A Case Study: The deployment of a novel *in situ* fluorimeter for monitoring biological contamination within the urban surface waters of Kolkata, India.

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Abstract:

The quality and health of many of our vital freshwater systems are poor. To tackle this with ever increasing pressures from anthropogenic and climatic changes, we must improve water quality monitoring and devise and implement more appropriate water quality parameters. Recent research has highlighted the potential for Peak T fluorescence (tryptophan-like fluorescence, TLF) to monitor microbial activity in aquatic systems. The VLux TPro (Chelsea Technologies Ltd., UK), an *in situ* real-time fluorimeter, was deployed in different urban freshwater bodies within Kolkata (West Bengal, India) during March 2019. This study is the first to apply this technology in surface waters within a densely populated urban area. Spot-sampling was also undertaken at 13 sampling locations enabling physicochemical analysis, bacterial enumeration and determination of nutrient (nitrate and phosphate) concentrations. This case study has demonstrated the ability of an *in situ* fluorimeter, VLux TPro, to successfully identify both biological contamination events and potential elevated microbial activity, related to nutrient loading, in complex surface freshwaters, without the need for expensive and time-consuming laboratory analysis.

Keywords

Biological contamination, Fluorescent organic matter, Freshwater, Surface waters, *In situ* sensors, Water quality

1 Introduction

Monitoring water quality globally is considered vital for human health and sustainable development, as well as ensuring aquatic ecosystem integrity (Firth, 1999; Postel, 2015). With increasing pressures on water sources due to population growth, industrialisation, agriculture, urbanisation and climatic changes (Khamis et al., 2017; Patil et al., 2012), it has become essential to manage our water sources effectively. To do this, we must be able to successfully monitor water quality with good spatio-temporal resolution (Postel, 2015).

The most frequently and routinely measured water quality parameters are physicochemical, chemical and biological in nature which rely on discrete sampling and lengthy, as well as expensive, laboratory testing (Blaen et al., 2016; Peleato et al., 2017). The measurement of such water quality parameters provides information relating to the structure of the aquatic ecosystem under investigation but does not provide information that usefully relate to the function of the monitored ecosystem (Matilainen et al., 2011; Patil et al., 2012). In particular, the routine measurement of the biological qualities of freshwater systems is performed by the measurement of biological indices that require macro-biological diversity assessment (surveys) and microbiological analysis via the culturing of specific indicator species (Bio-Science Division and Central Pollution Control Board, 2018; Central Pollution Control Board, 2017). The reliance on discrete sampling and laboratory testing limits the response needed to manage catchments that experience rapidly changing dynamic ecosystem interactions.

The formation of the Central Pollution Control Board in India has greatly benefitted the monitoring of Indian river systems at a catchment scale, providing a holistic approach to water quality assessment. Under the national water quality monitoring programme there are 9 core parameters monitored monthly: pH, temperature, conductivity, dissolved oxygen, BOD, nitrate, nitrite, faecal coliforms and total coliforms (Central Pollution Control Board, 2013). Alongside these core parameters, location-specific studies collect data for a range of field-based observations, general parameters, trace metals and pesticides (Central Pollution

Control Board, 2013). Biological water quality data is related to three parameters: saprobity index, a quantitative inventory of the presence of macro-invertebrate benthic fauna; diversity index, the evaluation of benthic fauna using the same animals collected for the estimation of the saprobic score; and production-respiration (P/R) ratio, ratio of gross production to total community respiration (Bio-Science Division and Central Pollution Control Board, 2018; Central Pollution Control Board, 2017, 2013). However, the latter parameter is infrequently used with biological water quality classification, commonly determined by combining the saprobic and diversity scores (Bio-Science Division and Central Pollution Control Board, 2018; Central Pollution Control Board, 2017).

Although improvements have been made, the assessment of these biological indices in freshwater biomes is still difficult to quantify with current approaches and practices. Furthermore, very little information or data is collected in real-time with biotic indices informing longer-term environmental conditions (Aazami et al., 2015; Santos et al., 2021). Whilst important to understand, the reliance on long-term biological changes for the assessment of pollution levels makes it difficult to implement timely intervention strategies especially within large and dynamic riverine systems (Santos et al., 2021). To combat this issue, there is the need to develop new monitoring parameters which enhance our understanding of water quality dynamics and processes, that take advantage of technological developments and real-time acquisition. Real-time technologies provide advantages in terms of streamlining the data collection process, reducing cost in the long-term and, critically, producing higher resolution data (Chowdury et al., 2019).

Water quality sensors are widely available for the monitoring of basic physicochemical parameters, but these sensors do not yet provide information regarding biotic parameters or biological processes that take place within aquatic systems. Fluorescence-based sensing technology has long been utilised for the identification of anthropogenic pollutants, such as polycyclic aromatic hydrocarbons (PAH) and optical brighteners (Cyr et al., 2019). Recently *in situ* real-time portable fluorimeters have been developed for sensing biological contamination.

Research concerning aquatic fluorescent organic matter (AFOM) has highlighted the potential of tryptophan-like fluorescence (TLF or Peak T; $\lambda_{ex}/\lambda_{em}$ 275/340-360 nm) for tracing microbial contamination events (Blaen et al., 2016; Carstea et al., 2020; Coble et al., 2014; Fox et al., 2017; Hudson et al., 2008; J.P.R. Sorensen et al., 2018; Sorensen et al., 2021, 2015b; Zhou et al., 2017). The widespread use of this technology for monitoring water quality has been limited by the inability to disentangle optical interferences caused by scattering (turbidity) and absorption (excess inorganic and organic material) (Khamis et al., 2015; Mendoza et al., 2020). Such optical interferences obviate the reporting of fluorescence in standardised units of measurements thus preventing the comparison of quantitative data for different aquatic sites. In this study we investigate if real-time Peak T values from an *in situ* fluorimeter (VLux TPro, Chelsea Technologies Ltd.), corrected for absorbance and turbidity, can be used to infer bacterial and nutrient contamination. The aim of this work is to deploy the VLux TPro for the first time to provide quantitative assessments of the biological properties of urban surface waters present in the densely populated city of Kolkata. This research reports the identification of wastewater contamination events from point sources. The benefit of utilising Peak T fluorescence to directly measure the "biological activity" in aquatic environments is demonstrated and the use of this new water quality information to inform biological contamination events in complex surface water matrices is discussed.

2 Materials and method

2.1 Study area

All field sites were located within the city of Kolkata, in the state of West Bengal, India. Kolkata has undergone various stages of expansion and urbanisation over the past 300 years, turning this marshland into a booming trading port, and now into a megacity (Bhattacharjee, 2014; Gangopadhyay and Patra, 2019). At the last census in 2011, Kolkata city (an area of 185 km^2) had a population of almost five million people, with over 14 million living in the Kolkata

Metropolitan Area (1851.41 km^2) (Bhattacharjee, 2014). The large population, thriving industry, and sustained and often unregulated urbanisation continues to put pressure on the water resources in Kolkata (Gangopadhyay and Patra, 2019; Gangopadhyay and Gupta, 2008). The field sampling locations were classified into sample types based on water course type: (1) The Hooghly River (Ganga); (2) Canals; (3) East Kolkata Wetlands.

