Science of the Total Environment 532 (2015) 14–19

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

To what extent can portable fluorescence spectroscopy be used in the real-time assessment of microbial water quality?

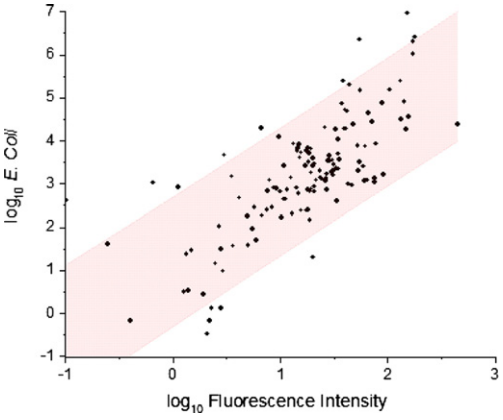
Andy Baker [a,*], Susan A. Cumberland [b], Chris Bradley [c], Chris Buckley [d], John Bridgeman [e]

[a] Connected Waters Initiative Research Centre, UNSW Australia, Sydney NSW2052, Australia
[b] School of Earth Sciences, University of Melbourne, Parkville, 3010 Melbourne, Victoria, Australia
[c] School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK
[d] Pollution Research Group, University of KwaZulu-Natal, South Africa
[e] School of Civil Engineering, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

HIGHLIGHTS

- Water fluorescence intensity in-situ is compared to its microbial water quality.
- Fluorescence intensity correlates with *E. coli* numbers over seven-log range.
- Portable fluorimetry can be an initial screening tool for microbial water quality.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 31 March 2015
Received in revised form 25 May 2015
Accepted 25 May 2015
Available online 5 June 2015

Editor: D. Barcelo

Keywords:
Organic matter fluorescence
Microbial water quality
Fluorimetry
River
Groundwater

ABSTRACT

The intrinsic fluorescence of aquatic organic matter emitted at 350 nm when excited at 280 nm correlates widely with water quality parameters such as biochemical oxygen demand. Hence, in sewage-impacted rivers and groundwater, it might be expected that fluorescence at these wavelengths will also correlate with the microbial water quality. In this paper we use a portable fluorimeter to assess the relationship between fluorescence intensity at this wavelength pair and *Escherichia coli* enumeration in contrasting river catchments of poor water quality: in KwaZulu-Natal, S. Africa and the West Midlands, UK. Across all catchments we demonstrate a log correlation ($r = 0.74$) between fluorescence intensity and *E. coli* over a seven-log range in *E. coli* enumerations on non-perturbed (unfiltered) samples. Within specific catchments, the relationship between fluorescence intensity and *E. coli* is more variable, demonstrating the importance of catchment-specific interference. Our research demonstrates the potential of using a portable fluorimeter as an initial screening tool for indicative microbial water quality, and one that is ideally suited to simple pollution scenarios such as assessing the impact of faecal contamination in river or groundwater at specific sites.

* Corresponding author.

http://dx.doi.org/10.1016/j.scitotenv.2015.05.114

1. Introduction

Microbiological waterborne disease is a significant concern for the water community globally, as pathogens (from human and animal wastes) in drinking water sources (streams, wells) cause ill health. One of the UN Millennium Development Goals (MDGs) seeks to improve access to safe water and to safe sanitation (WHO/UNICEF, 2014). The original target, monitored by a joint World Health Organisation and UNICEF programme (Bartram et al., 2014), was that by 2015, the proportion of people without safe access to drinking water and basic sanitation would be halved. While the target for access to safe drinking water was achieved in 2010, that for sanitation has yet to be met, although this difference has been shown to be partly an artefact of benchmarking differences (Cumming et al., 2014). It is estimated that by 2014, 2 billion people had gained access to a safe source of drinking water, but 700 million still lacked access (WHO/UNICEF, 2014) and it is envisaged that warmer temperatures associated with climate change will delay progress in meeting the MDGs (Hodges et al., 2014).

Globally diseases related to drinking water contamination are a significant burden on public health, with the main risk coming from the ingestion of pathogens from faecal sources associated, for example, with cholera, dysenteric and enteric fevers. Bain et al. (2014a) suggest that 1.8 billion people use a source of drinking water that suffers from some level of faecal contamination. Of particular concern is that water-related diseases disproportionally affect the poor. A recent meta-analysis of low- and middle-income countries demonstrated that faecal contamination of drinking water is more likely in low-income countries and in rural areas, and that even with 'improved' drinking water sources (e.g., boreholes, dug wells, protected springs, rainwater, standpipes and household connections), faecal contamination was observed in 38% of cases (Bain et al., 2014b). Faecal contamination of drinking water is most prevalent in Africa and South-East Asia (Bain et al., 2014a), caused mainly from practices of open defecation and damaged piped systems. However, the risk of water borne disease is greatly increased where the water supply and treatment infrastructure is damaged or poorly maintained, for example in areas of conflict, or in the aftermath of natural disasters.

