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Online fluorescence spectroscopy for the real-time evaluation of the microbial quality of drinking water

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ABSTRACT

We assessed the utility of online fluorescence spectroscopy for the real-time evaluation of the microbial quality of untreated drinking water. Online fluorimeters were installed on the raw water intake at four groundwater-derived UK public water supplies alongside existing turbidity sensors that are used to forewarn of the presence of microbial contamination in the water industry. The fluorimeters targeted fluorescent dissolved organic matter (DOM) peaks at excitation/emission wavelengths of 280/365 nm (tryptophan-like fluorescence, TLF) and 280/450 nm (humic-like fluorescence, HLF). Discrete samples were collected for *Escherichia coli*, total bacterial cell counts by flow cytometry, and laboratory-based fluorescence and absorbance. Both TLF and HLF were strongly correlated with *E. coli* ($\rho = 0.71–0.77$) and total bacterial cell concentrations ($\rho = 0.73–0.76$), whereas the correlations between turbidity and *E. coli* ($\rho = 0.48$) and total bacterial cell counts ($\rho = 0.40$) were much weaker. No clear TLF peak was observed at the sites and all apparent TLF was considered to be optical bleed-through from the neighbouring HLF peak. Therefore, a HLF fluorimeter alone would be sufficient to evaluate the microbial water quality at these sources. Fluorescent DOM was also influenced by site operations such as pump start-up and the precipitation of cations on the sensor windows. Online fluorescent DOM sensors are a better indicator of the microbial quality of untreated drinking water than turbidity and they have wide-ranging potential applications within the water industry.

1. Introduction

Enteric pathogens in drinking water pose a significant threat to public health globally (Baldursson and Karanis, 2011; Machdar et al., 2013; Reynolds et al., 2008). Worldwide regulation stipulates that the presence of enteric pathogens is inferred by the analysis of faecal indicator organisms, such as *Escherichia coli*, which are typically cultured over >18 h. Within the water industry, routine testing is typically conducted in off-site laboratories on discrete samples, collected on a daily to monthly basis (Cook et al., 2013; USEPA, 2013). However, the microbiological contamination of drinking water sources is inherently transient (Hynds et al., 2012; Kistemann et al., 2002; Worthington and Smart, 2017) and there is a shortage of suitable online detection methods that could be used to indicate contamination (Besmer and Hammes, 2016). Currently, online turbidity meters are commonly used as a simple, pragmatic approach to forewarn of microbiological risk during unexpected pollution events, despite turbidity not being considered a reliable indicator of microbiological contamination (Jung et al., 2014; Pronk et al., 2006; UKWIR, 2012).

An alternative approach for the water industry could be dissolved organic matter (DOM) characterisation by fluorescence spectroscopy. There is substantial research demonstrating that

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wastewater generally displays more intense DOM fluorescence than natural waters, especially at particular excitation/emission wavelength pairs (Baker, 2001; Baker and Inverarity, 2004; Carstea et al., 2016; Goldman et al., 2012; Lapworth et al., 2008; Reynolds and Ahmad, 1997; Roehrdanz et al., 2016). Hence, fluorescence spectroscopy could be a useful indicator of the wastewater contamination of natural waters used for drinking (Stedmon et al., 2011). Of particular interest is tryptophan-like fluorescence (TLF) at an excitation-emission ($\lambda_{ex}/\lambda_{em}$) peak of around 280/350 nm as it has been demonstrated to be positively correlated with BOD, COD and TOC (Cohen et al., 2014; Reynolds and Ahmad, 1997; Reynolds, 2002). More recently, it has also been shown that contaminated drinking water sources containing thermotolerant (faecal) coliforms (TTCs) have significantly higher TLF than those that are uncontaminated (Sorensen et al., 2015, 2016). There is also a strong positive correlation between TLF intensity and TTC concentration in both field studies of drinking water (Baker et al., 2015; Sorensen et al., 2015, 2016) and controlled laboratory conditions (Fox et al., 2017).

To date, studies investigating fluorescent DOM as a predictor of drinking water contaminated by faecal indicator organisms have been based on infrequent spot sampling. However, fluorescent DOM has the potential to be characterised online and *in-situ* by low-power, LED based fluorimeters. Such online deployments have predominantly focussed on studying dissolved organic carbon (DOC) dynamics in marine systems and rivers (Ruhala and Zarnetske, 2017). Shutova et al. (2016) provide the only example of deployment in the water industry, where online fluorimeters were used as an indicator of DOM during the water treatment process.

The aim of this study was to understand the utility of online fluorescence spectroscopy for the real-time evaluation of the microbial quality of raw drinking water at public supplies. We measured TLF and humic-like fluorescence (HLF) online at four groundwater-derived public water supplies and compared this to in-situ turbidity measurements, culture-based counts of *E. coli* and total bacterial counts measured by flow cytometry.

2. Methods

2.1. Study sites

Four groundwater-derived public water supply sites were selected in Southern England (Fig. 1). These sites all abstract from varying types of fractured limestone, which is the largest source of groundwater in the UK. These limestone aquifers can have rapid flow paths and the selected sites are all known to experience episodic pollution events that are generally linked to heavy rainfall. Site 1 comprises Portland Stone Formation boreholes, site 2 Chalk Group springs, site 3 Chalk Group boreholes, and site 4 Jurassic Limestone springs.

2.2. Online analysis

TLF was determined every two minutes on the raw water intake at each site for between 6 and 10 months. This measurement was undertaken using a telemetered UviLux fluorimeter targeting the excitation/emission peak at λ_{ex} 280 ± 15 nm and λ_{em} 365 ± 27.5 nm (Chelsea Technologies Group Ltd, UK). The minimum detection limit for the sensor is 0.17 ± 0.18 ppb dissolved tryptophan (Khamis et al., 2015). HLF was also measured every two minutes at Sites 1, 2 and 3 using a telemetered UviLux fluorimeter targeting the excitation-emission peak at λ_{ex} 280 ± 15 nm and λ_{em} 450 ± 27.5 nm (Chelsea Technologies Group Ltd, UK). This fluorimeter did not target the centres of the HLF peaks, typically observed at $\lambda_{ex} < 260$ and 320–360 nm (Fellman et al., 2010), but was aligned at the same excitation as TLF because of the optical overlap between the two regions. All fluorimeters were installed in flow-through cells, which excluded all natural light, connected to sample taps at each site. The temperature of water exiting each cell was monitored with a HOBO Tidbit® v2 (Onset Computer Corporation, USA).