The Hooghly River is a distributary of the Ganges River, and often referred to locally as the Ganga (Central Pollution Control Board, 2013; Gangopadhyay and Patra, 2019). It is of great cultural and social significance within India, being used for bathing and religious ceremonies and celebrations (Bio-Science Division and Central Pollution Control Board, 2018). It is also used as a shipping channel for goods boats and passenger ferries alike. This watercourse is subject to a range of pollution sources (Bio-Science Division and Central Pollution Control Board, 2018), receiving wastewater from 22 polluting industries within West Bengal, with the point sources in Kolkata contributing to 74% of this loading (Central Pollution Control Board, 2013). The water quality of the Hooghly River in Kolkata is classified as Class D (heavy pollution), although the biological water quality, based on saprobic and diversity score, of the Hooghly in Kolkata is classified as moderately polluted (Class C) (Bio-Science Division and Central Pollution Control Board, 2018). The Ganga Action Plan (GAP) aims to improve this to bathing water quality (Class B) (National River Conservation Directorate, 2009).

The canal system in Kolkata originated from channels used for local transport and trade, as well as an irrigation source (Gangopadhyay and Patra, 2019). As the city expanded, the canal system was also expanded to accommodate increased trade within the city and to transport goods beyond Kolkata (Bhattacharjee, 2014; Gangopadhyay and Patra, 2019). Over time, the canals began to deteriorate, mainly due to sedimentation, leading to a decline in their use. Some of the canals were repurposed to redirect storm water and sewage from east of the old city, into the River Bidvadhari. Many canals ceased to be used for transport or trade and were scarcely maintained (Bhattacharjee, 2014), effectively turning them in drainage channels for

storm waters and sewage (Gangopadhyay and Patra, 2019; Gangopadhyay and Gupta, 2008).

The East Kolkata Wetlands (EKW) are host to the world's largest sewage fed aquaculture system (EKWMA, 2016). Originally, these wetlands existed as fisheries to the east of the city and later as the location for diverted storm waters and sewage (Bhattacharjee, 2014; EKWMA, 2016). At present, the EKW not only act as the only sewage treatment for a third of the city's sewage, via filtration, sediment settlement and nutrient acquisition, but also provide food and a livelihood for many of the local population (Central Pollution Control Board, 2020; East Kolkata Wetlands Management Authority, 2019). To protect the area from rapid expansion, they were marked as a Ramsar site in 2002 and designated as one of the "Wetlands of International Importance" (EKWMA, 2016). The wetland ponds are surrounded by a network of small channels which are fed from the canal network, in particular Circular Canal (Bhattacharjee, 2014; Gangopadhyay and Patra, 2019)..

2.2 Sampling survey

The data presented in this case study was collected during a sampling survey which took place between 11th – 26th March 2019, prior to the monsoon (which usually begins at the start of June) and to avoid peak annual temperatures. The modelled average monthly flow rate for the Hooghly River in Kolkata throughout 2019 ranged from 20.27 – 937.83 m^3/s, with the lowest flow occurring in March and the highest in August (Sutanudjaja et al., 2018). The sampling survey analysed a total of 13 sampling sites, seven were located on the Hooghly River, four from the canal network and two samples from the East Kolkata Wetlands (EKW). A single sample was collected from each of the 13 sample sites. The locations for all sampling sites monitored are shown in Figure 1, and a table of all sampling location details (from GPS data) can be found in Supplementary Table 1.

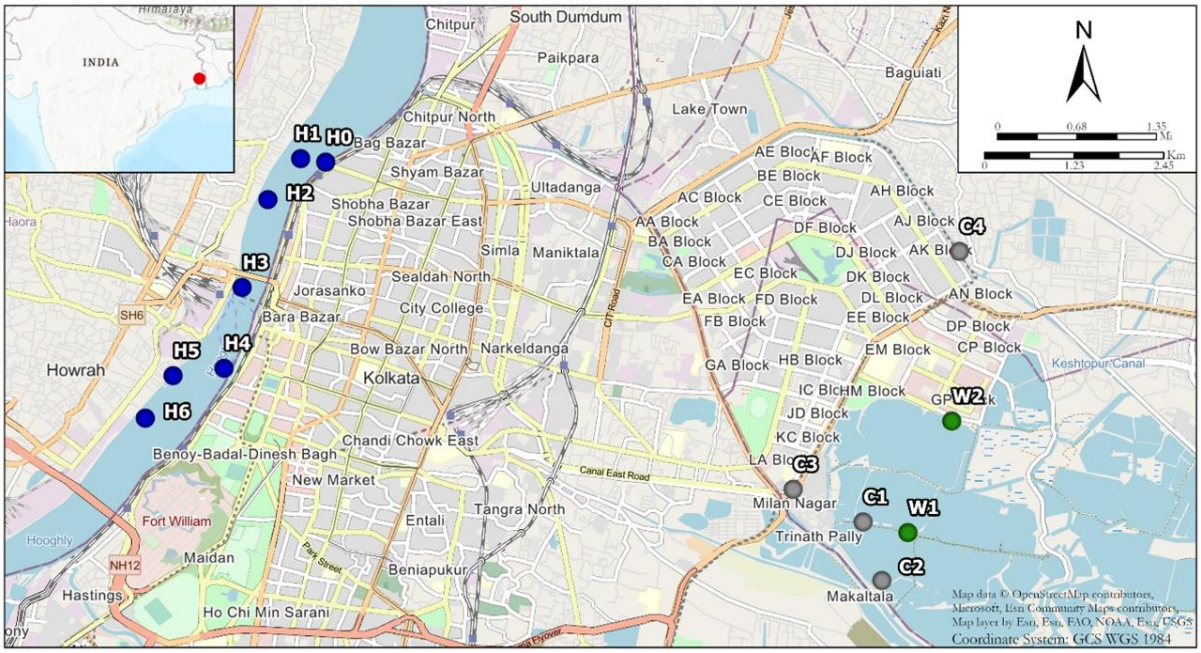


Figure 1: Study site map of sampling locations in urban Kolkata for the sampling survey conducted in March 2019. All field locations are labelled and identified using a coloured marker: Hooghly River [Hx] = blue; canal locations [Cx] = grey; and, East Kolkata Wetlands (EKW) [Wx] = green. An overview map is provided in the top left and scale information detailed in the top right. Map created in ArcGIS® Pro (ESRI™, California, US) using OpenStreetMap data (2021).