This critical public health issue is associated with a number of particular areas of concern, including improved pollution detection methods. Obtaining standard counts of faecal coliforms takes in excess of 30 h; it requires skilled technicians and the availability of appropriate laboratory conditions for sample preparation and analysis. Consequently, the use of faecal coliform counts in community water management and disaster relief scenarios, such as refugee and displaced peoples' camps, is problematic and infrequent, even though rapid drinking water quality checks are essential in preventing the rapid spread of disease and increased mortality. Also, these techniques are often beyond the reach of the poorest communities that are in urgent need of drinking water and sanitation improvements. These situations demonstrate that rapid science and engineering based indicators of safe drinking water are relevant and necessary for reducing poverty and developing sustainable livelihoods. It is also most likely that they will be widely adopted when delivered as part of a community-based drinking water solution (Anstiss and Ahmed, 2006).

Fluorescence characterisation of organic matter in aquatic systems has advanced significantly in recent years with technological improvements in optical instrumentation. In particular, the development of short-wave ultra-violet light emitting diodes (LEDs) has permitted the excitation of dissolved organic matter at ~280 nm using portable instrumentation (Cumberland et al., 2012). At this wavelength, the intensity of fluorescence emitted at ~350 nm (often referred to as peak T or peak T2) has been shown to relate to the water quality in sewage-impacted rivers (Baker, 2001). Peak T has been found to correlate with Biochemical Oxygen Demand (BOD) in rivers (Baker and Inverarity, 2004; Hudson et al., 2008; Hur et al., 2008; Hur and Cho, 2012; Knapik et al., 2014) and sewer systems (Hur et al., 2010). Peak T also correlates with BOD and chemical oxygen demand within the sewerage treatment process (Bridgeman et al., 2013; Yang et al., 2014); and with total bacteria using flow cytometry (Bridgeman et al., 2015).

The observations of a fluorescence peak T–BOD relationship led to the hypothesis that peak T measurement could also be used to quantify the presence of *Escherichia coli* (*E. coli*) through its remnant biomass containing fluorescent tryptophan residues. Cumberland et al. (2012) tested a portable fluorescence spectrometer to measure fluorescence intensity at these excitation and emission wavelengths and sought to determine whether there was a relationship with bacterial numbers. Their pilot-study, of 24 samples from four urban river and sewerage sites in the UK, demonstrated a linear correlation between fluorescence intensity and microbial water quality as determined by bacterial enumerations, over a three-log range of bacterial counts. These preliminary results suggest that this technique may have wider practical applications. Intrinsic fluorescence at ~280 nm excitation and ~350 nm emission in aquatic systems has many potential organic matter sources as this is the region where indole-containing compounds (Aiken, 2014; Baker et al., 2014) are excited. However, statistically significant correlations with BOD (Hudson et al., 2008), flow cytometry (Bridgeman et al., 2015) and microbial enumerations (Cumberland et al., 2012) suggest that, in at least the case of sewage-impacted riverine environments, tryptophan-like fluorescence from protein molecules derived from microbial matter, provides the dominant signal.

Previous research has demonstrated that, in riverine and sewerage systems, fluorescence intensity at 280 nm excitation and 350 nm emission correlates with BOD over the range 1 mg/l to 10,000 mg/l BOD (Baker et al., 2014). Where pollution by raw sewage is providing the source of BOD, it raises the prospect of a correlation between fluorescence intensity at the same wavelengths and microbial enumerations such as *E. coli* and total coliforms. Cumberland et al. (2012) demonstrate a reasonable correlation between fluorescence intensity and log *E. coli* ($r = 0.74$) in a small dataset of urban river samples and sewage effluents. Baker et al. (2014) report an earlier study investigating a cross-connected storm sewerage impacted small urban river and two sewage treatment works. In that study, an increase in the strength of the correlation between *E. coli* and fluorescence intensity with increasing *E. coli* numbers was observed. Furthermore, the correlation between fluorescence intensity and log *E. coli* was highly variable between individual storm sewers (correlations from 0.2 to 0.7), and was strongest in samples collected from within the sewage treatment process ($r = 0.84$).

This illustrates the potential for using fluorescence spectroscopy as a rapid measure of microbial water quality and provides the motivation for this paper. These studies suggest that a statistically significant correlation between fluorescence intensity and *E. coli* can occur, but investigations to date have been relatively small and have utilised relatively simple field experimental designs. Specifically we seek to test the wider use, and sensitivity of the portable fluorimeter (SMF-4, Safe Training Systems, Wokingham, UK) used by Cumberland et al. (2012) in more rigorous field applications, to determine the wider utility of this approach and its suitability for rapid water surveys. At present, the degree to which portable fluorometers can be used to assess microbial water quality in the field has yet to be firmly established, and there are two specific areas of concern. First, the significance of the sensitivity of fluorescence to environmental conditions requires further study as discussed in recent reviews (Hudson et al., 2007; Henderson et al., 2009; Baker et al., 2014). Particularly important in this respect are the effects of temperature (Baker, 2005), pH (Baker et al., 2007), and interactions with colloids (e.g., Khamis et al., 2015) and dissolved metals (Yamashita and Jaffe, 2008). Second, there is the likelihood that some of the fluorescence at the 280 nm excitation and 350 nm emission wavelength pair, may arise from microbiological processing of Natural Organic Matter (NOM) (for example, Hudson et al., 2008), affecting the strength of the correlation between fluorescence intensity and

E. coli. For these reasons, a large-scale, field, experiment is required to challenge the robustness of the fluorescence intensity–*E. coli* relationship, before the use of portable fluorescence can be established in the real-time enumeration of the microbial water quality of river and groundwater bodies.

