Calibration of an *in-situ* fluorescence based sensor platform for reliable BOD$_5$ measurement in wastewater

K. Khamis, C. Bradley, H. J. Gunter, G. Basevi, R. Stevens and D. M. Hannah

ABSTRACT

Reliance on Biochemical Oxygen Demand (BOD$_5$) as an indicator of wastewater quality has hindered development of efficient process control due to the associated uncertainty and lag-times. Surrogate measurements have been proposed, with fluorescence spectroscopy a promising technique. Yet, assessment of *in-situ* fluorescence sensors across multiple Wastewater Treatment Plants (WwTPs), and at different treatment stages, is limited. In this study a multi-parameter sonde (two fluorescence peaks, turbidity, temperature and electrical conductivity) was used to provide a BOD$_5$ surrogate measurement. The sonde was deployed at three WwTPs, on post primary settlement tanks (PST) and final effluent (FE). Triplicate laboratory measurements of BOD$_5$, from independent laboratories were used to calibrate the sensor, with high variability apparent for FE samples. Site and process specific sensor calibrations yielded the best results (R$_{cv}^2$ = 0.76–0.86; 10-fold cross-validation) and mean BOD$_5$ of the three laboratory measurements improved FE calibration. When combining PST sites a reasonable calibration was still achieved (R$_{cv}^2$ = 0.67) suggesting transfer of sensors between WwTPs may be possible. This study highlights the potential to use on-line optical sensors as robust BOD$_5$ surrogates in WwTPs. However, careful calibration (i.e. replicated BOD$_5$ measurements) is required for FE as laboratory measurements can be associated with high uncertainty.

Key words | final effluent, high frequency monitoring, humic-like fluorescence, post primary settlement, sensor calibration, tryptophan-like fluorescence, turbidity

K. Khamis (corresponding author)
C. Bradley
D. M. Hannah
School of Geography Earth and Environmental Science,
University of Birmingham,
Birmingham B15 2TT,
UK
E-mail: *k.khamis@bham.ac.uk*

H. J. Gunter
G. Basevi
R. Stevens
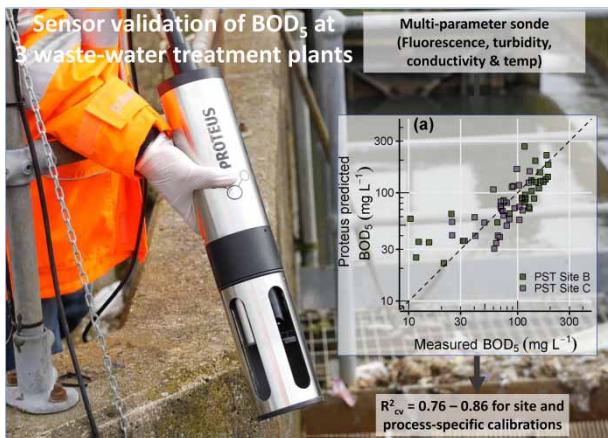RS Hydro Ltd,
Leask House, Hanbury Road, Stoke Prior,
Worcestershire B60 4JZ,
UK

HIGHLIGHTS

- A sensor calibration procedure for BOD$_5$ measurement was assessed at three wastewater treatment plants (WwTPs).
- Calibration for final effluent required replicated BOD$_5$ sampling to reduce uncertainty.
- Calibration for post settlement was robust and suggested sensor transfer between WwTPs is possible.
- Robust calibration for *in-situ* BOD$_5$ monitoring can improve process control and reduce pollution events.

GRAPHICAL ABSTRACT

INTRODUCTION

Each year, over 300 km^3 of wastewater is produced globally, of which ~60% is treated at wastewater treatment plants (WwTPs; Wu *et al.* 2019). The flow through WwTPs is typically characterised by high concentrations of organic matter (OM), but organic load and composition vary considerably across compressed spatiotemporal scales (Niku & Schroeder 1981; Ebrahimi *et al.* 2017). The spatial variability component is relatively predictable (i.e. is a function of treatment stage), while variability in time is generally associated with daily and seasonal trends in water use but can also respond to periodic additional inputs from stormwater and drainage inflow (Bridgeman *et al.* 2013). These variations in OM composition and flow volume can complicate wastewater treatment processes, and present difficulties in ensuring regulatory compliance. Some of these problems are scale dependent, for example diurnal variations in wastewater quality in small treatment plants can affect their ability to remove nitrogen (Raboni *et al.* 2013). However, the quality of influent (and effluent) wastewater can also vary considerably depending upon the water source, which complicates the treatment process (e.g. Imai *et al.* 2002). These problems present ongoing challenges, particularly in terms of how to improve water management systems, reduce nutrient fluxes, and advance sustainable solutions to increase the reuse of treated wastewater, particularly if there are high concentrations of recalcitrant OM.