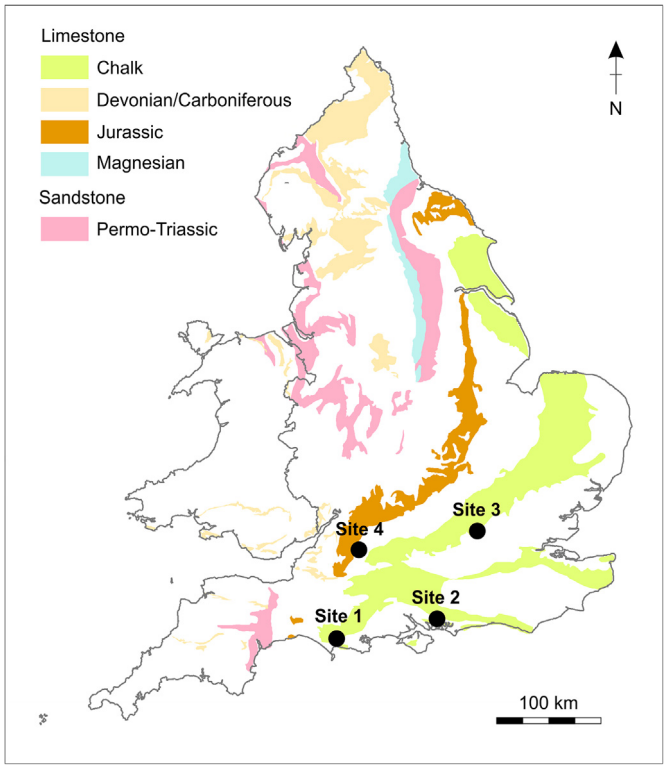

Fig. 1. Location of public water supplies and major aquifers of the UK. Note the Portland Stone Formation is too small to be visualised at this scale.

Factory calibrations were implemented on all UviLux sensors, which express TLF and HLF intensity in equivalent units of dissolved tryptophan and quinine sulphate units (QSU), respectively. The former unit is derived from direct calibration of the TLF sensor with tryptophan in deionised water. The latter unit is a standardised parameter that relates the fluorescence intensity at λ_{ex} 347.5 nm and λ_{em} 450 nm from 1 ppb of quinine sulphate dissolved in 0.105 M perchloric acid to direct calibration of the HLF sensor with pyrene tetrasulphonic acid in deionised water. For the calculation of a TLF:HLF ratio, TLF was converted to QSU by multiplication by 0.5365; this factor relates the fluorescence intensity of tryptophan to that of quinine sulphate.

At all sites abstracted raw groundwater is screened in real-time against defined turbidity thresholds, using nephelometric technology, before passing into a treatment plant prior to onward distribution. If water exceeds the defined threshold, usually 1 NTU, it is considered a potential threat to the provision of clean water, as high levels of microbial contamination may be present alongside particles that could reduce treatment efficacy (DWI, 2010). In this case, abstraction may cease or raw water may be directed to waste until turbidity returns to below the threshold. These instances were observed regularly at sites 1, 3 and 4 and all fluorescence data were disregarded during these periods that could last from hours to weeks. Only site 2 has a complete uninterrupted record of fluorescence data across the monitoring period.

2.3. Sample collection and laboratory analysis

2.3.1. Sample collection and storage

Duplicate water samples were retrieved from each site on a regular basis for comparison with the online sensors. One was collected and analysed for *E. coli* within 8 h following standard water industry compliance procedures (EA, 2009). The second was collected in sterile 15 ml polypropylene tubes, stored in the dark at 4 °C, and analysed for fluorescence and absorbance, and total bacterial cell counts by flow cytometry. These second samples were analysed within 48–96 h of collection. Sample stability tests were performed which confirmed the stability of samples over this timeframe, see Section 2.3.4.

2.3.2. Fluorescence and absorbance analysis

Fluorescence analysis was conducted on unfiltered samples at 20 °C with λ_{ex} between 200 and 400 nm (5 nm bandwidth) and λ_{em} between 280 and 500 nm (2 nm bandwidth) using a Varian™ Cary Eclipse fluorescence spectrometer (Agilent Technologies, USA). The scan rate was 9600 nm/min and the detector voltage was set to 900 V. All analysis was performed in a quartz cuvette with a path length of 1 cm. The Raman peak of ultrapure water at 348 nm was used to check for instrument stability prior to and following analysis, for blank correction, and to standardise the fluorescence excitation-emission matrices (EEMs).

Absorbance measurements were undertaken in a 1 cm quartz cuvette at 1 nm intervals from 800 to 200 nm using a Varian Cary 50 UV–Vis spectrophotometer (Agilent Technologies, USA). All absorbance spectra were referenced to a blank of ultrapure water. Mean absorbance was 0.011 cm^{-1} ($\sigma = 0.007$ cm^{-1}) at 280 nm. This indicates that inner-filter correction was not required, since the primary inner filter effect was <3.7% across all samples (Lakowicz, 2006).

2.3.3. Flow cytometry

It has been proposed that the total number of bacterial cells by flow cytometry could act as an indicator for drinking water contamination, as elevated counts could indicate contamination from external sources containing higher cell densities or the growth of bacteria within the distribution network (Lautenschlager et al., 2013). Consequently, total bacterial counts were analysed for on a BD Accuri C6 flow cytometer equipped with a 488 nm solid state laser (Becton Dickinson U.K. Ltd., Oxford, U.K.). Water samples (500 µl) were stained with SYBR Green I (Sigma-Aldrich, Gillingham, UK) at a final concentration of 1:200 v/v for 20 min in the dark at room temperature, before running on the Accuri at a slow flow rate (14 µL/min, 10 µm core) for 5 min and a detection threshold of 1500 on channel FL1. A single manually drawn gate was created to discriminate bacterial cells from particulate background, and cells per mL were calculated using the total cell count in 5 min divided by the reported volume run in µl. The mean absolute error (MAE) from 69 duplicate analyses across all sites was 6536 cells/mL.

2.3.4. Sample stability

A series of samples was collected sequentially from each of sites 1, 2, and 4. These samples were destructively analysed in triplicate on a daily basis, excluding weekends, over eleven days for fluorescence and nine days for total bacterial cell counts following the procedures listed above. For fluorescence, equivalent TLF and HLF wavelength pairs corresponding to the in-situ sensors were extracted from the excitation/emission matrix. The results are expressed in percentage change from the mean of three samples analysed after storage for one day (Fig. S1).

There was no consistent change in either TLF or HLF over 11 days, hence variation in storage time before analysis is unlikely to have influenced the results. Nonetheless, transformations during storage have been observed within the first 24 h, which have not been considered here. For example, Bieroza and Heathwaite (2016) demonstrated increases in TLF of 9–11% in unfiltered surface water samples stored at 10 °C over this timescale. However, potential changes in samples collected in this study are likely to be lower given the reduced storage temperature (4 °C) and because groundwater as a medium is likely to be more stable than river waters due to the generally lower concentrations of bacteria and nutrients. Of greater significance is the variability between samples analysed in triplicate on the same day (Fig. S1).

Bacterial cell counts were consistently lower after storage for one day (Fig. S1), with an overall mean decline of 12% between storage for one and two days across all samples. Nevertheless, there was no apparent change between two and four days. Therefore, it is considered that reported bacterial cells counts are marginally lower than at the time of sampling at sites 1, 2 and 4, but variation in storage time between 2 and 4 days is unlikely to have influenced the results.

2.4. Statistical analysis

Correlations were assessed by Spearman's Rank (Spearman, 1904). Differences in the median between two populations were assessed by Mann-Whitney tests and between multiple populations by the Kruskal-Wallis method (Kruskal and Wallis, 1952) followed by a pairwise multiple comparison using Dunn's Method (Dunn, 1964). *E. coli* data were classified into World Health Organisation (WHO) risk categories for some analyses which defines risks based on the plate count per 100 mL (WHO, 1997) as follows: Very Low (0 cfu), Low (1–9 cfu), Medium (10–99 cfu), High (100–999 cfu) and Very High (1000 + cfu). All these statistical analyses were non-parametric, due to the non-normal distribution of all datasets, and performed in SigmaPlot version 13.

