration likely due to better recovery of stressed cells using the liquid culture format (e.g. Hallas et al., 2008). This trend was observed at Qc3 in a previous study, although the strength of the correlation with GLUC activity was similar between membrane filtration (MI agar) and defined substrate technology (Colilert) ($r = 0.73$ and 0.77 , respectively) (Burnet et al., 2019a). In the present study, absence of parallel *E. coli* enumeration using membrane filtration and defined substrate technology for the other study sites prevents us from quantifying any potential assay-related bias.

Site-specific characteristics likely contributed most to the observed discrepancies in relationships between *E. coli* and GLUC activity. Site Qc3 displayed higher background GLUC activity compared to Qc1 and

Qc2 as reflected by its higher GLUC activity BAV of 6.0 mMFU/100 mL compared to 3.0 and 3.7 mMFU/100 mL for sites Qc1 and Qc2 respectively (Fig. 1). This can be explained by the direct influence of an upstream continuous release of UV-disinfected wastewater effluent (Burnet et al., 2019b), which is expected to generate non-culturable but still metabolically active *E. coli* cells that are detected with the enzymatic assay only (Villarino et al., 2000). Absence of apparent correlation between culturable *E. coli* and GLUC activity at Qc3 ($R^2 < 0.1$) is thus not surprising and deviations from a linear relationship between GLUC activity and *E. coli* for concentrations below 1000 CFU/100 mL have already been observed at this site (Burnet et al., 2019a). This consequently warrants caution on the use of direct enzymatic assays for the management of recreational water quality at Qc3, where only secondary contact recreation (fishing, kayaking) is currently allowed. The strength of the correlation between GLUC activity and culturable *E. coli* is assumed to depend on the number and type of samples and the diversity of pollution patterns reflected by these samples. In rural catchments characterized by agricultural runoff patterns, correlations were generally moderate (Ryzinska-Paier et al., 2014; Stadler et al., 2016), whereas it improved in urban settings under the influence of local untreated sewage discharges (Burnet et al., 2019a). In both rural and urban catchments, the strength of the correlation between culturable *E. coli* and GLUC activity increased during peak pollution events, compared to baseflow conditions (Burnet et al., 2019b; Ender et al., 2017; Stadler et al., 2016). As such, whereas assessment of GLUC activity and *E. coli* relationships at Qc2 ($R^2 = 0.64$) involved a large amount of samples collected also during peak contamination events, this was not the case for Qc1, Qc3 and NZ sites, which may in part