2.2.1 Collection of water samples

Water samples were collected from the centre of the water body where possible, or alternatively at margin locations with visual evidence of flow, to ensure mixing (Bowes et al., 2020). Samples were collected using a 500 mL PTFE bottle, rinsed with the sample three times prior to collection. The collection bottle was deployed to a depth of 30 cm, then decanted, unfiltered, into two 50 mL sterile falcon tubes, one 60 mL acid-washed (20% hydrochloric acid) Nalgene PTFE bottle, and two sterile 1.5 mL microcentrifuge tubes. One microcentrifuge tube was pre-prepared with glutaraldehyde, with a final concentration of 0.25%, to act as a fixative for storage. Subsequently, two 60 mL acid washed PTFE bottles were filled with a filtered subsample (0.45 µm sterile ThermoFisher cellulose nitrate membrane filters). All samples were kept in the dark and at 4°C when stored and kept chilled during transport.

182

183 *2.2.2 Physicochemical parameters*

184 The following key physicochemical parameters were measured in the field; dissolved oxygen
185 (DO), electrical conductivity (hereby referred to as conductivity), temperature and pH. DO was
186 measured immediately after sample collection using a handheld meter (HQ10, Hach, CO,
187 USA). Conductivity and temperature data were collected using a handheld Accumet™
188 conductivity meter (Fisherbrand, Pittsburgh, USA), and pH was measured with a handheld pH
189 meter (Jenway 570, Cole-Parmer, Illinois, USA).

190

191 *2.2.3 VLux TPro sensor*

192 Field trials were conducted using a prototype of the VLux TPro optical sensor (Chelsea
193 Technologies Ltd., UK) for measuring fluorescence intensity of Peak T, Peak C and
194 chlorophyll-α, as well as absorbance and turbidity (Fox, 2018). All fluorescence data is
195 measured at an excitation wavelength of 280 nm, with Peak T, Peak C and chlorophyll-α
196 fluorescence emission measured at 365 ±25 nm, 450 ±25 nm and 682 ±15 nm respectively.
197 The sensor has a dynamic range of 0-600 quinine sulphate units (QSU) and sensitivity of 0.02
198 QSU for the fluorescence channels. Absorbance was also measured at an excitation
199 wavelength of 280 nm, with a dynamic range of 0-3.5 optical density (OD) and sensitivity of
200 0.002 OD. The turbidity channel measured at an excitation and emission wavelength of 860
201 nm, in line with ISO 7027:1999(E) (ISO, 1999), and has a dynamic range of 0-1000 Formazin
202 Nephelometric Units (FNU) and sensitivity of 0.01 FNU (Fox, 2018). All VLux TPro
203 fluorescence sensing data output is corrected for optical interferences using real-time and *in*
204 *situ* absorbance and turbidity measurements. All corrections are applied through internal
205 software algorithms, reporting all fluorescence measurements in quinine sulphate units (QSU).
206 A QSU value of 1 is equivalent to 1 ppb of quinine sulphate standard in 0.1 M perchloric acid
207 (Starna Cells, USA) at $\lambda_{ex}/\lambda_{em}$ 347.5/450 nm (Fox, 2018).

The VLux TPro was deployed to a depth of 30 cm at all monitoring sites, as per collected water samples. Data collected was logged using a custom-made data logger, using a Raspberry Pi 3 B+ (The Raspberry Pie Foundation, UK) in conjunction with a custom logging general user interface (GUI) created in Python™ (Python Software Foundation).

2.3 Laboratory analysis of water samples

2.3.1 Microbiological analysis

Microbiological analysis for *Escherichia coli* and coliform enumeration was undertaken within 6 hours of sample collection. A standard dilution series using sterilised de-ionised water was prepared and each dilution filtered onto a sterile 0.2 µm membrane filter (Whatman® 0.2 µm nitrocellulose membrane filters, GE Healthcare, UK), and placed on Membrane Lactose Glucuronide Agar (MLGA) plates, as per the coliform membrane filtration method (Sartory and Howard, 1992; The Environment Agency, 2008). This method allows for *Escherichia coli* and coliforms to be differentiated and enumerated.

Analysis for total bacteria cell count was conducted via flow cytometry, using a Gallios flow cytometer (Beckman-Coulter, UK). An aliquot of 0.5 mL from each glutaraldehyde fixed sample was stained with SYBR Green I (Sigma-Aldrich, UK) at a final concentration of 1:1000 for 30 minutes at room temperature in the dark. An addition of 2.0 µL of 1 µm diameter beads (Life Technologies, UK) to each sample was used as a calibration and counting standard. Each sample was run for 1 minute at a low flow rate using excitation with a 488 nm laser. Gates were manually drawn in Kaluza 1.2 software (Beckman-Coulter, UK) to distinguish and count bacterial cells from background noise and non-bacterial particles**.**

2.3.2 Nutrient analysis

Samples were analysed for nutrient concentrations at the UK Centre for Ecology & Hydrology (UKCEH). All analyses were carried out immediately on return to the UK, which was between

2 and 18 days after sample collection. Concentrations for total phosphorus, total dissolved phosphorus, soluble reactive phosphorus, and dissolved ammonium were determined as described by Bowes et al. (2020). Dissolved organic carbon (DOC) was analysed by thermal oxidation using an Elementar Vario Cube (Elementar Analysensysteme GmbH, Germany) (Bowes et al., 2020). Major dissolved anion concentrations (fluoride, chloride, nitrite, nitrate and sulphate) were determined using ion chromatography (Dionex AS50, Thermo Fisher Scientific, USA) (Bowes et al., 2020). Aquacheck quality control standards (LGC Standards, UK) were used for all analyses.

2.4 Data analysis

The statistical significance of variation between water quality parameters for the three different urban water types was obtained using a one-way ANOVA. Linear regression was also used to identify and characterise the relationships between the water quality parameters. The R^2 value was used to determine the strength of the correlation, as well as to determine the significance ($p < 0.05$). All statistics were performed in Prism 9 (GraphPad, CA, USA).

3 Results

3.1 Water quality within Kolkata city

Average values of key physicochemical and chemical water quality parameters, for the different water types (Hooghly River, Canals and EKW), are shown in Table 1, as well as the optical data obtained from the VLux TPro sensor deployment.

Table 1: Physicochemical and chemical water quality data, and optical data (from the VLux TPro sensor) for the Hooghly River, Canal and the East Kolkata Wetland (EKW) water samples. Data shown is the parameter average for each water type ± 1 standard deviation: six field sites on the Hooghly River; four canal water field sites; and two field sites in the East Kolkata Wetlands (EKW).