Parallel factor analysis (PARAFAC) was undertaken on blank corrected fluorescence EEMs from all sites to extract, model and quantify the spectrally overlapping components. PARAFAC uses a least-squares algorithm to decompose the data; for further details on the method and approach see Stedmon et al. (2003). The analysis was undertaken using the 'DOMFluor v1.7' toolbox (Stedmon and Bro, 2008) in Matlab version 9.1. Rapid model convergence was obtained and validated using independent split-half analysis of the dataset following removal of outlier data which had high leverage (Stedmon and Bro, 2008).

3. Results and discussion

3.1. Online indication of E. coli

There are strong correlations between TLF and *E. coli* ($\rho = 0.71$, $p < 0.001$, $n = 134$) and HLF and *E. coli* ($\rho = 0.77$, $p < 0.001$, $n = 122$), but only a moderate correlation between turbidity and *E. coli* ($\rho = 0.48$, $p < 0.001$, $n = 134$). When grouping the *E. coli* data into WHO risk categories, TLF, HLF and turbidity all vary significantly between categories (Kruskal-Wallis, $p < 0.001$) (Fig. 2). Significant differences exist between all categories for TLF (Dunn's Method). However, for turbidity and HLF (HLF was only monitored at sites 1–3), Low and Medium categories are not significantly different, although both are significantly different from the Very Low category. There were insufficient data to robustly include the High risk category in the analysis ($n = 5$), although all variables are generally most elevated in this category (Fig. 2).

Consideration of the collated dataset as a whole is important for evaluating the universal applicability of these indicators. Ideally each indicator would also be assessed at each site individually to

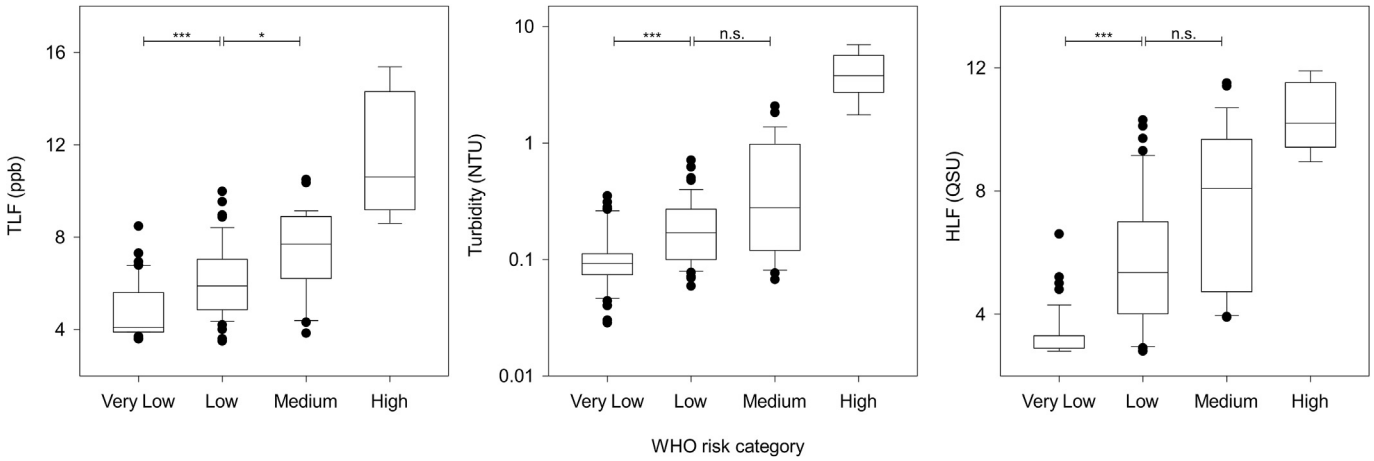

Fig. 2. Boxplots of TLF, turbidity and HLF against WHO risk categories for *E. coli*. Boxes illustrate median and interquartile range (IQR), whiskers indicate 5th and 95th percentile, and all outliers are shown. Results of non-parametric Dunn's Method tests are displayed for Very Low to Medium categories: not significant = n.s = , p = 0.05 (*), $p < 0.001$ (***) = . Sample sizes were Very Low = 53, Low = 47, and Medium = 29 for TLF and turbidity, and Very Low = 49, Low = 44, and Medium = 24 for HLF.

confirm that any collated analysis is not biased by site-to-site differences. However, there is a limited spread of data across risk categories at each individual site with which to undertake such analyses (Fig. S2). Where $n > 5$, there is a significant difference between Low and Medium risk categories at site 1 for TLF (Mann-Whitney, p = 0.04) and turbidity (Mann-Whitney, p = 0.01), but not HLF (Mann-Whitney, p = 0.09); there are no significant differences between Very Low and Low categories at site 2 for any variable (Mann-Whitney, p = 0.49–0.91). Similarly, correlations are only present at site 1 for all indicators (0.49–0.54, $p < 0.001$, n = 53), which has the largest number of samples that are spread across all risk categories.

At all sites, TLF remains in excess of the proposed threshold of 1.3 ppb demonstrated by Sorensen et al. (2018) to indicate the presence of faecal contamination in untreated drinking water. Indeed, all sites experience *E. coli* contamination, but this ranges from commonplace occurrence at site 1 (93% of samples) to episodic occurrence at site 3 (12% of samples). TLF always remains above the threshold because the sites appear to have a reasonably consistent TLF baseline of around 4–8 ppb (Fig. 3). Comparatively, previous groundwater studies linking TLF to faecal indicator organisms in boreholes have suggested a zero TLF baseline (Sorensen et al., 2015, 2016).

3.2. Online indication of total bacterial cell counts

A strong positive non-linear correlation is observed between TLF and total bacterial cell counts, although there is noticeable scatter (Fig. 4). This positive correlation holds at several individual sites: site 1 ($\rho = 0.82$, n = 17, $p < 0.001$), site 2 ($\rho = 0.68$, n = 91, $p < 0.001$), and site 4 ($\rho = 0.70$, n = 5, $p = 0.23$). No correlation is evident at site 3 ($\rho = 0.11$, n = 11, $p = 0.73$) because there is limited variation in TLF ($\sigma = 0.1$ ppb) and bacterial cell counts ($\sigma = 5800$ cells/mL). In fact, if site 3 is removed from the overall analysis, the overall correlation