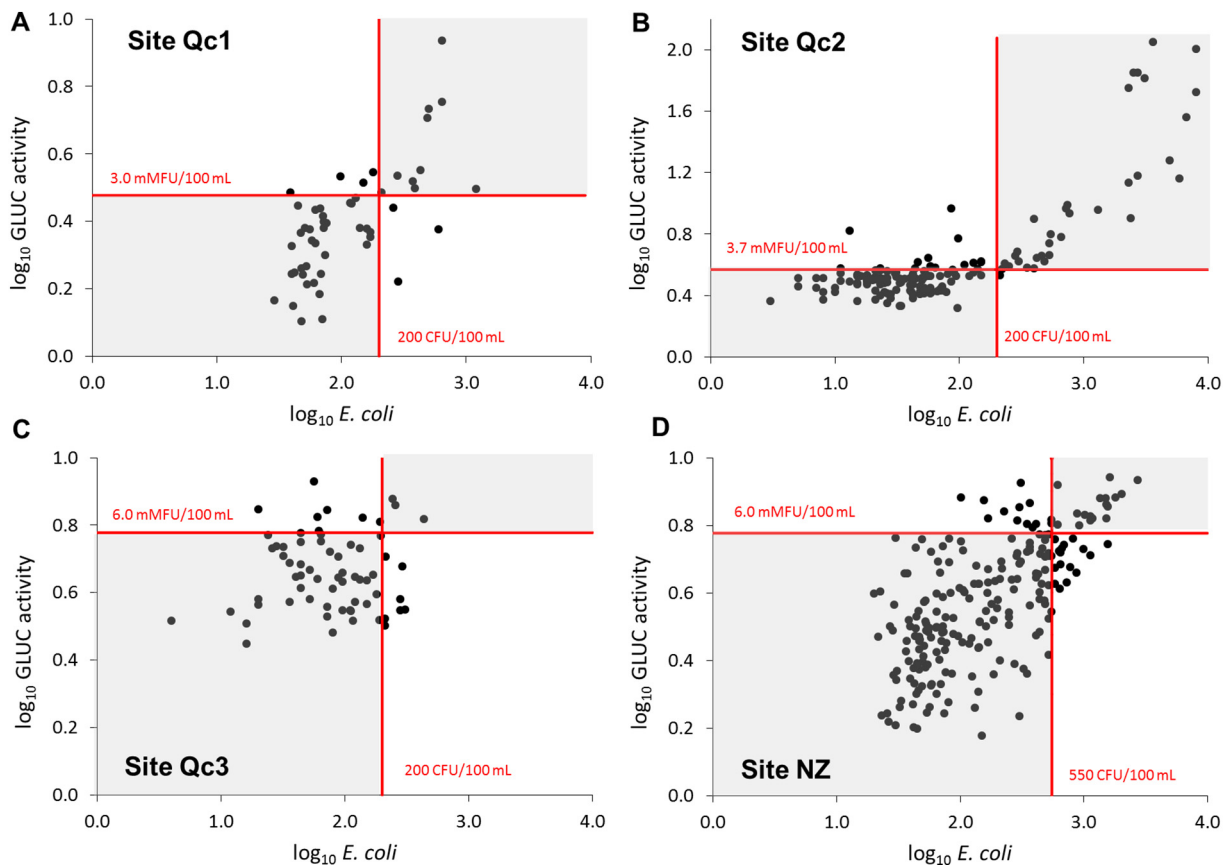


Fig. 1. Relationships between \log_{10} -transformed GLUC activity and \log_{10} -transformed concentrations of culturable *E. coli* for study sites Qc1-3 and NZ using part of the site-specific paired routine and event-based (when available) samples. Vertical red lines represent *E. coli* beach action values (BAVs) of 200 CFU/100 mL and 550 CFU/100 mL for QC and NZ sites, respectively. Horizontal red lines represent the site-specific adjusted GLUC activity BAV, and values expressed in mMFU/100 mL are indicated for each site. Shaded (grey) areas represent situations where both methods would lead to same decisions based on respective BAVs. A: Qc1 ($n = 54$), $y = 0.35x - 0.31$, $R^2 = 0.51$; B: Qc2 ($n = 155$), $y = 0.36x + 0.05$, $R^2 = 0.63$; C: Qc3 ($n = 65$), $y = 0.03x + 0.61$, $R^2 = 0.01$; D: NZ ($n = 220$), $y = 0.24x + 0.05$, $R^2 = 0.45$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

explain the comparatively weaker relationships at the latter study sites ($R^2 \leq 0.5$) (Fig. 1).

As *E. coli* concentrations increased in surface water samples, the proportion of GLUC activity signal per culturable *E. coli* decreased for all sites investigated in our study (Fig. 2). This trend has been reported by others and reflects the varying proportion of a subset of the *E. coli* population that has lost its ability to grow on culture media while still being metabolically active (Petit et al., 2000; Garcia-Armisen et al., 2005). High ratios of GLUC activity per culturable *E. coli* were observed in low-contaminated environments; this was explained by more stressful conditions in these environments due to better light penetration and lack of nutrients (Farnleitner et al., 2001; George et al., 2000; Garcia-Armisen and Servais, 2004; Servais et al., 2009). Converse et al. (2012) also showed that the ratio of culture to qPCR-based *Enterococci* counts was lowest for diffuse pollutions and closer to unity in highly contaminated settings. Interestingly, a subset of Qc2 samples deviated from the linear relationship for *E. coli* counts >2000 CFU/100 mL and paired GLUC activities >13 mMFU/100 mL (Fig. 2). These samples were collected during the rising limb and peak of a contamination episode following two major rainfall events (24 h-cumulated rain >20 mm) that triggered untreated sewage discharges from local (<1 km proximity) CSO outfalls. The comparatively higher proportion of GLUC activity per culturable *E. coli* may have resulted from inputs of aged faecal deposits within the sewershed, which could have been resuspended during the CSO event (Passerat et al., 2011). Moreover, extracellular GLUC (e.g. from lysed *E. coli*) is also detected by the enzymatic assay used in the present study and the enzyme is more persistent than the culturable cells in the environment (Fiksdal and Tryland, 2008). However, it was also shown that free enzymes only represent a small percentage of the measured enzymatic signal (Farnleitner et al., 2001). Similarly, previous tests at Qc3 showed that the GLUC activity signal from the extracellular fraction (<0.22 μ m) only represented up to 15% of the total signal (Burnet et al., 2019b). Another potential issue with GLUC determination is that non-*E. coli* bacteria also produce β -D-glucuronidase however, they must occur at significant concentrations to interfere with the signal of target bacteria (Tryland and Fiksdal, 1998). Additional investigations are therefore needed to encompass a larger diversity of catchments and pollution sources, and to better understand if (and how) metabolic pathways may be prioritized by *E. coli* according to the level of contamination and the type of aquatic environment.