	Hooghly	Canals	EKW
PHYSICOCHEMICAL DATA			
DO (mg/L)	5.375 ±0.392	1.135 ±0.589	13.285 ±3.174
pH	7.982 ±0.074	7.160 ±0.492	8.950 ±0.381
Conductivity (µS/cm)	343.017 ±19.854	1192.650 ±303.249	788.500 ±36.062
Temperature (°C)	29.37 ±1.61	28.875 ±0.767	31.000 ± 1.556
CHEMICAL DATA			
Soluble reactive phosphorus (mg/L)	0.005 ±0.003	0.834 ±0.581	0.010 ±0.057
Total phosphorus (mg/L)	0.044 ±0.011	1.290 ±0.335	0.070 ±0.037
Dissolved fluoride (mg/L F)	0.273 ±0.004	0.214 ±0.128	0.408 ±0.042
Dissolved chloride (mg/L Cl)	21.588 ±2.581	212.539 ±83.756	182.944 ±22.267
Dissolved ammonium (mg/L NH_4)	0.159 ±0.363	13.500 ±3.122	0.032 ±0.023
Dissolved nitrite (mg/L NO_2)	0.060 ±0.068	0.000 ±0.000	0.000 ±0.000
Dissolved nitrate (mg/L NO_3)	1.572 ±0.563	0.154 ±0.065	0.156 ±0.012
Total dissolved nitrogen (mg/L N)	1.018 ±0.196	12.887 ±2.806	1.472 ±0.321

Dissolved sulphate (mg/L SO_4)	22.237 ±0.926	22.847 ±4.922	23.336 ±4.667
Dissolved organic carbon (mg/L)	2.734 ±0.198	9.296 ±2.528	13.008 ±0.606
OPTICAL DATA			
Chlorophyll-α (QSU)	13.498 ±0.878	23.554 ±2.673	112.907 ±49.080
Peak T (QSU)	11.163 ±1.250	216.009 ±73.926	345.900 ±232.760
Peak C (QSU)	11.983 ±1.577	134.818 ±51.430	94.745 ±12.658
Absorbance	0.174 ±0.028	0.333 ±0.077	0.908 ±0.351
Turbidity (FNU)	94.361 ±11.164	50.133 ±7.973	219.347 ±201.200

The physicochemical data shown in Table 1 demonstrates the diversity of the water bodies studied. The canal waters exhibit properties that are characteristic of waters impacted by untreated sewage wastewater, with very low dissolved oxygen (DO) concentrations in comparison to the Hooghly River, alongside high conductivity values, phosphorus (both soluble reactive phosphorus and total phosphorus), dissolved ammonium and dissolved organic carbon (DOC) concentrations (Table 1). One-way ANOVA analyses for all these parameters demonstrate significant differences ($p < 0.05$) for all these parameters between the different water types. The canal and EKW waters also demonstrate high chloride concentrations while all three water types contain <0.5 mg/L of fluoride (Table 1), comparable with other natural freshwaters (Chilton et al., 2006). The EKW exhibits the highest pH, DO and DOC values, but significantly lower phosphorus and dissolved ammonium concentrations ($p < 0.05$) than those observed in the canal and Hooghly River waters which feed this wetland system (Table 1). The dissolved sulphate concentrations are consistent across the different waters assessed, with no significant differences between the waters identified.

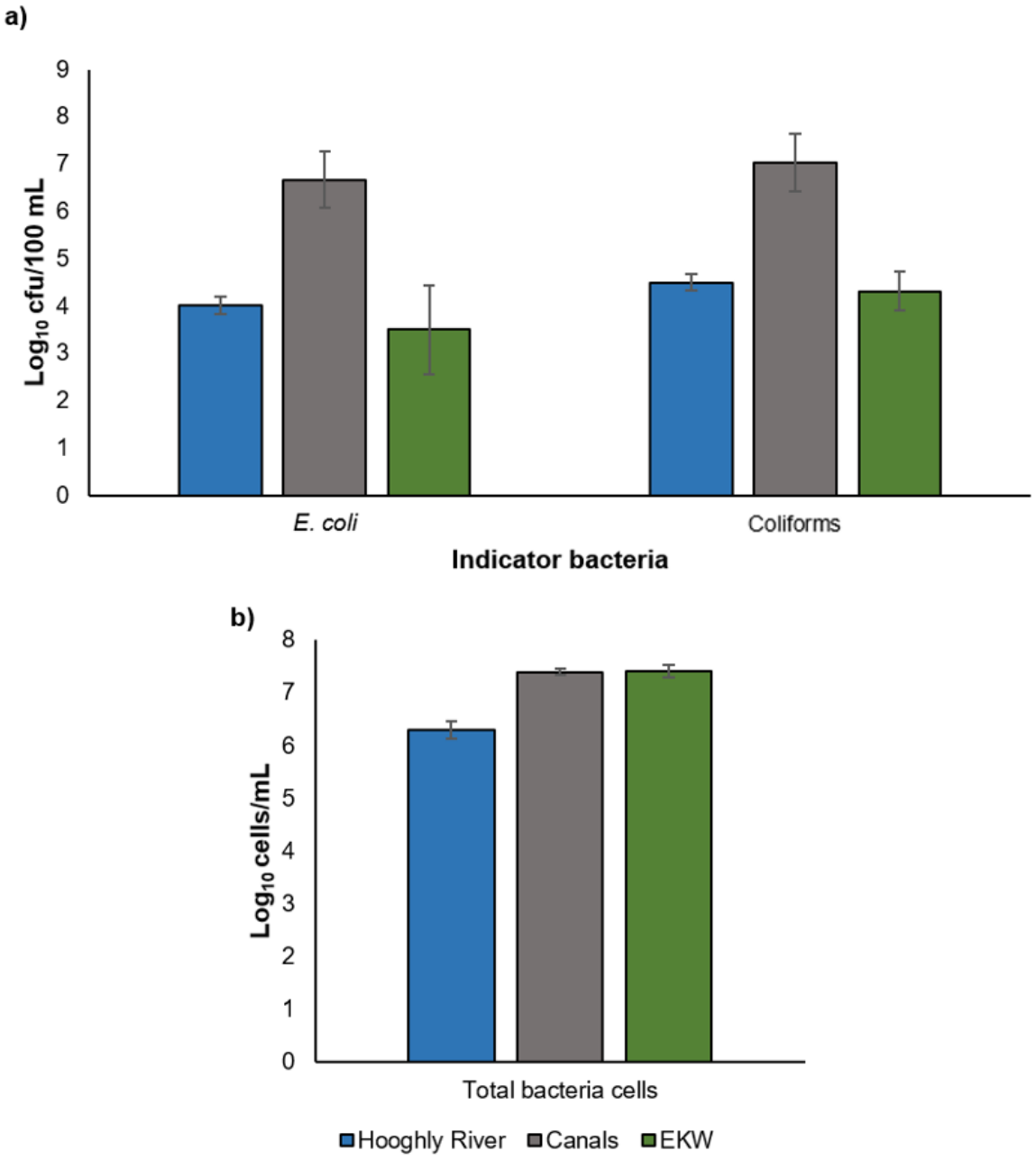
The VLux TPro sensor data, shown in Table 1, also shows that the canal and EKW waters exhibit significantly higher Peak T (tryptophan-like fluorescence, TLF, $\lambda_{ex}/\lambda_{em}$ 280/365(±25) nm), and Peak C fluorescence (associated with humic-like natural organic matter, $\lambda_{ex}/\lambda_{em}$

280/450(±25) nm), by an order of magnitude, than the Hooghly River ($p < 0.05$). Surprisingly, this difference in water quality of the three water types is not clearly identified within the turbidity data ($p > 0.05$), largely due to the high variability of the turbidity within and between the water body types. The EKW waters also demonstrate chlorophyll-α fluorescence intensity that is an order of magnitude greater than that of the other urban water bodies assessed here, with a one-way ANOVA identifying significant differences ($p < 0.05$) identified between the chlorophyll-α intensity for each water type.