In order to address this challenge, we have undertaken field campaigns to assess water quality in the province of KwaZulu-Natal, South Africa. Working with local water providers, sites were identified where there were known microbial water quality concerns and complex water quality issues, and for which rapid microbial enumeration methods would be of practical benefit. Our experimental design was one that would deliberately challenge a fluorescence intensity–*E. coli* relationship and also be of practical application: we chose to analyse non-perturbed, unfiltered samples. Here, we present the results of these field campaigns and the strength of the fluorescence intensity–*E. coli* relationship on unfiltered water samples across multiple catchments.

2. Methods and site description

2.1. Site descriptions

We analysed water samples from two contrasting catchments in KwaZulu Natal, South Africa (Fig. 1a). The larger ~4432 km^2 Umgeni River catchment (also known as the Mgeni, Mngeni and uMngeni) in KwaZulu-Natal was sampled in the region around Pietermaritzburg along the Msunduzi (also known as the Dusi, Duzi and uMsunduzi) River and its tributaries. Water samples were collected from the Dorpspruit, Slangspruit and Bynespruit tributaries as well as the main stem of the Msunduzi River.

Water samples were taken at regular spatial intervals from the main-stem of the rivers during routine water quality sampling (Fig. 1b). The sites were predominantly located upstream of Pietermaritzburg, through the city, and immediately upstream and downstream of the Darvill Waste Water Works (the main sewage treatment plant for the Pietermaritzburg area serving over 300,000 people and treating 75 Ml/day). The Msunduzi and its tributaries join the Umgeni River, which is the main water supply to the Inanda Dam. The latter supplies 300 Ml/day of raw water to Durban Heights and Wiggins potable water treatment works, which supplies potable water to the greater Durban area.

Our second catchment was the smaller Umbilo River, situated within Durban (Fig. 1c). The river was again sampled at regular spatial intervals down the main stem, following a gradient from the relatively pristine catchment headwaters to reaches downstream where industrial and residential areas predominate. Water samples were collected weekly (Pietermaritzburg) or twice-weekly (Durban) in January and February 2011 (following the annual summer rainy season), yielding up to eight replicate samples per site and sixty-nine (Umgeni catchment) and sixty-six (Umbilo catchment) water samples in total.

Both catchments are impacted by poor water quality. The causes of this poor quality are numerous, and include unconsented and consented industrial discharges, consented treated wastewater effluent, regular illegal dumping of contaminants, sewage contamination from informal settlements, failing septic tanks, contaminants in stormwater runoff, and direct defecation.

2.2. Methods

In the Umgeni catchment, we worked with staff from Umgeni Water, Pietermaritzburg laboratory, who routinely monitor river samples for water quality including microbial enumeration. *E. coli* was determined in the laboratory using standard Colilert methods. Split water samples were analysed in the laboratory for their fluorescence intensity following the methods of Cumberland et al. (2012). In all cases the water samples were non-perturbed. Samples were not filtered to retain the microbial fraction, which can be present both as free material in the <0.1 μm as well as in larger colloidal (>0.1 μm) size fractions. These conditions also emulate worst-case field applications where filtration of the water samples is not possible.

We used a portable fluorimeter (SMF-4, Safe Training Systems, Wokingham, UK) similar to that described by Cumberland et al.