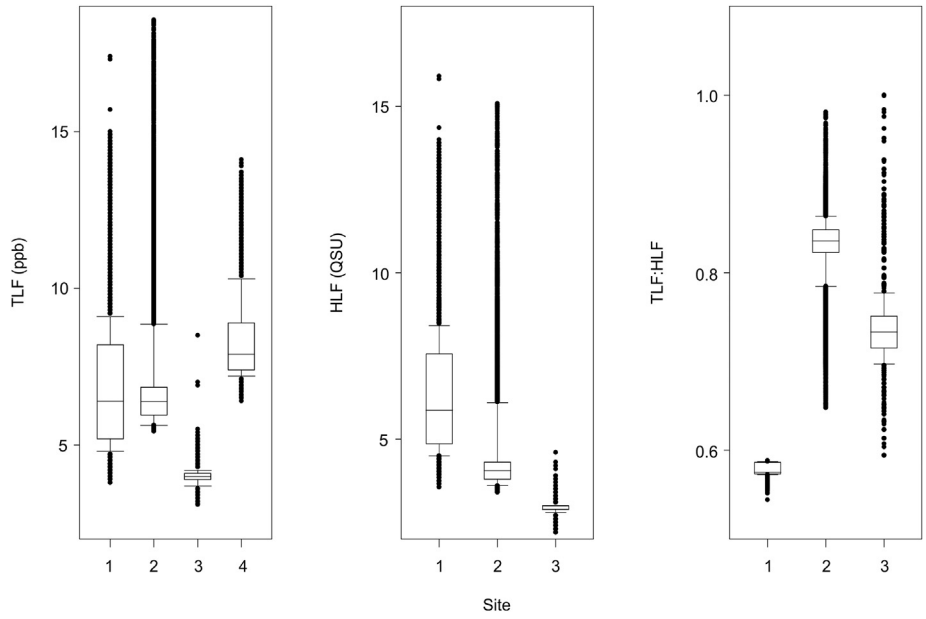

Fig. 3. Boxplots of online TLF, HLF and TLF:HLF ratio subsampled to 15 min resolution data at relevant sites.

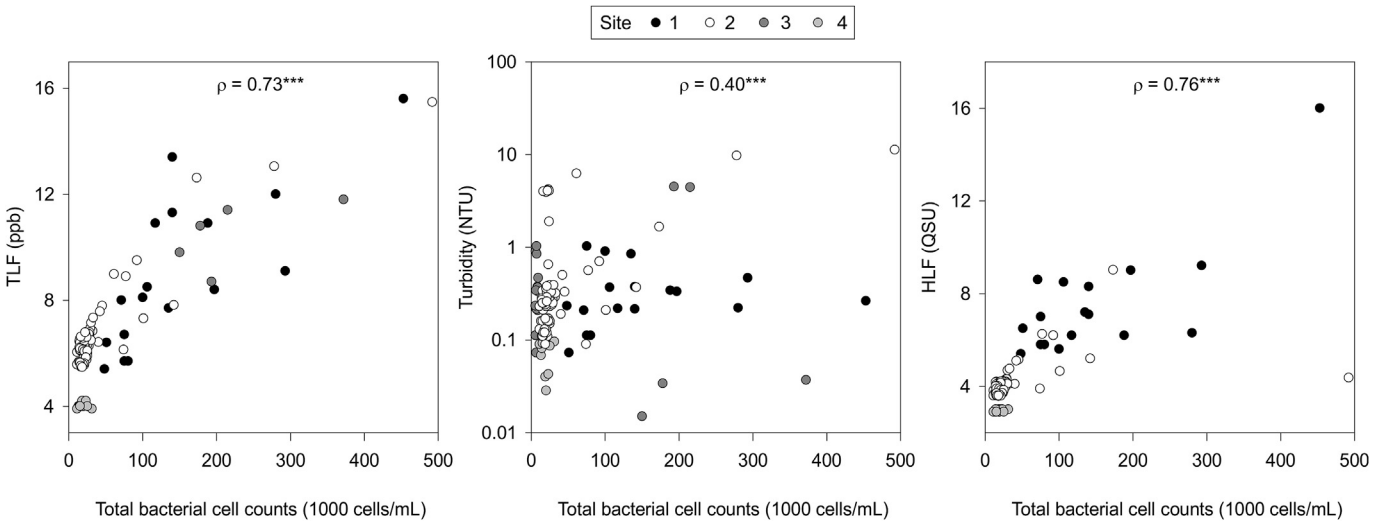

Fig. 4. Scatterplots of TLF, turbidity and HLF against total bacterial cell counts for all sites. Spearman's rank correlation coefficients (ρ) and significance are displayed (*** indicates $p < 0.001$), n = 124 for TLF and turbidity and n = 119 for HLF.

becomes even stronger (ρ = 0.86, n = 113, $p < 0.001$). It is also noted that there is a discrepancy in the order of 2 ppb TLF at similar low bacterial cell counts between sites 2 and 3.

HLF is strongly correlated with total bacterial cell counts in a collated dataset from sites 1–3 (Fig. 4). Again, this positive correlation is observed at site 1 (ρ = 0.77, n = 17, $p < 0.001$) and site 2 (ρ = 0.65, n = 91, $p = <0.001$), but not site 3 where there is limited variation in counts. The correlation coefficients for sites 1 and 2 are very similar to the correlation coefficients between TLF and total bacterial cell counts.

Only a weak correlation is noted between turbidity and total bacterial cell counts across all sites (Fig. 4). The only statistically significant correlation is actually observed at site 2 (ρ = 0.72, n = 91, $p < 0.001$), with no relationships at the other sites (ρ = 0.01 to −0.18).

3.3. Complete time series of online indicators at site 2

The complete dataset from site 2 shows the relationship between online indicators and bacteriological variables (Fig. 5). There is a consistent TLF baseline (IQR = 0.9 ppb) where *E. coli* are generally absent, or few in number, and total bacterial cell counts are reasonably stable (IQR = 10 000 cells/mL). Following rainfall, increases in TLF, *E. coli* and total bacterial cell counts are noted. These increases are greatest during the main groundwater recharge season ending in March, and include several smaller peaks between late-May and mid-July. HLF displays a near identical relationship to TLF and bacteriological variables over the time period. Examination of the TLF:HLF ratio demonstrates it is reasonably stable at around 0.85, except during events when there is a decrease in the ratio due to a proportionally greater increase in HLF. There is a noticeable decline in baseline TLF and HLF of around 0.8 ppb and 0.4 QSU, respectively, over the record.

Turbidity increases appreciably during events in the main groundwater recharge season, but returns back to baseline more quickly than the bacteriological and fluorescence variables. This partially mirrors the results of Pronk et al. (2006) who demonstrated that following rises in turbidity, DOC and *E. coli* counts in springs after rainfall, turbidity rapidly returned to baseline values rapidly whereas both DOC and *E. coli* remained elevated for several more days. These observations may result from turbidity predominantly being associated with larger particles, which rapidly settle out, whereas smaller particles also associated with bacteria and fluorescent DOM continue to be transported through the system. It is also noted that turbidity changes during late Spring and Summer rainfall events, are minimal despite sizeable increases in bacteriological variables. These observations indicate why turbidity is an inferior indicator of both *E. coli* and total bacterial cells compared to fluorescence at site 2.

3.4. HLF bleed-through into the TLF spectral region

The sensor data from sites 1 to 3 do not indicate a clear TLF peak, with relatively constant TLF:HLF ratios (IQR = 0.01–0.03) that never exceed 1.0 (Fig. 3). Mean laboratory fluorescence data also do not indicate a visual TLF peak (Fig. 6). The TLF region appears completely masked by the neighbouring HLF peak centred at λ_{em} 420–460 nm, which bleeds through and causes an apparent amplified TLF signal. Deconvoluting the EEMs statistically using PARAFAC analysis demonstrates that a two component model, based on HLF peaks (Fig. S3), explains 98.3% of the variability. A validated three component model, including an additional TLF component (Fig. S3), provides only a very marginal improvement to 98.6%. It is, hence, unsurprisingly that HLF and TLF were similarly correlated to both *E. coli* and total bacterial cells.