Correlations between Peak T fluorescence and the physicochemical and chemical data were explored using linear regression. Significant, albeit weak, correlations were identified between Peak T fluorescence and conductivity ($R^2 = 0.34$, $p < 0.05$), and dissolved nitrate concentrations ($R^2 = 0.39$, $p < 0.05$). A strong significant relationship was identified between Peak T and DOC ($R^2 = 0.74$, $p < 0.001$). Peak T fluorescence did not demonstrate significant linear relationships ($p > 0.05$) with any of the other field physicochemical parameters or chemical data when assessing all water types combined.

3.1.1 Biological water quality within Kolkata city

The biological enumeration data highlights the heavy biological contamination within the canal waters, with these waters demonstrating the highest *E. coli* and coliforms counts of the three water types (Figure 2a). The total bacterial cell counts demonstrate the high levels of bacteria in the canals, but also in the EKW (Figure 2b). However, this data does not determine the species or viability of the cells present.



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301 **Figure 2:** Average bacterial enumeration data (± SD) for the three urban water types; Hooghly River
302 (blue), canals (grey) and East Kolkata Wetlands (green): a) *E. coli* and coliform enumeration data
303 ($_{log}$cfu/100mL); b) total bacteria cells ($_{log}$cells/mL). Data shown is the parameter average for each water
304 type with error bars representing ± 1 standard deviation: six field sites on the Hooghly River; four canal
305 water field sites; and two field sites in the East Kolkata Wetlands (EKW).

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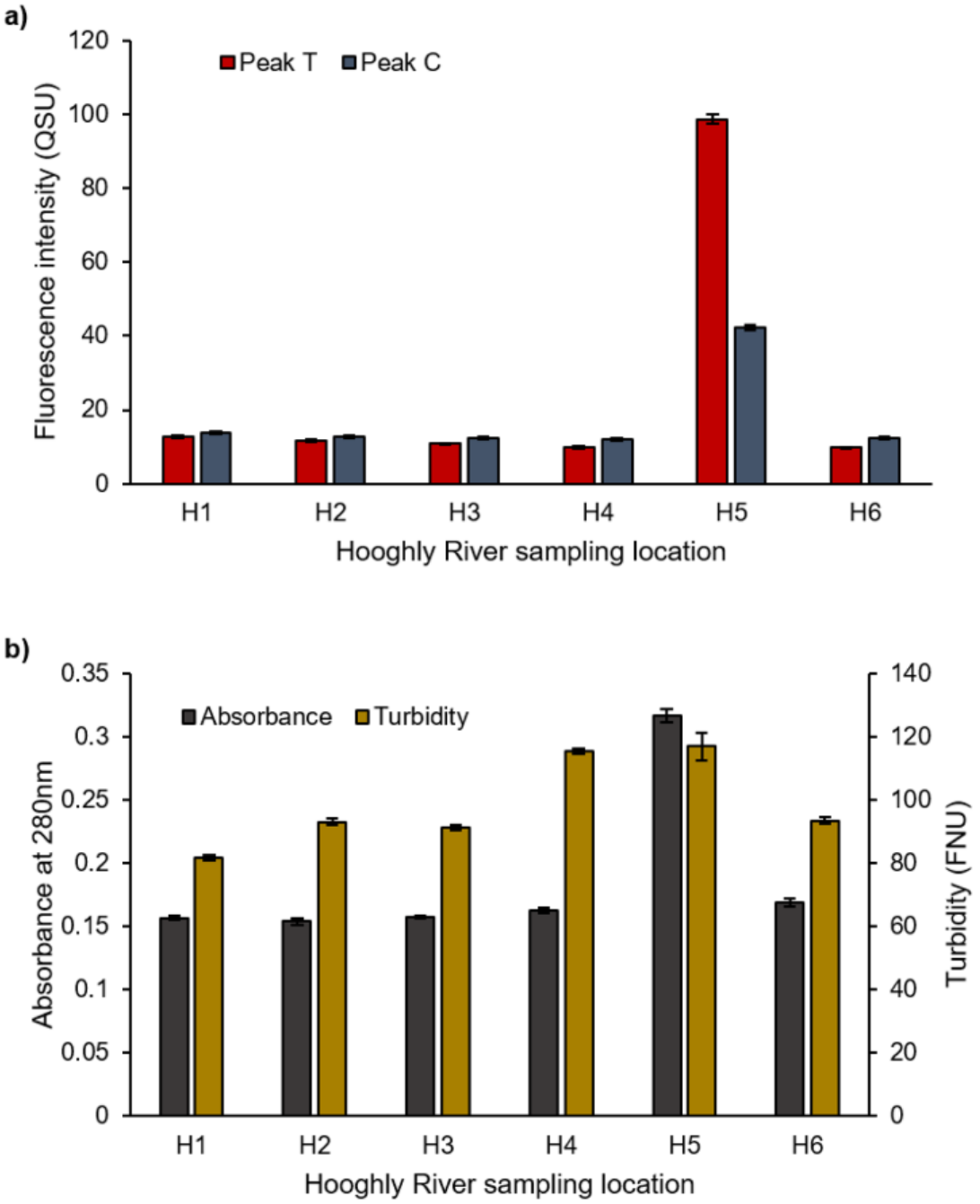
A moderate significant correlation, determined using linear regression, was identified between Peak T fluorescence intensity and the total bacteria cell count across all samples (R^2 = 0.51, $p < 0.01$). No significant correlations were identified between Peak T and *E. coli* or coliform enumeration ($p > 0.05$) for all water types.

3.2 The identification of a contamination event

The VLux TPro sensor was deployed in the Hooghly River to assess the ability of the sensor to identify bacterial pollution events in a complex surface water. Figure 3 shows the VLux TPro data from a boat study which was conducted across six sampling sites, on a 3 km stretch of the Hooghly River, over a two-hour monitoring period; the study site map is shown in the section 2.2 (Figure 1).