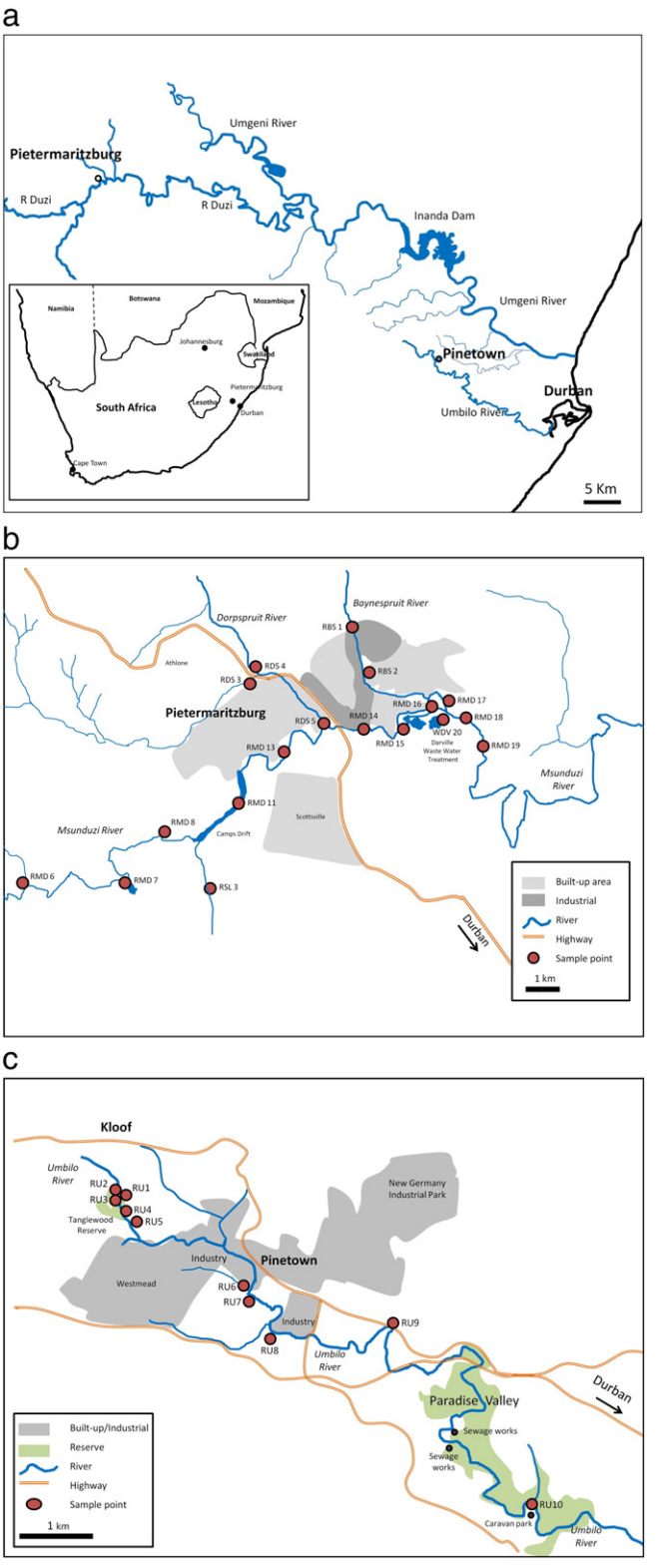


Fig. 1. (a) Overview map of the two catchments, KwaZulu-Natal, South Africa. (b) Water sampling locations on the Msumduzi catchment, Pietermaritzburg. (c) Water sampling locations on the Umbilo catchment, Durban.

(2012). This comprised an LED light source powered by four AA batteries with fixed excitation wavelength (λ_{ex}) of 280 nm. Emitted fluorescence was centred on 360 nm (slit width of 10 ± 4 nm). Data were automatically background subtracted by taking the sum of 16 readings (from binary multiples) without the LED excitation and subtracting it from the sum of 16 readings from the LED-excited sample. The displayed reading was taken as the sample reading. Sample analysis was undertaken using a 1 cm pathway quartz cuvette (QG Helma). In all cases the samples were blank subtracted using a deionised water blank. Prior to fieldwork in South Africa, the SMF4 instrument (Cumberland et al., 2012) was compared to the SMF4 (this study) by a series of dilution experiments using tryptophan as a standard. Data on three of the five tests were also collected from a bench top instrument (Varian Cary Eclipse). Three tests were performed on pure tryptophan in deionised water at a range of 10–100 ppb, a test on diluted river water only, and a test where tryptophan was spiked into river water at a concentration of 10–100 ppb. For each instrument three to five measurements were recorded from which standard deviations were calculated. All correlations between instruments were >0.97 (Pearson's correlation coefficient). Detection limits (DL) were 0.9 ppb tryptophan, calculated as three-times the standard deviation of the blank. All data reported here as tryptophan standard equivalents.

Umbilo River water sampling points were selected based on sites routinely accessed by eThekwini Water and Sanitation (Durban Government), supplemented by additional sampling points in the catchment headwaters (Tanglewood Farm Nature Reserve). Fluorescence readings were taken on site and water samples collected and stored on ice in new sterile 50 ml tubes with no headspace. These samples were analysed in the Pollution Research Group Laboratories, at the University of KwaZuluNatal (UKZN), Durban. On return to the laboratory, samples were immediately processed for *E. coli* enumeration, under sterile conditions using identical methods as in the Umgeni catchment: the Colilert (IDEXX) method was used with appropriate dilutions. Trays were incubated at 37 °C and read the following day after 18 h. Blanks were prepared incubated for every 10 samples and were always negative.

Interferences for aquatic organic matter fluorescence are relatively well understood, and include variations in temperature, solution pH, turbidity and colour (Hudson et al., 2007; Downing et al., 2012; Khamis et al., 2015). In order to assess the influence of possible interferences on the fluorescence data, water samples were also measured for turbidity, suspended solids, pH and temperature. Turbidity was measured in NTU using a portable meter (Hach DR 890). Temperature, pH and electrical conductivity were determined using a Hanna combined pH and electrical conductivity meter (H198129). Other potential interferences (e.g., colour and absorbance) were not measureable given limited resource availability, and our intention to focus on rapid field assessment.