In other studies, HLF has proven to be similarly or better correlated than TLF: to BOD at wastewater treatment works (Cohen et al., 2014), to total coliforms and *E. coli* at springs (Frank et al., 2018) and to *E. coli* concentration during controlled laboratory experiments (Fox et al., 2017). Therefore, a HLF fluorimeter alone may suffice for monitoring the microbial quality of untreated drinking water sources in many instances. Alternatively, a sensor that excites at λ_{ex} 280 nm and has a broader emission filter of λ_{em} 335–500 nm could also provide comparable performance. The advantage of this device would be a reduction in cost due to the replacement of an expensive narrow bandpass filter.

The ratios of fluorescent peaks are commonly used to address the issue of optical bleed-through (e.g. Baker, 2002; Lapworth et al., 2008). Variability in TLF:HLF ratios between sites demonstrates that there is not a consistent ratio in the absence of a clear TLF peak at these study sites (Fig. 3). This observation arises because fluorescence intensity is always low and relatively consistent at

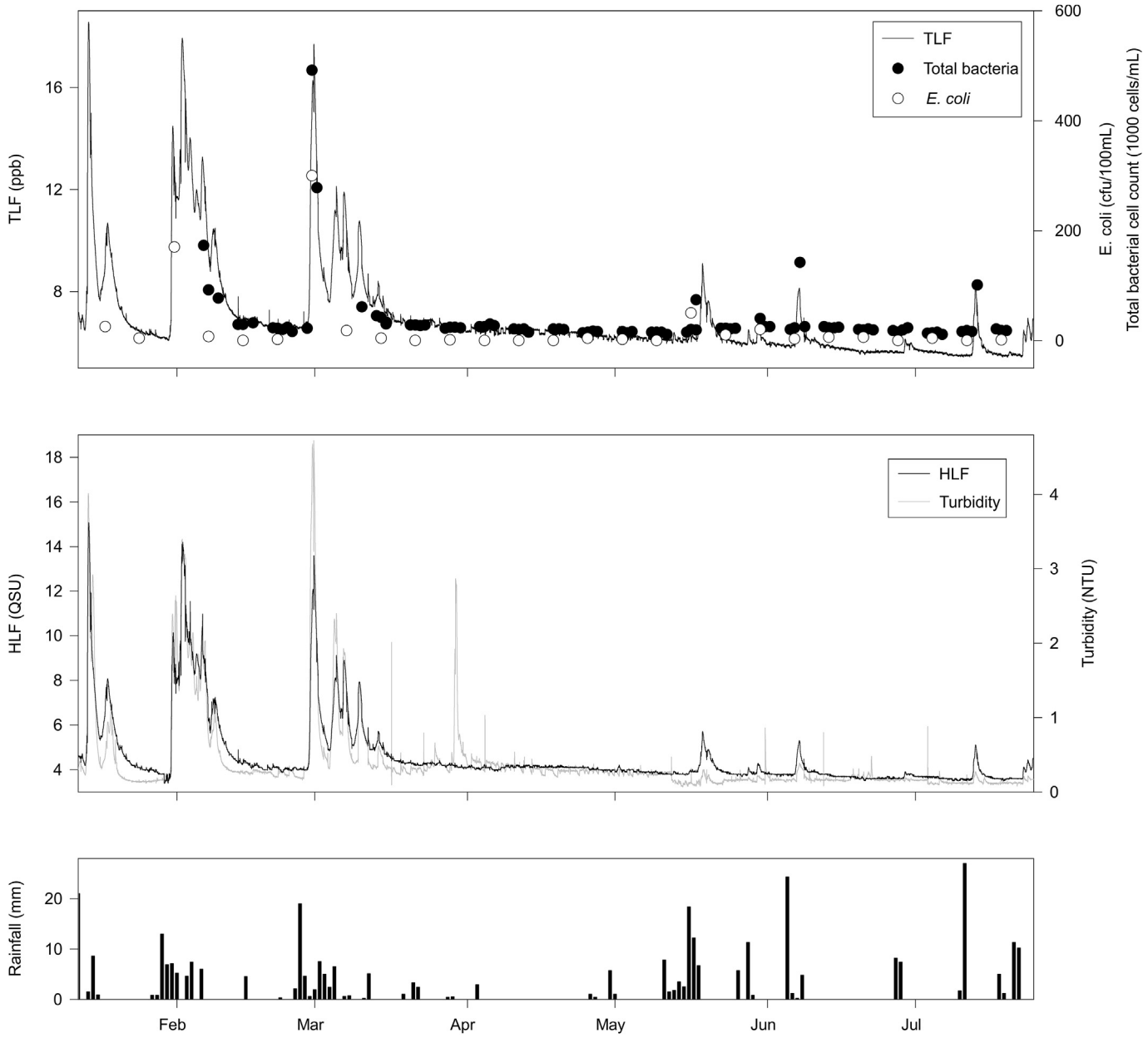

Fig. 5. Comparison of TLF, HLF, and turbidity with bacteriological variables at site 2. All data are displayed at 15 min resolution. No samples were taken for total bacterial cell counts prior to 6 Feb and between 1 and 10 Mar. Daily rainfall values are from Havant, Hampshire.

wavelengths slightly shorter than the TLF region (λ_{em} 320–340 nm); therefore, an intense HLF peak will typically result in a lower TLF:HLF ratio as the recession in fluorescence intensity from the peak is steeper (Fig. 6b). Hence, site 1 with a relatively intense HLF peak has a median ratio of 0.58, as opposed to a median ratio of 0.73 at site 3 which has the lowest intensity HLF peak. Consequently, using TLF:HLF ratios does not allow comparisons between sites, although it may indicate relative changes in the fluorescent components at an individual site.

The lack of a clear TLF peak indicates the sites are not likely to have been appreciably impacted by wastewater during the monitoring period, which is the most likely attribution of TLF in groundwater. HLF is generally considered to be of allochthonous origin (Coble et al., 2014) and is likely to derive from the near-surface in groundwater. Therefore increases in fluorescence during rainfall events are indicative of water from the near-surface rapidly impacting the sites.

3.5. Operational constraints

At all water company sites, operating procedures impacted fluorescence monitoring. Borehole pump start-up resulted in spikes in fluorescence as well as turbidity at both borehole sites. These spikes typically lasted for minutes to hours until returning to expected levels. However, fluorescence remained elevated for longer periods (up to several days) when the primary water source was rotated to another borehole at the same site that had remained on standby for days/weeks. Boreholes contain elevated concentrations of planktonic bacteria cells and DOC compared with the surrounding aquifer when they are not pumped due to the

accumulation of organic material (Sorensen et al., 2013). The longer a borehole is not pumped, the greater the accumulation of this organic material within the borehole and adjacent aquifer. Consequently, a greater load is mobilised by pump start-up and it takes longer for fluorescence readings to stabilise. Furthermore, there is likely to be build-up of organic material within the pipe network with time when there is no active flow that would then only begin to clear upon pump start-up.