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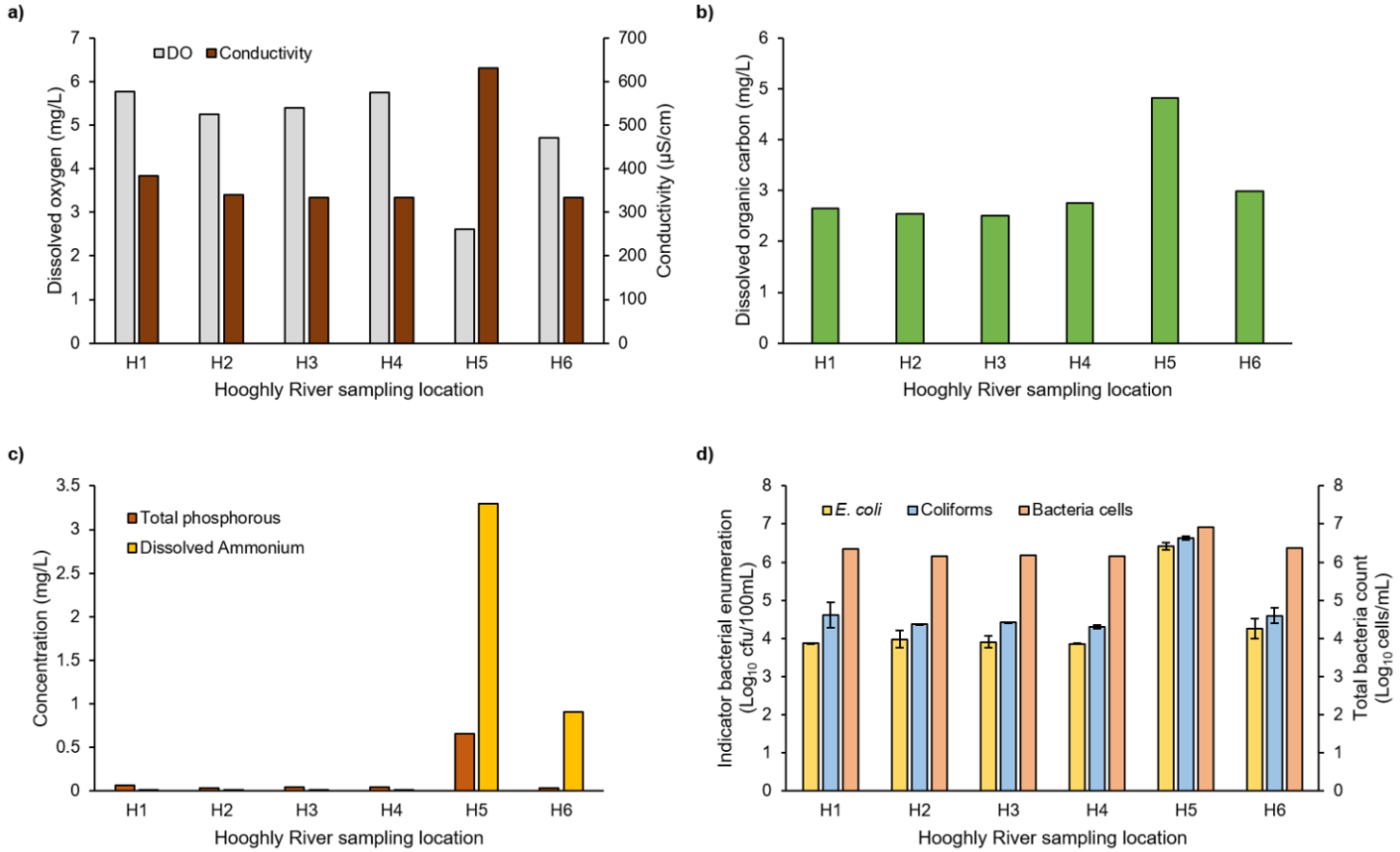
320 **Figure 3:** VLux TPro fluorescence data for the Hooghly River boat sampling survey, demonstrating a
321 biological contamination event (from an urban drain) at sampling location H5: a) Peak T fluorescence
322 intensity (QSU); b) Peak C fluorescence (QSU). Data is averaged over a minute of sampling (n = 60),
323 error bars represent ± 1 standard deviation.

324

From this data, a contamination event can clearly be identified at sampling location H5, where there was a visually fast-flowing wastewater drain (almost 4 m wide) approximately 50 m upstream of the sampling point. This contamination event at site H5 is easily seen in the VLux TPro Peak T data (Figure 3a), which shows almost an order of magnitude increase (98.67 ±1.25 QSU) in comparison to upstream sampling sites H1-H4 (9.77-12.71 QSU). This is also reflected, to a lesser extent, in the Peak C data which demonstrates almost a fourfold increase in intensity from 11.98-13.85 QSU upstream to 42.35 ±0.78 QSU at H5. The VLux TPro absorbance data also reflects the contamination event, demonstrating a doubling from the upstream sampling locations (Figure 3b). However, this event is not clearly identified by the turbidity data, with comparable elevated turbidity at sites H4 and H5 (Figure 3b).

Figure 4 shows physicochemical, chemical and microbiological data for the samples collected from the Hooghly River boat survey. The contamination event at sampling location H5 is identifiable from the physicochemical and chemical data at H5 compared to the upstream sampling locations (H1-H4): increases, by two orders of magnitude, in total phosphorus (from 0.031-0.062 mg/L upstream to 0.656 mg/L at H5) and dissolved ammonium concentration (from 0.008-0.015 mg/L upstream to 3.3 mg/L at H5); DOC almost doubles from 2.51-2.76 mg/L upstream to 4.83 mg/L at H5; the DO concentration halves from 5.26-5.78 mg/L upstream to 2.61 mg/L at H5; conductivity doubles from 346-389 µS/cm upstream to 664 µS/cm at H5; at least a two-log increase in bacterial enumeration is seen from 4.63×10^3 - 2.79×10^4 *E. coli* Log_{10} cfu/100 mL upstream to 3.07×10^6 *E. coli* Log_{10} cfu/100 mL, and 4.24×10^4 - 4.35×10^5 coliforms (cfu/mL) upstream to 1.15×10^7 coliforms (Log_{10} cfu/100 mL) at H5; and an increase in total bacteria cell counts is also seen at H5. This is indicative of pollution from a raw sewage source.



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349 **Figure 4:** Hooghly River data demonstrating a biological contamination event (from an urban drain) at H5: a) Concentration of total phosphorus (mg/L) and
350 dissolved ammonium (mg/L); b) Dissolved organic carbon (DOC) concentration (mg/L); c) Field conductivity (µS/cm) and dissolved oxygen (mg/L) data; d)
351 bacterial enumeration data for *E. coli* and coliforms (Log_{10} cfu/100mL), replicated in triplicate (n = 3) with error bars representing ± 1 standard deviation, and
352 total bacteria cell counts (Log_{10} cells/mL).