2) Agreement between beach management decisions based on *E. coli* and GLUC activity BAVs

Because GLUC activity BAVs are not defined in water quality regulations, their performance was challenged against *E. coli* BAVs considered as the gold standard. GLUC activity BAVs were defined in a way to

minimize the rate of failures to act while maintaining false alarms as low as possible (Table S1). Optimized GLUC activity BAVs were 3.0, 3.7, 6.0 and 6.0 mMFU.100 mL⁻¹ for site Qc1, Qc2, Qc3 and NZ, respectively. The higher threshold value at Qc3 reflected the stronger GLUC activity background signal caused by an expected higher proportion of non-culturable cells at that site as explained in the previous section. A similarly high GLUC activity BAV was found for NZ site, but this is explained by the higher regulatory *E. coli* BAV of 550 CFU/100 mL compared to the 200 CFU/100 mL prescribed in Quebec.

False-alarms were more frequent than failures to act (Table 1) because we chose a conservative GLUC activity threshold that minimized failures to act (Fig. S1) and they were in the range of those observed in studies involving culture and qPCR (Dorevitch et al., 2017; Griffith and Weisberg, 2011; Raith et al., 2014). Most of these studies compared Day 0 culture and Day 0 qPCR ("same sample" results) in absence of temporal series of daily measurements. In addition, they did not consider the possibility of agreements to be the result of chance. To our knowledge, only Dorevitch et al. (2017) compared Day 0 culture and Day 1 qPCR. The authors found that apparently high BAV exceedance agreements ($>70\%$) between both methods were largely due to chance and concluded that the culture method may not be appropriate given the 18 to 24-h delay that hampers same-day decision making. Our findings lead to similar conclusions for Qc1 and Qc3 sites, as apparent exceedance agreements between the GLUC activity and *E. coli* BAVs were entirely due to chance (Cohen's kappa between -0.1 and 0.2) (Table 3). Moderate associations between both methods were found at Qc2 and NZ (Cohen's kappa between 0.47 and 0.65), with only a slightly better performance for GLUC activity and *E. coli* exceedance results from a same sample (Table 3). Although additional observations and study sites are warranted, our results suggest that apparent agreements between Day 0 culture and Day 1 GLUC activity are limited and essentially due to chance.

Point pollution sources generating rapid changes in *E. coli* concentrations are expected to contribute to discrepancies in BAV exceedances between culture and GLUC activity given the 24-h delay in results communication for culture assays. Our data illustrate how rainfall-induced discharges of combined sewer overflow outfalls at Qc2 caused strong hourly fluctuations in *E. coli* concentrations at the beach (Fig. 3). In practice, beach managers can only access Day 0 culture results after 24 h, which could thus lead to erroneous management decisions and unnecessary beach closures on Day 1, while bathers would have been exposed to potential health risks on Day 0.

3) Online monitoring of GLUC activity at Qc2 and NZ sites

The exceedance agreement analyses performed in this study involved two different techniques that fundamentally differ in terms of measurement throughput and sample-to-result time. As such, enzyme-based online monitoring represents an attractive alternative to the culture-based approach because it reports the rapid changes in recreational water quality (Fig. 3). For instance, we identified BAV exceedances at Qc2 as a result of CSO discharges, and they were characterized by a duration of 3 to 34 h and maximal GLUC activities of 110 mMFU.100 mL⁻¹ (corresponding to $>10^3$ CFU.100 mL⁻¹). Although high frequency sampling for culturable *E. coli* alongside online monitoring of GLUC activity was performed for only 2 events, strong associations between both parameters were observed (Fig. 3a, b), suggesting that GLUC activity is suitable for identifying potential health risks in these field settings.