The resultant fluorescence intensity, *E. coli* and water quality dataset was analysed using IBM SPSS Statistics 22 and Microcal OriginPro 2015.

3. Results and discussion

A summary of the water quality data is provided in Table 1. The KwaZulu-Natal river data has a seven-log range in *E. coli* and four-log range in fluorescence intensity. *E. coli* enumerations vary between catchments, with a greater range and variability observed in some catchments (e.g., the Umbilo) compared to others (e.g., the Msumduzi). The pH of all samples were circum-neutral, all 7 ± 1 pH units. pH variations within this range are not expected to create significant optical interferences at the wavelengths of interest (Baker et al., 2007). Mean turbidity varied from 8 ± 6 (two sites on the Umbilo) to 61 ± 95 NTU (the Dorpspruit catchment), with the highest observed value of 290 NTU. Turbidity variations in this range have been demonstrated to increase the apparent fluorescence signal at the wavelengths of interest due to increased light scattering (Khamis et al., 2015). The temperatures are those of the water samples at the time of laboratory analysis and ranged between 20 and 26 °C. The effects of temperature on fluorescence intensity at these wavelengths have been previously documented (Baker, 2005), with fluorescence quenched with increasing temperature.

The relationship between fluorescence intensity and *E. coli* for the whole dataset is shown in Fig. 2. Fig. 2 also includes previously published river and sewage treatment data from the West Midlands, England (from Cumberland et al., 2012). The log correlation between fluorescence intensity and *E. coli* for the KwaZulu-Natal dataset is 0.55 ($n = 114$, Pearson's product correlation). This is weaker than that previously reported for the West Midlands dataset ($n = 24$, $r = 0.81$). The log correlation between *E. coli* and fluorescence intensity for the combined dataset is 0.74 ($n = 136$). We also performed a stepwise least squares regression on the KwaZulu-Natal dataset to investigate correlations between *E. coli* and all measured field parameters, which demonstrated that the field parameters provided no additional statistical power.

The overall correlation between fluorescence intensity and *E. coli* for the KwaZulu-Natal dataset masks the highly variable strength of the fluorescence intensity–*E. coli* linear correlation for the individual catchments. In general, this variability in the strength of correlation reflects both the sample size, and the range and variability in *E. coli* enumerations, in each catchment. On the Umbilo, $r = 0.71$ ($n = 53$, significant at 95% confidence level); on the Dorpspruit $r = -0.24$ ($n = 9$, not significant), on the Baynespruit ($r = 0.06$, $n = 12$, not significant), on

Table 1
Summary water quality data.

River	Number of samples	Temperature (°Celsius)		pH		Turbidity (NTU)		Fluorescence Intensity (ppb tryptophan)		*E. coli* (CFU/100 ml)	
		Mean	sd	Median	sd	Mean	sd	Mean	sd	Mean	sd
Baynespruit	9	nd	nd	7.8	0.1	16	19	85	137	7740	8524
Dorpspruit	9	nd	nd	7.8	0.0	61	95	17	6	3889	2536
Msumduzi	38	nd	nd	7.6	0.2	41	25	33	19	10,515	39,949
Sangspruit	6	nd	nd	7.9	0.2	20	9	34	8	44,807	77,749
Umbilo RU1	6	21.1	1.4	7.0	0.5	10	6	16	15	8976	15,879
Umbilo RU2	6	19.9	0.1	6.7	0.2	8	6	8	7	696	406
Umbilo RU3	6	19.9	0.1	6.7	0.2	9	5	8	6	1139	1762
Umbilo RU4	6	22.1	1.2	7.1	0.1	11	4	15	4	2525	3208
Umbilo RU5	7	20.6	0.6	7.3	0.5	8	6	9	7	2081	4433
Umbilo RU6	6	23.5	0.7	7.6	0.0	18	2	44	7	65,875	60,120
Umbilo RU7	6	23.6	0.6	7.2	0.2	23	15	196	82	9,204,500	
Umbilo RU8	6	23.7	0.7	7.5	0.0	21	10	132	47	967,767	1,089,707
Umbilo RU9	7	24.8	1.2	7.7	0.1	15	3	62	11	462,130	978,300
Umbilo RU10	8	26.0	1.4	7.7	0.1	25	28	137	14	68,425	89,826
West Midlands	24	nd	nd	nd	nd	nd	nd	6	10	146	294