Improvements in wastewater treatment and optimisation of the treatment process requires real-time information on water quality. There have been a range of potential monitoring solutions proposed, including biosensors, microbial cells, bioreactors and optical sensors (Bourgeois *et al.* 2001; Jouanneau *et al.* 2014). While improvements in bioreactors have been apparent over the last decade (e.g. BIOX-1010) the maintenance requirements are still significant and measurement frequency is often hours rather than minutes (Yu *et al.* 2013). Optical sensors can be used to provide surrogates of OM quality and quantity (Ulliman *et al.* 2020) in aerobic and anaerobic wastewater treatment processes (Mesquita *et al.* 2017). Commercial UV-VIS absorbance sensors (e.g. Spectrolyser [s::can GmbH], Opus [Trios GmbH]) have been used successfully in wastewater treatment plants for a number of years reducing the need for manual sampling and laboratory analyses (Chow *et al.* 2018). However, individual installations may require site-specific calibrations and complications from matrix effects have been observed (Rieger *et al.* 2004).

Recently, there has been considerable interest in wastewater applications of fluorescence spectroscopy as the technology yields immediate results, is relatively inexpensive, reagentless and potentially has a lower minimum detection limit than absorbance (Henderson *et al.* 2009; Carstea *et al.* 2016). There have been examples of successful wastewater applications using laboratory fluorescence spectroscopy based on excitation-emission matrix spectroscopy. These have included characterisation of removed OM fractions through derivatives of synchronous fluorescence spectroscopy (Yu *et al.* 2013) and size exclusion chromatography (Ignatev & Tuhkanen 2019), the characterisation of

Uncorrected Proof

3   K. Khamis *et al.* | Calibration of an in-situ sensor for wastewater monitoring   **Water Science & Technology** | in press | 2021

effluent degradable or non-degradable derived OM (Choi *et al.* 2017; Ulliman *et al.* 2020) and prediction of soluble organic load (Goffin *et al.* 2018). However, field deployable or online applications remain limited despite the potential to be used as a proxy for standard water quality assessment metrics (Carstea *et al.* 2020).

There is particular interest in reliable online surrogates for Biochemical Oxygen Demand (BOD$_5$; Reynolds & Ahmad 1997; Hudson *et al.* 2008) a bioassay test widely used in the water industry to quantify the concentration of biodegradable organic compounds. While *in-situ* fluorescence monitoring has the potential to address the need for real-time BOD$_5$ data (Carstea *et al.* 2020), their applications in WwTPs remains challenging and has been limited to date. For example, bio-fouling is problematic for wastewater sensors in general requiring ongoing maintenance to ensure data quality (Brito *et al.* 2014; Carstea *et al.* 2018). Moreover, short-term variations in turbidity, OM and sediment composition may require the development of site-specific algorithms with further complications from matrix interference (Khamis *et al.* 2015; Khamis *et al.* 2017). There is the additional requirement (for BOD$_5$ estimates) to calibrate sensor output against a parameter (BOD$_5$) which is itself subject to considerable error (Bridgeman *et al.* 2013), thus violating the assumption that reference measurements are free of bias (Rieger *et al.* 2005). Furthermore, the potential errors associated with transferring calibration procedures and coefficients (in this case BOD$_5$) to different locations within a treatment works (e.g. post primary settlement to final effluent), and between treatment works, is also uncertain.

Given the above, there is clearly a need to assess rigorously the viability of commercially available multi-wavelength fluorescence sensors for *in-situ* BOD applications. Particularly if the need for time consuming and expensive laboratory determinations of BOD$_5$, which also have significant associated error, can be reduced (Bourgeois *et al.* 2001). There are, however, several key research questions that constrain applications of *in-situ* fluorescence spectroscopy for monitoring organic matter in WwTPs. In particular how does uncertainty in laboratory BOD$_5$ estimation impacts on optical sensor calibration (i.e. spatial variability)? How transferable sensor calibrations are between WwTPs of differing size and treatment process? Can modular monitoring platforms with additional measurement sensors (e.g. temperature, turbidity, etc.) account for confounding matrix effects such as turbidity?

In this article we investigate these research questions by (i) quantifying the uncertainty in laboratory determinations of BOD$_5$ for final effluent and settled sewage from three WwTPs; (ii) creating robust calibration(s) for an *in-situ* BOD sensor system and assessing the transferability of the calibration(s) between WwTPs; (iii) Optimising the calibration procedure (i.e. no. of samples/sampling occasions); and (iv) Assessing the potential to use the sensor system for treatment process control (i.e. Activated Sludge Process), and as an online warning system of pollution (i.e. threshold exceedance for final effluent).