Variations in the active pumping rate within a borehole are also likely to impact fluorescence monitoring. A higher flow rate increases flow velocities and shear stress within the borehole and aquifer, increasing disturbance, entrainment, and shearing of biofilms. Previous research has demonstrated an increase in DOC mobilisation and bacterial cell counts at higher pumping rates (Graham et al., 2015; Kwon et al., 2008). A higher pumping rate through the pipe network could similarly increase biofilm mobilisation (Cloete et al., 2003), with a resultant increase in TLF and HLF.

Minor sensor drift of 0.3–0.8 ppb TLF over 6–10 months was evident at all sites and is the underlying cause of the change in baseline fluorescence over time at Site 2. Sensor drift was evaluated by testing the fluorimeters in ultrapure water and tryptophan standards before installation and following removal at the end of the project. There was no visible sign of material on any sensor window, but the apparent drift could be corrected for by cleaning the windows with a lens cloth. It is recommended that suppliers supplement these optical sensors with self-cleaning wipers on the windows for online installations. This should resolve the problem, but infrequent manual maintenance will still be required to validate the sensor readings. More problematic was an installation at a riverbank filtration site that was not included in this paper. Here, there was a dramatic >40% loss of signal in the first two weeks following installation (Fig. S4). The sensor was cleaned and reinstalled on multiple occasions, but similar rates in signal loss were always observed. Hydrochemical analysis of deposits removed from the windows indicated a build-up of metals, most notably iron. The sensor windows are constructed from silica, which has a surface comprising mainly silanol (Si-O-H) groups. Any loss of the proton could allow the windows to become negatively charged, attracting cations, and there could also be exchange of the proton with cations in the sample.

There are a range of other variables that interfere with fluorescence spectroscopy measurements (Hudson et al., 2007), notably temperature and turbidity (Khamis et al., 2015). Turbidity was generally very low with the 95th percentile being 1.0, 0.1, 0.1 and 1.6 NTU at sites 1 to 4, respectively. Temperature was similar at all sites, with median values of between 10.6 and 12.2 °C, with variations typically limited to only 1–2 °C, and only very occasional greater variation. Therefore, neither temperature nor turbidity are likely to have had appreciable influence on the fluorescence results. It is possible to generate correction factors to account for variations in temperature and turbidity, which could be automated online (e.g. Shutova et al., 2016), and these may be necessary for surface water sources. However, Khamis et al. (2015) demonstrated only marginal improvements by correcting groundwater TLF data and suggest that corrections factors are likely to be unnecessary for groundwater sources universally, as generally turbidity is very low and temperature is perennially stable.

3.6. Beyond an indicator of microbial quality in untreated water

Online fluorescence has viable applications beyond purely as an indicator of the microbial quality of untreated drinking water. Bridgeman et al. (2015) identified a correlation between TLF and total bacterial cell counts within the treated drinking water distribution network; albeit slightly weaker ($r^2 = 0.56$) than in this study. The correlations between fluorescence and total bacterial counts, observed herein and in Bridgeman et al. (2015), indicate that online fluorescence spectroscopy could represent a more practical, cheaper, and robust alternative to flow cytometry for monitoring total bacteria throughout the water supply network. However, flow cytometry has the potential to record other properties of bacterial communities, including cell size, viability (the proportion of live and dead cells) and the metabolically active proportion of the community (Hammes and Egli, 2010).

Within the treated water distribution network it has been demonstrated that HLF is relatively stable (Heibati et al., 2017) so online fluorescence would be a potentially sensitive indicator of any water quality changes. Potential changes could include cross-connection detection, which many researchers have highlighted as a future use for online fluorescence (e.g. Hambly et al., 2010), or failure in the water treatment system. Further uses could be strategic deployments at the outlets of service reservoirs and large diameter trunk mains, which can both suffer from structural integrity issues. Nevertheless, the use of online fluorescence spectroscopy for treated water is likely to be complicated by water treatment processes that remove fluorescent DOM to various degrees (Carstea et al., 2016). Further work is need to appraise the utility of the technique on treated water.

Fluorescence targeting HLF ($\lambda_{ex}/\lambda_{em}$ of around 350/450 nm) has been correlated with DOC (Shutova et al., 2016) and TOC (Bieroza et al., 2009; Bridgeman et al., 2015; Stedmon et al., 2011) in untreated and treated drinking water. If peak C is related to the total organic matter load in water, then online HLF fluorimeters could be useful to forewarn of the generation of harmful amounts of carcinogenic by-products during certain types of disinfection such as trihalomethanes (Yang et al., 2015).

Fluorescence spectroscopy could also be a useful indicator for the water industry, or other abstractors, for rapidly characterising the vulnerability of groundwater sources to contamination. The higher the fluorescence (either TLF or HLF) then the stronger the likely link with the near-surface, which is the source of the majority of microbial and chemical contaminants. Therefore, fluorescence could be considered an instantaneous assessor of risk for groundwater sources.

4. Conclusions

- Online tryptophan-like fluorescence (TLF) and humic-like fluorescence (HLF) were both strongly correlated with *E. coli* concentration and total bacterial cell counts at public water supplies.
- The current commonly employed microbial indicator in the UK, turbidity, was more weakly correlated with both bacterial variables. Nevertheless, turbidity is still an essential indicator of suspended solids in raw water that can affect treatment efficacy.
- Monitoring both TLF and HLF was unnecessary in this environment with minimal change in the ratio. A HLF sensor alone would be sufficient for evaluating when water from the near-surface was impacting a groundwater supply.
- Fluorescence data were strongly influenced by pump start-up at borehole sources and, at one site, continuous build-up of ferric deposits on the sensor reduced intensity by up to >40% within two weeks. There is a need for manufacturers to supply fluorimeters that are more resistant to fouling, for example through the provision of wiper systems.
- Online fluorescence could be a more practical approach for monitoring total bacterial cell counts than flow cytometry.
- Online fluorescence is an effective indicator of the microbial quality of untreated drinking water and could be effective