The contrasting contamination loads and temporal dynamics of GLUC activity (and *E. coli*) between Qc2 and NZ sites reflect a combination of different faecal pollution sources, land use practices and local hydrometeorological conditions. Whereas 12 CSOs of various sizes discharge untreated water along a 10 km-stretch upstream of Qc2, pollution sources at NZ are mostly diffuse (Table 2). In addition, the NZ

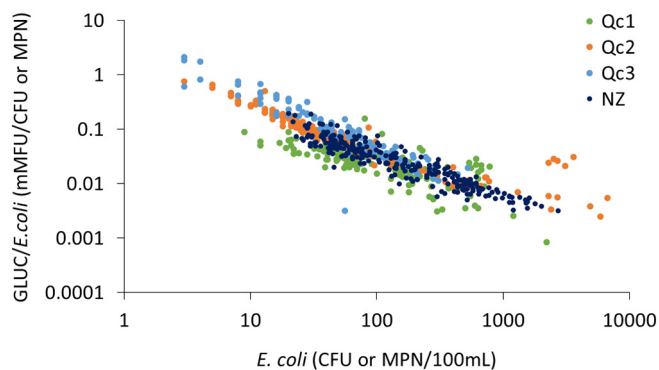


Fig. 2. Fluctuation of GLUC activity to culturable *E. coli* ratios as a function of culturable *E. coli* concentrations. For each site, all samples were pooled to yield a total of 167, 194, 100 and 257 samples for Qc1, Qc2, Qc3 and NZ sites, respectively.

Table 3

Percentages (and beachdays) of false-alarms and failures to act for GLUC activity and culture-based on beach management decisions. Sites Qc1, Qc2 and NZ are recreational areas with direct contact, while only indirect contact activities are allowed at Qc3.* Levels of Cohen's kappa (κ) agreement as proposed by McHugh (2012), 95% Lower and Upper Confidence Levels are given in brackets.

Site	GLUC activity day 1 vs <i>E. coli</i> day 0 ("same day" result)				GLUC activity day 0 vs <i>E. coli</i> day 0 ("same sample" result)			
	Qc1	Qc2	Qc3	NZ	Qc1	Qc2	Qc3	NZ
% Failure to act (n)	9.5 (2)	2.6 (1)	5.9 (2)	3.1 (1)	9.5 (2)	2.6 (1)	2.9 (1)	3.1 (1)
% False-alarm (n)	19.0 (4)	2.6 (1)	32.3 (11)	0 (0)	14.3 (3)	0	29.4 (10)	0 (0)
κ^*	−0.17 – None (−0.94, 0.59)	0.47 – Moderate (−0.24, 1.18)	−0.11 – None (−0.59, 0.36)	0.64 – Moderate (−0.04, 1.32)	−0.15 – None (−1.01, 0.71)	0.65 – Moderate (−0.02, 1.32)	0.06 – None (−0.40, 0.52)	0.65 – moderate (−0.03, 1.32)

river is regulated by successive dams, thereby buffering the catchment response to precipitation events. In addition to the analysis of various catchments parameters, automated high frequency monitoring of faecal pollution through GLUC activity can provide valuable information on catchment microbial dynamics (Stadler et al., 2019) and identify

dominant sources of faecal pollution (Burnet et al., 2019b). Consequently, in addition to providing information to beach managers to support near real-time decision making, online monitoring could further help stakeholders prioritize investments in remediation actions to improve water quality in recreational areas.