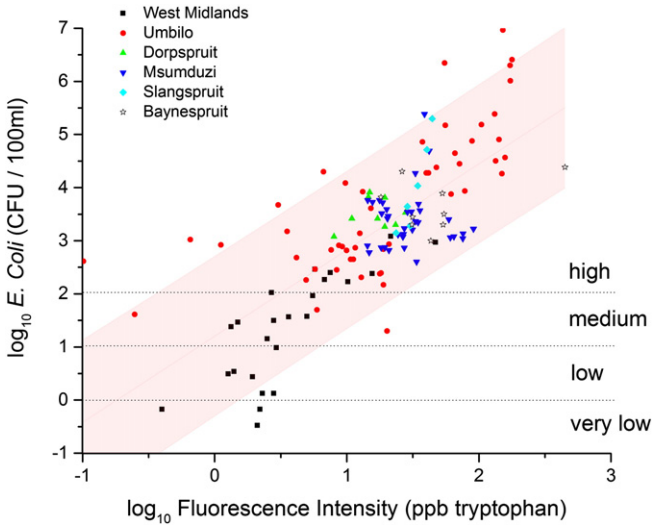


Fig. 2. Relationship between log fluorescence intensity and log *E. coli* for KwaZulu-Natal and UK datasets combined. 90% prediction and confidence limits are shown by the shaded areas, and the WHO risk thresholds for *E. coli* numbers shown by dotted horizontal lines.

the Msumduzi r = 0.05 (n = 36, not significant) and on the Slangspruit (r = 0.95 n = 6, significant at 95% confidence level). Further studies, increasing the sample size, may help strengthen the fluorescence intensity–*E. coli* relationship.

We investigated this variability in the strength of the fluorescence intensity–*E. coli* relationship further: performing a principal components analysis (PCA) on the normalised dataset (Fig. 3). We used the parameters pH, turbidity, fluorescence intensity and *E. coli*. Temperature was not included in the analysis as this variable was the laboratory temperature, and as such was relatively invariant as well as being incomplete (Table 1). PCA analysis identified two eigenvectors with a score greater than one (Fig. 3). In principal component one (PC1), all water quality parameters positively covaried and this component explained 37% of the variance in the dataset. River water samples associated with this component would have high *E. coli* associated with a high fluorescence intensity, turbidity and pH. Fluorescence intensity and *E. coli* correlated in Principal Component two (PC2) (28% of variance in the dataset), in this case with an inverse relationship with pH and turbidity.

For the individual catchments, the PCA analysis reveals some differences in the relationship between the water quality variables. On the Umbilo, there was a clear separation between sample sites upstream (RU1–RU5) and downstream (RU6–RU10) of Pinetown in principal component 1 (Fig. 3). Downstream samples are therefore characterised by higher *E. coli*, fluorescence intensity, pH and turbidity. In contrast, an upstream–downstream relationship was not observed on the Msumduzi and its tributaries at Pietermaritzburg (data not shown). All Msumduzi catchment samples have positive loadings on the first principal component, a similar loading to the samples downstream of Pinetown on the Umbilo, and indicative of samples with high *E. coli*, fluorescence intensity, pH and turbidity. With the exception of three outlying samples on the Dorpspruit and Baynespruit with high loadings, the principal component analysis demonstrates relatively low variance between the water quality parameters on the Msumduzi.

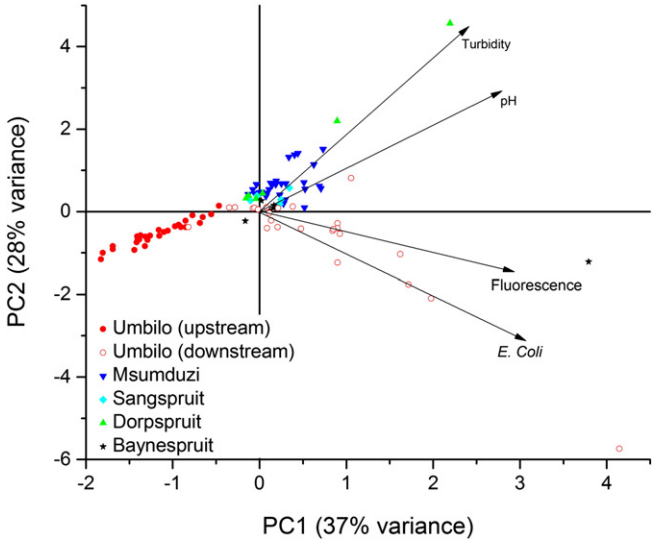


Fig. 3. Principal component analysis of the KwaZulu-Natal dataset.