When analysing the data for the Hooghly River only, strong significant correlations (R^2 = >0.85, $p < 0.01$) were seen between Peak T fluorescence intensity and many of the physicochemical and chemical parameters measured (Figures 3 and 4). Total phosphorus and soluble reactive phosphorus demonstrated very significant strong correlations with Peak T ($R^2 = 0.999$, $p < 0.0001$), as well as dissolved ammonium also demonstrating a strong positive correlation ($R^2 = 0.92$, $p < 0.001$), as shown by Figure 3a. DOC concentrations (Figure 4b) also correlated strongly with Peak T fluorescence ($R^2 = 0.94$, $p < 0.001$), with a weaker linear relationship between Peak C and DOC ($R^2 = 0.90$, $p < 0.01$). DO and conductivity also demonstrate strong significant linear correlations with Peak T fluorescence, $R^2 = 0.89$ ($p < 0.01$) and $R^2 = 0.98$ ($p < 0.0001$) respectively. Strong significant correlations between Peak T and bacterial enumeration data, *E. coli*, coliforms and total bacteria cell counts, are seen for the Hooghly River samples; $R^2 = 0.999$, $p < 0.0001$, $R^2 = 0.999$, $p < 0.0001$ and $R^2 = 0.91$, $p < 0.001$ respectively.

A more limited study was also undertaken in the post-monsoon season (December 2019); field locations shown in Supplementary Figure 1 and detailed in Supplementary Table 2, with summary data presented in Supplementary Table 3. Initial observations show little differences in the sensor data, with some seasonal variation in the physicochemical parameters. Further work to map this over space and time, and to further assess the potential application of Peak T as a novel water quality parameter, is needed.

4 Discussion

Single channel *in situ* fluorescence sensors have been used in freshwater systems to assess the quality of urban surface freshwaters (Khamis et al., 2017; Mendoza et al., 2020). However, these studies have been limited by optical interferences and the inability to collect quantitative fluorescence data, related to unit standardisation. This study presents the first freshwater field deployment of a multichannel *in situ* fluorescence sensor which provides internally corrected fluorescence data in standard units (QSU). The sensor deployment occurred as part of a sampling survey, conducted in March 2019, of different urban waters within Kolkata, India. By monitoring physicochemical, chemical and biological water quality parameters alongside the VLux TPro sensor, this study has been able to obtain a truly quantitative Peak T data set. This data sets provides the ability to evaluate the relationship of *in situ* Peak T data with a suite of field and laboratory water quality parameters, and assess the benefit of using fluorescence within water quality and water management.

4.1 Water quality of the Hooghly River within Kolkata city

The Hooghly River at Kolkata is classified by the Central Pollution Control Board of India as Class D (heavily polluted), although it's designated as Class B (slight pollution); where waters are designated for use for organised outdoor bathing (Central Pollution Control Board, 2013; National River Conservation Directorate, 2009). The pH and DO data for the Hooghly River in this study, shown in Table 1, demonstrates compliance with the water quality standards for Class B waters, where the pH must be between 6.5-8.5 and the DO >5 mg/L waters (Central Pollution Control Board, 2013; National River Conservation Directorate, 2009), which aligns with others studies (National River Conservation Directorate, 2009) and the post-monsoon survey conducted in December 2019 (Supplementary Table 3). However, the Hooghly River in Kolkata fails to meet the Class B criteria for Biochemical Oxygen Demand (BOD), <3 mg/L, and total coliforms, <500 most probable number (MPN)/100 mL (National River Conservation

Directorate, 2009). This failure was also seen in the bacterial enumeration in this study where the total coliforms (combined *E. coli* and coliform counts) are more than two-log higher (Figure 2) than permitted maximum enumeration (2500 MPN/100mL), but in agreement with other monitoring data for this stretch of the Hooghly River (Central Pollution Control Board, 2013). It is these biological failures that cause the water quality status of the Hooghly River in Kolkata to be classified as Class D (Table 1) (National River Conservation Directorate, 2009). Conversely, the biological water quality status of this section of the river is classified as Class C (moderately polluted) (Bio-Science Division and Central Pollution Control Board, 2018; Central Pollution Control Board, 2017). This is based on the saprobic and diversity scores of macro-invertebrate benthic fauna (Bio-Science Division and Central Pollution Control Board, 2018).

4.2 Peak T: a new water quality parameter

Many physicochemical and chemical water quality parameters can now be monitored *in situ* using real-time sensors and report data remotely via telemetry systems. At present, both microbiological and biological water quality monitoring requires time consuming discrete sampling surveys, yet it is evident from the data in this study, and others (Central Pollution Control Board, 2013; National River Conservation Directorate, 2009), that biological water quality is essential for determining the aquatic ecosystem health and long-term effects of pollutants (Bio-Science Division and Central Pollution Control Board, 2018; Central Pollution Control Board, 2017). The use of Peak T fluorescence has, until now, been hindered by technological issues, such as how to account for optical interferences (Khamis et al., 2017; Mendoza et al., 2020), as well as understanding what water quality criteria Peak T relates to. With technological advances and improved scientific knowledge, the use of *in situ* Peak T fluorescence could provide information regarding biological activity and underpinning system health.

Previously, Peak T has been correlated with faecal indicator species, such as *E. coli*, leading to the notion that Peak T fluorescence could act as a proxy for bacterial indicator species counts (Carstea et al., 2020; J.P.R. Sorensen et al., 2018; James P.R. Sorensen et al., 2018; Sorensen et al., 2016, 2015a). Significant correlations between Peak T fluorescence intensity and *E. coli* or coliform enumeration were not identified across all water types in this study. However, a significant correlation was seen between Peak T and total bacteria cell count (R^2 = 0.51, $p < 0.01$). This indicates that Peak T fluorescence, a ubiquitous fluorescence signature across multiple bacterial species (Elliott et al., 2006; Fox et al., 2017; Goto et al., 2020), could be used to provide information regarding the overall microbial community activity and metabolism, rather than being species specific, particularly in varying and complex water matrices (Fox et al., 2019, 2017; Goto et al., 2017; Lønborg et al., 2009; Paerl et al., 2003; Romera-Castillo et al., 2011). When analysing the Hooghly River data only, very strong significant correlations between Peak T and the microbiological data are identified (R^2 = >0.91, $p < 0.001$). This suggests that grouping water types and understanding system baseline values and fluctuations can enhance the benefit of Peak T fluorescence for water quality monitoring, but that there is still value in this parameter without having this prior knowledge.