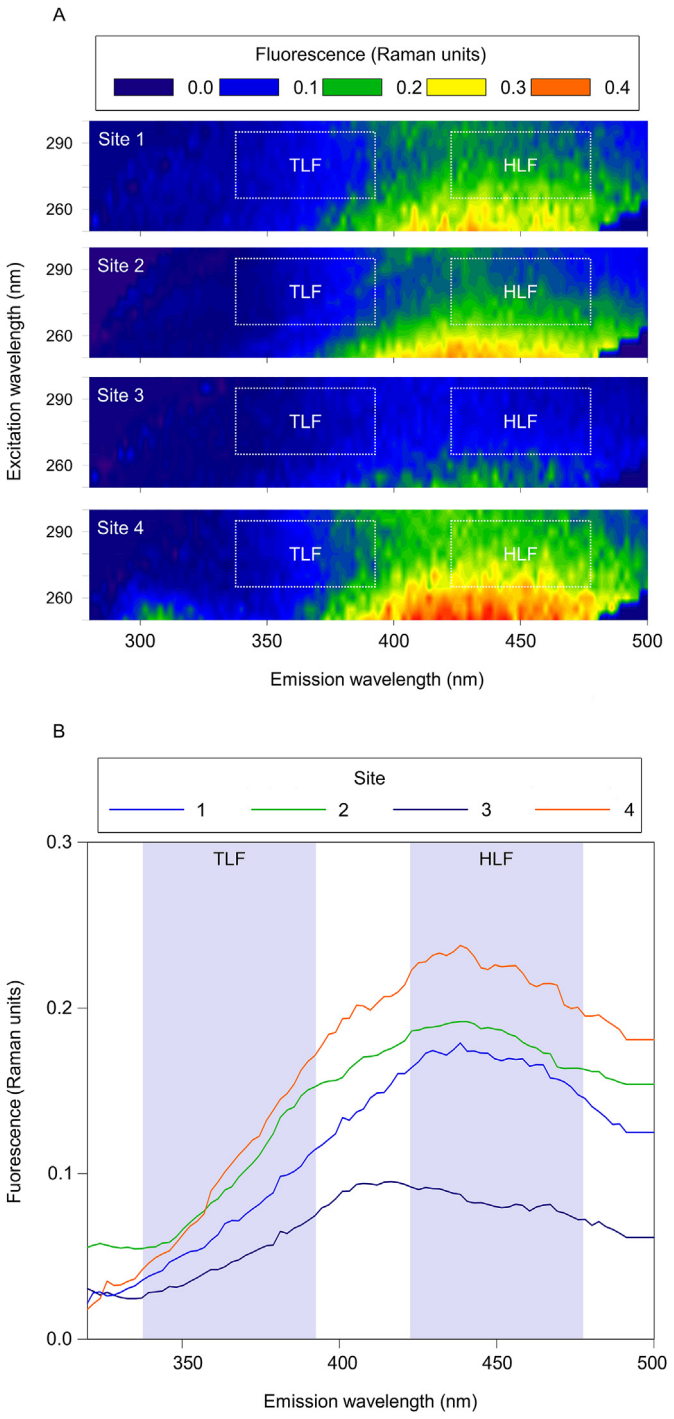

Fig. 6. A) Mean fluorescence excitation/emission matrix between λ_{ex} 250–300 nm and λ_{em} 280–500 nm for all sites with TLF and TLF sensor spectral regions indicated; B) Mean fluorescence intensity at λ_{ex} 280 nm, λ_{em} 320–500 nm for all water samples collected at sites 1 to 4.

throughout the water supply chain for identifying changes in water quality that could signify the presence of enteric pathogens.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.watres.2018.03.001.

References

Baker, A., 2001. Fluorescence excitation-emission matrix characterization of some sewage-impacted rivers. Environ. Sci. Technol. 35 (5), 948–953.
Baker, A., 2002. Fluorescence properties of some farm wastes: implications for water quality monitoring. Water Res. 36 (1), 189–195.
Baker, A., Cumberland, S.A., Bradley, C., Buckley, C., Bridgeman, J., 2015. To what extent can portable fluorescence spectroscopy be used in the real-time assessment of microbial water quality? Sci. Total Environ. 532, 14–19.
Baker, A., Inverarity, R., 2004. Protein-like fluorescence intensity as a possible tool for determining river water quality. Hydrol. Process. 18 (15), 2927–2945.
Baldursson, S., Karanis, P., 2011. Waterborne transmission of protozoan parasites: review of worldwide outbreaks–an update 2004–2010. Water Res. 45 (20), 6603–6614.
Besmer, M.D., Hammes, F., 2016. Short-term microbial dynamics in a drinking water plant treating groundwater with occasional high microbial loads. Water Res. 107, 11–18.
Bieroza, M., Baker, A., Bridgeman, J., 2009. Relating freshwater organic matter fluorescence to organic carbon removal efficiency in drinking water treatment. Sci. Total Environ. 407 (5), 1765–1774.
Bieroza, M.Z., Heathwaite, A.L., 2016. Unravelling organic matter and nutrient biogeochemistry in groundwater-fed rivers under baseflow conditions: uncertainty in in situ high-frequency analysis. Sci. Total Environ. 572, 1520–1533.
Bridgeman, J., Baker, A., Brown, D., Boxall, J., 2015. Portable LED fluorescence instrumentation for the rapid assessment of potable water quality. Sci. Total Environ. 524, 338–346.
Carstea, E.M., Bridgeman, J., Baker, A., Reynolds, D.M., 2016. Fluorescence spectroscopy for wastewater monitoring: a review. Water Res. 95, 205–219.
Cloete, T., Westaard, D., Van Vuuren, S., 2003. Dynamic response of biofilm to pipe surface and fluid velocity. Water Sci. Technol. 47 (5), 57–59.
Coble, P.G., Lead, J., Baker, A., Reynolds, D.M., Spencer, R.G., 2014. Aquatic Organic Matter Fluorescence. Cambridge University Press.
Cohen, E., Levy, G.J., Borisover, M., 2014. Fluorescent components of organic matter in wastewater: efficacy and selectivity of the water treatment. Water Res. 55, 323–334.
Cook, C., Prystajecky, N., Ngueng Feze, I., Joly, Y., Dunn, G., Kirby, E., Özdemir, V., Isaac-Renton, J., 2013. A comparison of the regulatory frameworks governing microbial testing of drinking water in three Canadian provinces. Can. Water Resour. J. 38 (3), 185–195.
Dunn, O.J., 1964. Multiple comparisons using rank sums. Technometrics 6 (3), 241–252.
DWI, 2010. Guidance on the Implementation of the Water Supply (Water Quality) Regulations 2000 (as amended) in England.
EA, 2009. The Microbiology of Drinking Water (2009) - Part 4-Methods for the Isolation and Enumeration of Coliform Bacteria and Escherichia coli (Including E. coli O157:H7).
Fellman, J.B., Hood, E., Spencer, R.G., 2010. Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: a review. Limnol. Oceanogr. 55 (6), 2452–2462.
Fox, B., Thorn, R., Anesio, A., Reynolds, D., 2017. The in situ bacterial production of fluorescent organic matter; an investigation at a species level. Water Res. 125, 350–359.
Frank, S., Goeppert, N., Goldscheider, N., 2018. Fluorescence-based multi-parameter approach to characterize dynamics of organic carbon, faecal bacteria and particles at alpine karst springs. Sci. Total Environ. 615, 1446–1459.
Goldman, J.H., Rounds, S.A., Needoba, J.A., 2012. Applications of fluorescence spectroscopy for predicting percent wastewater in an urban stream. Environ. Sci. Technol. 46 (8), 4374–4381.
Graham, P., Baker, A., Andersen, M., 2015. Dissolved organic carbon mobilisation in a groundwater system stressed by pumping. Sci. Rep. 5, 18487.
Hambly, A., Henderson, R., Storey, M., Baker, A., Stuetz, R., Khan, S., 2010. Fluorescence monitoring at a recycled water treatment plant and associated dual distribution system–Implications for cross-connection detection. Water Res. 44 (18), 5323–5333.
Hammes, F., Egli, T., 2010. Cytometric methods for measuring bacteria in water: advantages, pitfalls and applications. Anal. Bioanal. Chem. 397 (3), 1083–1095.
Heibati, M., Stedmon, C.A., Stenroth, K., Rauch, S., Toljander, J., Säve-Söderbergh, M., Murphy, K.R., 2017. Assessment of drinking water quality at the tap using