METHODS

Site description

In-situ monitoring and sampling were conducted at three WwTPs in the West Midlands, UK (~52.3°N, 1.9°W), between March 2019 and January 2020. The WwTPs have been anonymised and will be referred to herein as Sites A, B and C. herein. Site A (WwTP A) was the smallest of the three sites (p.e. = 360) and primarily received household waste mixed with surface runoff. Site B (WwTP B) received sewage from a catchment with a population equivalent (p.e) of ~25,250. Flow into the site was derived from a variety of sources, including household waste, surface runoff and industrial waste. At site B the treatment pipe-line consisted of primary settlement, followed by trickling filters, humus tanks and occasionally sand filtration before the effluent was discharged into the local watercourse. Treatment after screening consisted of twin rotating biological contactors before discharge into a local river. Site C (WwTP C) was a large works (p.e = 93,500) which received water from a variety of sources, including tankered waste from other works. After screening and primary settlement the flow was split to either a treatment track consisting of trickle bed-humus tanks-sand filter or activated sludge treatment- sand filter. No disinfection (e.g. chlorination) occurred before any of the final effluent monitoring locations. Discharge consents for the sites (i.e. flow and quality) are outlined in Table 1.

Sensors and sampling

A multiparameter sonde configured for BOD measurement was used for all *in-situ* monitoring (*Proteus*, *Proteus Instruments*, Worcestershire, UK). The *Proteus* had five sensing elements comprising: (1) a fluorometer for measuring tryptophan-like fluorescence (excitation wavelength [λ_{ex}] ~280 nm, emission wavelength [λ_{em}] ~350 nm), (2) a fluorometer for measuring humic-like fluorescence, herein

Uncorrected Proof

4 | K. Khamis *et al.* | Calibration of an in-situ sensor for wastewater monitoring | **Water Science & Technology** | in press | 2021

Table 1 | Information on treatment works size, flow and discharge permit

Variable	Site A	Site B		Site C	
Population equivalent	360	25,050		93,500	
Dry weather flow (m³ d⁻¹)	75	5,797		27500	
BOD$_5$ permit (mg L⁻¹)	25	25 (60)		15 (50)	
Mean daily Flow (L s⁻¹)	2.04 (1.08–4.99)	101 (61.7–169.1)		446 (299–629.4)	
Location	FE	FE	PST	FE	PST
Mean BOD$_5$ (mg L⁻¹)	7.9 ± 2.5 (14.5–4.5)	13.2 ± 4.0 (20–6)	109.2 ± 60.0 (191.7–10.3)	5.7 ± 3.8 (15–1.8)	77.2 ± 28.9 (129.5–25)
Mean turbidity (FNU)	8.9 ± 11.5 (44.5–1.8)	63.6 ± 45.3 (175.4–25)	84.7 ± 39.8 (160.3–20.8)	34.7 ± 20.0 (96.7–6.8)	80.9 ± 29.4 (135.7–35)
Mean TRYP$_{20}$ (ppb)	80 ± 13.7 (94.5–36.5)	66.8 ± 26.3 (96.1–26.6)	114.7 ± 91.1 (308.8–30.5)	46.2 ± 14.7 (64.6–14.6)	61.8 ± 21.9 (101.3–28.8)
Mean fDOM$_{20}$ (ppb)	186.4 ± 20.5 (205.5–118.4)	134.9 ± 24.3 (166.1–95.7)	123.8 ± 23.5 (172–78.4)	109.7 ± 17.4 (132.1–54.4)	103.5 ± 23.3 (152.7–62.4)
Mean EC (µS cm⁻¹),	794.8 ± 117.2 (852.8–410.1)	974.5 ± 274 (1344–631.4)	1150.8 ± 93.7 (1,353–1,015)	768.1 ± 45.7 (850.9–648)	797.3 ± 187.6 (1,018–395.2)
Mean Tw (°C)	12 ± 0.7 (13–10.5)	20.5 ± 0.9 (22–19.1)	17.2 ± 3.1 (19.9–12.3)	13.8 ± 1.7 (15.8–11.3)	15.9 ± 0.4 (16.7–15.1)