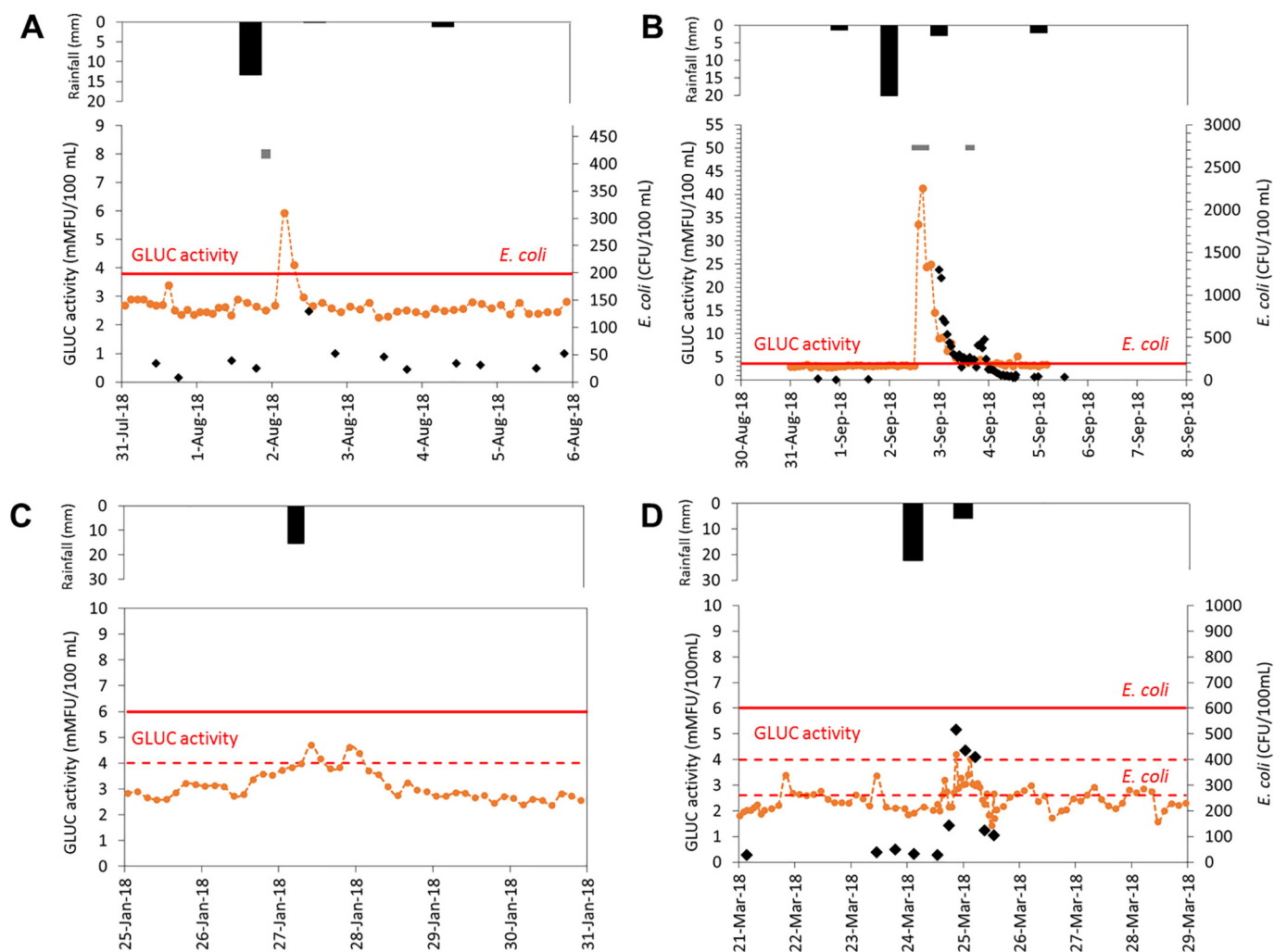


Fig. 3. Online near real-time monitoring of GLUC activity (orange dots) at Qc2 (A, B) and NZ (C, D) sites. Parallel routine or event-based enumeration of culturable *E. coli* (black dots) following rain episodes (24 h cumulative rainfall in black histograms) is represented in A, B and D. In C, GLUC activity measurements were carried out in absence of *E. coli* monitoring. For Qc2 (A and B), dark grey dots/horizontal lines illustrate the duration of upstream combined sewer overflows. For all site, the red full lines represent the regulatory beach action values (BAVs) in Quebec and New Zealand regulations (200 and 550 CFU/100 mL, respectively) and the adjusted GLUC activity-based BAVs; the red dotted lines represent the "alert" threshold as determined in the New Zealand regulation only (260 CFU/100 mL) and/or the adjusted GLUC activity-based threshold. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4) Does online monitoring of GLUC activity offer new insights for beach management?