The PCA confirms a general correlation between *E. coli* and fluorescence intensity, which agrees with the relationship that is observed in Fig. 2. However, at an individual catchment scale, both the PCA and Fig. 2 demonstrate a more variable fluoresce intensity–*E. coli* relationship. Optical interferences, such as pH, thermal quenching, and scattering of emitted fluorescence in turbid samples, will all affect the *E. coli*–fluorescence intensity relationship. However, their effect on fluorescence intensity over the observed range of pH, temperature and turbidity is not in itself sufficient to explain the 2.5 log range in the 90% prediction limits (Fig. 2). The effects of pH quenching of fluorescence intensity have been reported as <40% for a 4 pH unit change (Baker et al., 2007) and therefore likely to explain less than 20% of the variability in this dataset. Thermal quenching will produce a ~30% change in fluorescence intensity over a 35 °C temperature range (Baker, 2005) and therefore is likely to explain less than 5% of the variability in the dataset. Khamis et al. (2015) report up to 100% increase in fluorescence intensity with turbidity in the range of 0–300 NTU, although for different instrumentation. This potentially has the greatest effect on the data reported here, with a doubling of observed fluorescence possible for a few turbid samples. These optical interference therefore cannot explain the variability in the fluorescence intensity–*E. coli* relationship, and other factors must be important. Our data suggests that these are likely to be catchment specific, such as (1) microbial activity that increases fluorescence intensity without the presence of *E. coli*. These might be observed in eutrophicated systems not impacted by sewage contamination (2) pollution sources, unrelated to faecal pollution, which emit fluorescence in the region of interest. Numerous candidates can be proposed, including oil spills (Carstea et al., 2010) and polycyclic aromatic hydrocarbons (Baker et al., 2014).

4. Conclusions

The overall log correlation between *E. coli* and fluorescence intensity across several catchments and two continents yields a global relationship of r = 0.74 over seven-log *E. coli* numbers and four-log fluorescence intensity, with log (*E. coli*) = 1.201 + 1.622 log (Fluorescence Intensity), over a range of pH from 6.6 to 7.8 and turbidity of 8 to 61 NTU. This strength of correlation demonstrates a limit to the prediction (at a 90% prediction level) of *E. coli* using portable fluorescence intensity to 2.5-log at the regional catchment scale. Given the number of compounds that can potentially fluorescence in this optical region (Aiken, 2014; Baker et al., 2014), and the number of potential interferences to fluorescence, such a limit to predicting *E. coli* numbers at this scale is unexpected.

We undertook this research to challenge the use of a handheld fluorescence instrument as a practical method of obtaining an immediate estimate of *E. coli* numbers. We deliberately chose a complex scenario, with catchments of poor water quality and multiple pollution sources, and unfiltered water samples, as a worst-case for the use of this technology. Even in this scenario, our results suggest promise. For example, in our dataset, if we used fluorescence to detect whether water samples were of 'high risk' (defined as *E. coli* of >100 CFU/100 ml, see Fig. 2) and chose a cut-off fluorescence intensity of 3 ppb tryptophan equivalent,

then fluorescence would give a prediction in 94% of all or samples (120 out of 128). This level of successful prediction is partly a result of our relatively small dataset (n = 128), and other cut-off values for *E. coli* and fluorescence give weaker results. In less challenging water environments with fewer potential optical interferences, such as in groundwater and water distribution systems, we anticipate the best prediction of *E. coli*.

Our intended application of the technology remains disaster relief situations, where the rapid displacement of large numbers of people to locations with poor sanitation requires a quick and simple assessment method for rapid and continuous screening of surface and groundwater quality. It is in applications such as this that we feel our results have the greatest potential application. In such situations, presuming faecal contamination is the dominant source of poor water quality, then we believe that handheld fluorescence intensity measurements is a suitable screening tool that can prevent the use of contaminated water and which ultimately can help save lives. Fluorescence screening for *E. coli* in such situations has a number of advantages including its ease of use by non-experts, simple training requirements, the generation of immediate results and its portability and low voltage battery power.

Acknowledgements

This research was funded by the UK EPSRC Grant EP/H003061/1. It would not have been possible without the support of Merlien Reddy at UKZN, Narrosha Reddy and Ian Bailey at Umgeni Water. We also thank the laboratory staff at Umgeni Water. Chris Fennimore at eThequini Water and Sanitation, Durban provided data for Fig. 1. We thank Mariann Saebo for field support and Caryl Combrinck for field site access (Tanglefoot Reserve).

References

Aiken, G., 2014. Fluorescence and dissolved organic matter: a chemist's perspective. In: Coble, P.G., et al. (Eds.), Aquatic Organic Matter Fluorescence. Cambridge University Press.

Anstiss, R.G., Ahmed, M., 2006. A conceptual model to be used for community-based drinking-water improvements. J. Health Popul. Nutr. 24, 262–266.

Bain, R., Cronk, R., Hossain, R., Nonjour, S., Onda, K., Wright, J., Yang, H., Slaymaker, T., Hunter, P., Prüss-Ustün, A., Bartram, J., 2014a. Global assessment of exposure to faecal contamination through drinking water based on a systematic review. Trop. Med. Int. Health 19, 917–927.

Bain, R., Cronk, R., Wright, J., Yang, H., Slaymaker, T., Bartram, J., 2014b. Fecal contamination of drinking-water in low- and middle-income countries: a systematic review and meta-analysis. PLoS Med. 11, e1001644.

Baker, A., 2001. Fluorescence excitation–emission matrix characterisation of some sewage impacted rivers. Environ. Sci. Technol. 35, 948–953.

Baker, A., 2005. Thermal fluorescence quenching properties of dissolved organic matter. Water Res. 39, 4405–4412.

Baker, A., Inverarity, R., 2004. Protein-like fluorescence intensity as a possible tool for determining river water quality. Hydrol. Process. 18, 2927–2945.