fluorescence spectroscopy. Water Res. 125, 1–10.
Hudson, N., Baker, A., Reynolds, D., 2007. Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters—a review. River Res. Appl. 23 (6), 631–649.
Hynds, P.D., Misstear, B.D., Gill, L.W., 2012. Development of a microbial contamination susceptibility model for private domestic groundwater sources. Water Resour. Res. 48 (12).
Jung, A.-V., Le Cann, P., Roig, B., Thomas, O., Baurès, E., Thomas, M.-F., 2014. Microbial contamination detection in water resources: interest of current optical methods, trends and needs in the context of climate change. Int. J. Environ. Res. Publ. Health 11 (4), 4292–4310.
Khamis, K., Sorensen, J., Bradley, C., Hannah, D., Lapworth, D.J., Stevens, R., 2015. In situ tryptophan-like fluorometers: assessing turbidity and temperature effects for freshwater applications. Environ. Sci. Process. Impacts 17 (4), 740–752.
Kistemann, T., Claßen, T., Koch, C., Dangendorf, F., Fischeder, R., Gebel, J., Vacata, V., Exner, M., 2002. Microbial load of drinking water reservoir tributaries during extreme rainfall and runoff. Appl. Environ. Microbiol. 68 (5), 2188–2197.
Kruskal, W.H., Wallis, W.A., 1952. Use of ranks in one-criterion variance analysis. J. Am. Stat. Assoc. 47 (260), 583–621.
Kwon, M.J., Sanford, R.A., Park, J., Kirk, M.F., Bethke, C.M., 2008. Microbiological response to well pumping. Groundwater 46 (2), 286–294.
Lakowicz, J.R., 2006. Principles of Fluorescence Spectroscopy. Springer.
Lapworth, D.J., Gooddy, D., Butcher, A., Morris, B., 2008. Tracing groundwater flow and sources of organic carbon in sandstone aquifers using fluorescence properties of dissolved organic matter (DOM). Appl. Geochem. 23 (12), 3384–3390.
Lautenschlager, K., Hwang, C., Liu, W.-T., Boon, N., Köster, O., Vrouwenvelder, H., Egli, T., Hammes, F., 2013. A microbiology-based multi-parametric approach towards assessing biological stability in drinking water distribution networks. Water Res. 47 (9), 3015–3025.
Machdar, E., Van Der Steen, N., Raschid-Sally, L., Lens, P., 2013. Application of quantitative microbial risk assessment to analyze the public health risk from poor drinking water quality in a low income area in Accra, Ghana. Sci. Total Environ. 449, 134–142.
Pronk, M., Goldscheider, N., Zopfi, J., 2006. Dynamics and interaction of organic carbon, turbidity and bacteria in a karst aquifer system. Hydrogeol. J. 14 (4), 473–484.
Reynolds, D., Ahmad, S., 1997. Rapid and direct determination of wastewater BOD values using a fluorescence technique. Water Res. 31 (8), 2012–2018.
Reynolds, D.M., 2002. The differentiation of biodegradable and non-biodegradable dissolved organic matter in wastewaters using fluorescence spectroscopy. J. Chem. Technol. Biotechnol. 77 (8), 965–972.
Reynolds, K.A., Mena, K.D., Gerba, C.P., 2008. Reviews of Environmental Contamination and Toxicology. Springer, pp. 117–158.
Roehrdanz, P.R., Feraud, M., Lee, D.G., Means, J.C., Snyder, S.A., Holden, P.A., 2017. Spatial models of sewer pipe leakage predict the occurrence of wastewater indicators in shallow urban groundwater. Environ. Sci. Technol. 51 (3), 1213–1223.
Ruhala, S.S., Zarnetske, J.P., 2017. Using in-situ optical sensors to study dissolved organic carbon dynamics of streams and watersheds: a review. Sci. Total Environ. 575, 713–723.
Shutova, Y., Baker, A., Bridgeman, J., Henderson, R., 2016. On-line monitoring of organic matter concentrations and character in drinking water treatment systems using fluorescence spectroscopy. Environ. Sci. Water Res. Technol. 2 (4), 749–760.
Sorensen, J., Lapworth, D., Marchant, B., Nkhuwa, D., Pedley, S., Stuart, M., Bell, R., Chirwa, M., Kabika, J., Liemisa, M., 2015. In-situ tryptophan-like fluorescence: a real-time indicator of faecal contamination in drinking water supplies. Water Res. 81, 38–46.
Sorensen, J., Sadhu, A., Sampath, G., Sugden, S., Gupta, S.D., Lapworth, D., Marchant, B., Pedley, S., 2016. Are sanitation interventions a threat to drinking water supplies in rural India? An application of tryptophan-like fluorescence. Water Res. 88, 923–932.
Sorensen, J.P., Baker, A., Cumberland, S.A., Lapworth, D.J., MacDonald, A.M., Pedley, S., Taylor, R.G., Ward, J.S., 2018. Real-time detection of faecally contaminated drinking water with tryptophan-like fluorescence: defining threshold values. Sci. Total Environ. 622, 1250–1257.
Sorensen, J.P., Maurice, L., Edwards, F.K., Lapworth, D.J., Read, D.S., Allen, D., Butcher, A.S., Newbold, L.K., Townsend, B.R., Williams, P.J., 2013. Using boreholes as windows into groundwater ecosystems. PLoS One 8 (7), e70264.
Spearman, C., 1904. The proof and measurement of association between two things. Am. J. Psychol. 15 (1), 72–101.
Stedmon, C.A., Bro, R., 2008. Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. Limnol Oceanogr. Meth. 6, 572–579.
Stedmon, C.A., Markager, S., Bro, R., 2003. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. Mar. Chem. 82, 239–254.
Stedmon, C.A., Seredyńska-Sobecka, B., Boe-Hansen, R., Le Tallec, N., Waul, C.K., Arvin, E., 2011. A potential approach for monitoring drinking water quality from groundwater systems using organic matter fluorescence as an early warning for contamination events. Water Res. 45 (18), 6030–6038.
UKWIR, 2012. Turbidity in Groundwater: Understanding Cause, Effect and Mitigation Measures. Report Ref. No. 12/DW/14/5.
USEPA, 2013. Revised Total Coliform Rule (RTCR) 78 FR 10269, vol. 78. No. 30.
WHO, 1997. Guidelines for Drinking-Water Quality, second ed. WHO Press, Geneva, Switzerland.
Worthington, S.R., Smart, C.C., 2017. Transient bacterial contamination of the dual-porosity aquifer at Walkerton, Ontario, Canada. Hydrogeol. J. 1–14.
Yang, L., Kim, D., Uzun, H., Karanfil, T., Hur, J., 2015. Assessing trihalomethanes (THMs) and N-nitrosodimethylamine (NDMA) formation potentials in drinking water treatment plants using fluorescence spectroscopy and parallel factor analysis. Chemosphere 121, 84–91.