Peak T fluorescence is again seen to show few significant correlations with the chemical data across all samples, with DOC demonstrating the only strong correlation with Peak T (R^2 = 0.74, $p < 0.001$). Similar relationships between DOC and Peak T fluorescence have been reported previously, with Peak T being associated with the labile DOC fraction (Carstea et al., 2020; Coble et al., 1993; Judd et al., 2006; Yang et al., 2014). Surprisingly, no significant correlation is identified between Peak T and DO, a physicochemical water quality parameter that has long been associated with Peak T due to the use of DO, and the biochemical oxygen demand (BOD), to infer biological activity levels (Ahmad and Reynolds, 1999; Baker and Inverarity, 2004; Hudson et al., 2008; Khamis et al., 2021; Reynolds and Ahmad, 1997; Yang et al., 2014). No significant correlation between Peak T and turbidity is observed. Previous research has reported the use of turbidity measurements as an indicator of bacterial

contamination (Jung et al., 2014). However the presence of high sediment and pollution loading within complex surface waters (Allen et al., 2008), such as the Hooghly River, means that turbidity measurements cannot be used as a reliable indicator of bacterial contamination. Peak T data for the Hooghly River does exhibit a strong significant correlation ($R^2 = 0.85$, $p < 0.01$) with the total number of bacterial cells (determined by flow cytometry). This suggests that Peak T provides a potential insight into microbial activity within freshwater ecosystems, encompassing many frequently measured parameters and, therefore, providing a clear benefit for using this *in situ* and real-time parameter for high spatio-temporal resolution water quality monitoring.

Comparing Peak T to standard water chemistry can be useful for exploring the relationship of Peak T fluorescence with other water quality parameters. However, this study demonstrates that Peak T fluorescence data is best used independently to other water quality parameters as it can provide information that directly relates to the microbiological activity within the aquatic system under investigation. At present such information cannot be provided by current monitoring parameters. None of the physicochemical or biological data collected as part of time constrained discrete sampling surveys provide insight into the daily real-time activity of the aquatic microbial community. It is also clear from the data here that Peak T fluorescence can be utilised to identify contamination events or microbial activity in complex water matrices. As such, deploying the VLux TPro sensor could greatly improve the monitoring of biological contamination hotspots and point-source pollution from sewage inputs into river systems. Through improved resolution of water quality measurements through time and space, both quality and resources can be better managed.

4.3 Using Peak T fluorescence *in situ* to identify contamination events

This study uses *in situ* Peak T fluorescence to identify pollution events in the Hooghly River, within the densely populated urban area of Kolkata. A pollution event is clearly identified within

477 the quantitative Peak T data, obtained from the VLux TPro sensor, as shown by Figure 3a.
478 The contamination event at sampling site H5 is easily identified from the background Peak T
479 fluorescence, without the need for prior knowledge of the baseline and diurnal or seasonal
480 variations. This contamination event is also identified within the other water quality parameters
481 monitored here (Figures 3 and 4), obtained during the field survey or from laboratory analysis
482 of collected water samples. This data suggests the pollution is likely to be derived from a raw
483 sewage source. Whilst many of the physicochemical and chemical water quality parameters
484 measured can be monitored *in situ,* a suite of sensors would be required to obtain information
485 which can allow the diagnosis of contamination. This can be extremely costly, hindering the
486 uptake of such multiparameter *in situ* monitoring and limiting the spatio-temporal resolution of
487 the data collected. Ultimately, this reduces the identification of contamination events and
488 prevents improved monitoring and management of water quality. *In situ* Peak T fluorescence
489 provides a tool for us to monitor and identify contamination hotspots more readily than current
490 practices, and could provide an insight into ecosystem health, via microbial activity, which
491 would otherwise only be available by adopting a multiparameter approach.

492 Although further work is required to determine the true implications of this, such a water quality
493 parameter could be of great benefit for water quality management. This highlights the benefit
494 of *in situ* real-time Peak T fluorescence for the identification of a range of pollution sources
495 and events.

496

497 **4.4 Potential application of the VLux TPro sensor and future work**

498 By understanding the signatures of different water types monitored in this study (Table 1), the
499 contamination event at sampling location H5 can be identified as wastewater contamination
500 (Figures 3 and 4). This highlights the benefit of detailed and in-depth analysis for monitoring
501 contamination events. However, such a comprehensive data set is time consuming, expensive
502 and difficult to obtain with good spatio-temporal resolution. The transient nature of the

503 contamination event demonstrates the difficulty in monitoring such large systems via discrete
504 sampling only. To improve the identification of such contamination events, the VLux TPro
505 could be deployed on the 57 existing water quality monitoring stations on the Ganges/Hooghly
506 system, 10 of which are within the state of West Bengal and 2 are within Kolkata (Central
507 Pollution Control Board, 2013). Alternatively, these robust sensors could be deployed on
508 shipping or passenger boats. This would greatly improve spatio-temporal resolution of water
509 quality monitoring and further explore locations of frequent contamination.

510 Further exploratory work is required to assess the application of the VLux TPro, and to assess
511 the ability of this novel fluorimeter to perform, as reported here, in different systems and to
512 assess the impact of seasonality on sensor application. A limited study using the VLux TPro
513 was also undertaken within Kolkata in the post-monsoon season (December 2019). Initial
514 observations show little differences in the sensor data, with some seasonal variation in the
515 physicochemical parameters. However, further deployments to assess both spatial and
516 temporal variations are required to provide a more detailed understanding of the application
517 of Peak T as a novel water quality parameter.

518 In addition, fluorescence characterisation of different industrial effluents, which are known to
519 add to the pollution of the Hooghly River, should be assessed to provide added benefit to the
520 inclusion of such multi-channel fluorimeters into regular water quality monitoring. This would
521 aid tracing of pollution events and origins within freshwater systems, enabling more effective
522 management of pollution sources.

523 The data derived from the EKW waters demonstrates effective nutrient sequestration within
524 the wetland system, when compared to the canal waters which feed this wetland system
525 (Table 1). Interpreting the EKW data is limited by the number of sample sites of ‘clean’ waters
526 in this system. Further work on wetland systems, and the relationship of Peak T and
527 chlorophyll-α with algal populations, is needed to fully explore the potential of the VLux TPro
528 sensor for monitoring eutrophic waters. However, monitoring the EKW system does allow us

to understand how we may be able to identify highly eutrophic inputs to the main river system, perhaps from tributaries or eutrophication events related to land use practices.

5 Conclusions

- This study provides the first *in situ* fluorescence case study of its kind using a sensor with in-built corrections for turbidity and absorbance, reporting fluorescence data quantitatively in standardised units, QSU. These technological advances allow Peak T fluorescence data to be collected and compare a range of urban waters systems.
- Peak T fluorescence provides *in situ* information regarding biological water quality, biological contamination events and microbial activity, something previously limited to time consuming and expensive discrete sampling surveys.
- Peak T fluorescence can identify wastewater contamination in real-time, within a polluted and complex urban surface water matrix.

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Data availability

The data required to reproduce the above findings are available to download from https://doi.org/10.5285/9bc3dce7-7c2b-49dd-9b76-819267d7a352 (Fox et al., 2022).

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