Baker, A., Elliott, S., Lead, J.R., 2007. Effects of filtration and pH perturbation on organic matter fluorescence. Chemosphere 67, 2035–2043.

Baker, A., Andersen, M.S., Marjo, C.E., Zainuddin, N.S., Rutlidge, H., Graham, P.W., Henderson, R.K., 2014. Investigation of pollution in rivers and groundwater by fluorescence. Encyclopedia of Analytical Chemistrypp. 1–14.

Bartram, J., Brocklehurst, C., Fisher, M.B., Luyendijk, R., Hossain, R., Wardlaw, T., Gordon, B., 2014. Global monitoring of water supply and sanitation: history, methods and future challenges. Int. J. Environ. Res. Public Health 11, 8137–8165.

Bridgeman, J., Baker, A., Carliell-Marquet, C., Carstea, E., 2013. Determination of changes in wastewater quality through a treatment works using fluorescence spectroscopy. Environ. Technol. 34, 3069–3077.

Bridgeman, J., Baker, A., Brown, D., Boxall, J.B., 2015. Portable LED fluorescence instrumentation for the rapid assessment of potable water quality. Sci. Total Environ. 524–525, 338–346.

Carstea, E.M., Baker, A., Bieroza, M., Reynolds, D.M., 2010. Continuous fluorescence excitation–emission monitoring of river organic matter. Water Res. 44, 5356–5366.

Cumberland, S., Bridgeman, J., Baker, A., Sterling, M., Ward, D., 2012. Fluorescence spectroscopy as a tool for determining microbial quality in potable water applications. Environ. Technol. 33, 687–693.

Cumming, O., Elliott, M., Overbo, A., Bartram, J., 2014. Does global progress on sanitation really lag behind water? An analysis of global progress in community- and household-level access to safe water and sanitation. PLoS One 9, e114699.

Downing, B.D., Pellerin, B.A., Bergamaschi, B.A., Saraceno, J.F., Kraus, T.E., 2012. Seeing the light: the effects of particles, dissolved materials, and temperature on in situ measurements of DOM fluorescence in rivers and streams. Limnol. Oceanogr. Methods 10, 767–775.

Henderson, R.K., Baker, A., Murphy, K.R., Hambly, A., Stuetz, R.M., Khan, S.J., 2009. Fluorescence as a potential monitoring tool for recycled water systems: a review. Water Res. 43, 863–881.

Hodges, M., Belle, J.H., Carlton, E.J., Liang, S., Li, H., Luo, W., Freeman, M.C., Liu, Y., Gao, Y., Hess, J.J., Remais, J.V., 2014. Delays in reducing waterborne and water-related infectious diseases in China under climate change. Nat. Clim. Chang. 4, 1109–1115.

Hudson, N.J., Baker, A., Reynolds, D., 2007. Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters — a review. River Res. Appl. 23, 631–649.

Hudson, N.J., Baker, A., Ward, D., Brunsdon, C., Reynolds, D., Carliell-Marquet, C., Browning, S., 2008. Fluorescence spectrometry as a surrogate for the BOD_5 test in water quality assessment: an example from South West England. Sci. Total Environ. 391, 149–158.

Hur, J., Cho, J., 2012. Prediction of BOD, COD, and total nitrogen concentrations in a typical urban river using a fluorescence excitation–emission matrix with PARAFAC and UV absorption indices. Sensors 12, 972–986.

Hur, J., Hwang, S.-J., Shin, K.-K., 2008. Using synchronous fluorescence technique as a water quality tool for an urban river. Water Air Soil Pollut. 191, 231–243.

Hur, J., Lee, B.M., Lee, T.H., Park, D.H., 2010. Estimation of biological oxygen demand and chemical oxygen demand for combined sewer systems using synchronous fluorescence spectra. Sensors 10, 2460–2471.

Khamis, K., Sorensen, J.P.R., Bradley, C., Hannah, D.M., Lapworth, D.J., Stevens, R., 2015. In situ tryptophan-like fluorometers: assessing turbidity and temperature effects for freshwater applications. Environ. Sci. Process. Impacts 17, 740–752.

Knapik, H.G., Fernandes, C.V.S., de Azevedom, J.C.R., Porto, M.F.D., 2014. Applicability of fluorescence and absorbance spectroscopy to estimate organic pollution in rivers. Environ. Eng. Sci. 31, 653–663.

WHO/UNICEF, 2014. Progress on Sanitation and Drinking-Water — 2014 Update. WHO, Geneva 978924 150724 0.

Yamashita, Y., Jaffe, R., 2008. Characterizing the interactions between trace metals and dissolved organic matter using excitation–emission matrix and parallel factor analysis. Environ. Sci. Technol. 42, 7374–7379.

Yang, L., Shin, H.-S., Hur, J., 2014. Estimating the concentration and biodegradability of organic matter in 22 wastewater treatment plants using fluorescence excitation emission matrices and parallel factor analysis. Sensors 14, 1771–1786.