Short-term contamination peaks that occur at urban beaches due to upstream point pollution sources are usually not detected under most regulatory monitoring frameworks. Even if detected through daily sampling, any notification would be delayed by at least 24 h using culture-based standard assays. Whereas qPCR generates same-day results for mid-day beach management decisions, its implementation for routine notification remains a challenge though (Griffith and Weisberg, 2011). The combination between high-throughput *in situ* measurements (hourly results) and the rapidity of results communication (<30 min) using enzyme-based online monitoring should thus ultimately improve recreational water quality management and foster public engagement through transparent and real-time communication of water quality data. The combination of online GLUC activity data with sewershed, hydrometeorological and catchment-related data will also provide valuable inputs for water quality prediction models. While being more user-friendly, a limitation of the automated online GLUC activity measurement technology remains its comparatively high capital costs though (Burnet et al., 2019a). Also, studies are needed to better understand the impact of non-*E. coli* and extracellular GLUC, as well as possible inhibitors of the enzymatic signal (Fiksdal and Tryland, 2008). Given the absence of regulatory requirements and standardisation, GLUC activity BAVs cannot be used by beach managers to trigger advisories or reopen an impaired beach. Nevertheless, online GLUC activity monitoring alongside regulatory sampling frameworks (as well as high-frequency analysis of culturable *E. coli* following rainfall episodes) can greatly help understand the fine temporal dynamics of faecal pollution at the beach while further contextualizing any regulatory grab sample, especially if the beach is impacted by intermittent upstream point pollution sources.

Further improvements can be brought to this study. Firstly, additional bathing seasons and sites are needed to encompass more water quality impairment episodes and further challenge the proposed GLUC activity BAVs. Secondly, the time series measured at Qc2 reveal the existence of secondary GLUC activity peaks following the main contamination peak. For example, a 2 h-exceedance of the GLUC activity threshold occurred on Sept 3rd (Fig. 3b) in absence of exceedance in *E. coli* BAV. Identifying the nature and origin of such peak events warrants more investigations. One possibility could be that aged contamination conveying non-culturable *E. coli* cells from further upstream sources reached the beach following the precipitation episode. Any decision making based on such intermittent BAV exceedances in GLUC activity thus remains doubtful. Finally, as waterborne pathogens can be detected in water in absence of culturable *E. coli* (Wu et al., 2011), it would be particularly important to assess the value of GLUC activity as a conservative biochemical parameter for the prediction of pathogen occurrence (enteric viruses, protozoan parasites) at the beach. This would be particularly needed in the case of GLUC activity BAV exceedances that occur in absence of concomitant increases in *E. coli* counts. Similar epidemiological studies as those performed for the definition of qPCR-based *Enterococci* BAVs (Wade et al., 2006) should thus be designed to assess the indicator value of GLUC activity for gastrointestinal illness risk.

4. Conclusions

- Online monitoring of GLUC activity was compared to culturable *E. coli* at 3 recreational sites in Quebec and 1 site in New Zealand to develop site-specific GLUC activity BAVs equivalent to the regulatory *E. coli* BAVs.
- Fitted GLUC activity BAVs ranged between 3.0 and 6.0 mMFU.100 mL⁻¹, and depended on the national regulatory threshold as well as on the background GLUC activity signal.
- Rates of false alarms (0 to 32%) and failures to act (3 to 10%) were site-dependent but comparable to those reported in recent studies on

qPCR-based BAVs.

- Threshold exceedance agreements between Day 0 *E. coli* and Day 0 GLUC activity BAVs (same sample) and between Day 0 *E. coli* and Day 1 GLUC activity (same result day) were weak for Qc1 and Qc3 but stronger for Qc2 and NZ sites. Overall though, decision agreements were essentially due to chance.
- Results demonstrate the potential of automated online GLUC activity monitoring for near real-time detection of BAV exceedances. The gathered high-resolution datasets on faecal pollution dynamics should support informed decision making towards safer access to recreational waters.

CRediT authorship contribution statement

Margot Cazals: Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Rebecca Stott:** Funding acquisition, Supervision, Investigation, Validation, Writing - review & editing. **Carole Fleury:** Formal analysis, Investigation, Writing - review & editing. **François Proulx:** Formal analysis, Investigation, Writing - review & editing. **Michèle Prévost:** Funding acquisition, Supervision, Methodology, Writing - review & editing. **Pierre Servais:** Resources, Validation, Writing - review & editing. **Sarah Dorner:** Funding acquisition, Supervision, Methodology, Writing - original draft. **Jean-Baptiste Burnet:** Funding acquisition, Supervision, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.137303>.

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