The mean ± SD (max – min) recorded for measured water quality parameters, including BOD$_5$, are displayed for each monitoring location. Specifically, mean laboratory measured BOD (mg L⁻¹), mean daily flow recorded at each site during the monitoring period (L s⁻¹). Turbidity (FNU), TRYP$_{20}$ = Temperature corrected tryptophan like fluorescence, (ppb), fDOM$_{20}$ = temperature corrected humic-like fluorescence (ppb), EC = electrical conductivity (µS cm⁻¹), Tw = Water temperature (°C). The BOD$_5$ permit (mg L⁻¹) represents the concentration permitted during dry weather conditions, the stormflow permit is displayed in parentheses (note this does not change for site A). The maximum permitted dry weather flow is also provided for each site (m³ d⁻¹), FE = final effluent, PST = post primary settlement tanks.

referred to as fDOM, (λ_{ex} ~325 nm, λ_{em} ~470 nm) (3) an electrical conductivity sensor, (4) a turbidity sensor, (λ 850 nm), and, (5) a thermistor for temperature measurement (temperature was only used to correct fluorescence measurements). Prior to installation at each site, sensors were cleaned using mild detergent and ultrapure water before calibration following manufacturer specifications. Further details of optical sensor properties (including linear range and detection limits) are outlined in Table S1 (supplementary material).

Sites B and C had suitable deployment and sampling locations after the Primary Settlement Tanks (PSTs) and Final Effluent (FE) with monitoring only possible for the FE at Site A. At each location the sensor was attached to metal railings using a stainless steel chain and all sensing elements were submerged to a minimum depth of 0.3 m at a location that was well mixed with a representative flow velocity. An auto sampler (Teledyne ISCO 6712, Nebraska, USA for the first three sampling events, and Teledyne ISCO 3700 thereafter) was used to collect discrete water samples from the PST or FE. The inlet tube was situated in the flow at the same depth as the sensors and a coarse strainer was fitted to ensure no blockage of the tubing occurred during sample collection. Measurements were taken using the *Proteus* every 5 minutes and the information was relayed to a remote server through a custom telemetry setup.

The auto sampler was synchronised with the *Proteus* (internal clocks cross-referenced on each deployment) and programmed to start sampling at 17:00 (UTC) on the sampling day, and to continue at two hourly intervals until 09:00 the following morning, giving a total of 24 samples (3 × 1 L sample bottles for each sample time). The auto sampler was emptied at ~09:00 on the day the auto sampler program finished. Additional samples were obtained manually when technical staff were on site. Samples were kept on ice and sent to three independent laboratories (10 samples per laboratory) where BOD$_5$ was determined according to a standard methodology (Young *et al.* 1981). Full chain of custody was ensured with samples temperature controlled during transport and BOD$_5$ analysis initiated within 24 h. The sampling process was repeated three times per sampling site to capture three examples of the diurnal demand cycle (Table S2, supplementary material). The choice to use three independent laboratories for BOD$_5$ quantification enabled us to decrease the influence of random lab errors on the calibration model, which was particularly important as a quality reference dataset is central to robust sensor calibration (Rieger *et al.* 2006).

Uncorrected Proof

5   K. Khamis *et al.* │ Calibration of an in-situ sensor for wastewater monitoring   **Water Science & Technology** │ **in press** │ **2021**

Statistical analysis

The raw tryptophan-like fluorescence (TRYP) and fDOM signals were temperature corrected using an equation outlined in Khamis *et al.* (2017):

$$F_{ref} = \frac{F_{mes}}{1 + \rho(T_{mes} - T_{ref})} \tag{1}$$

where F is the fluorescence signal (i.e. TRYP or fDOM) T is temperature (°C) and subscripts *mes* and *ref* represent the measured and reference values respectively. Following previous studies a reference temperature of 20 °C was chosen, thus $T_{ref} = 20$ °C and F_{ref} is the fluorescence signal at 20 °C. The temperature compensation factors (ρ) were chosen based on Downing *et al.* (2012) and Khamis *et al.* (2015).

The laboratory data were screened for outliers using graphical tools (Zuur *et al.* 2010) and methods outlined by Rieger *et al.* (2005). In total 36 samples (8% of total) were removed due to significant variation from the mean (i.e. 20% following Lepot *et al.* (2013)) or trending in the opposite direction to other samples (see Table S2 for more information). To assess the accuracy of the BOD measurements, readings from individual laboratories (Lab A, B and C) were compared to the mean. A suite of goodness of fit metrics were calculated using the *HydroGoF* package (Zambrano-Bigiarini 2014) for each site and location, including:

1. Pearson's correlation coefficient (r)
2. Square of the correlation coefficient (coefficient of determination; R^2)
3. Root mean square error (RMSE)

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(\hat{x}_i - x_i)^2}{n}} \tag{2}$$

4. The coefficient of variation (CV %)

$$CV\% = 100 \times \frac{RMSE}{\bar{x}} \tag{3}$$

5. The slope of the Ordinary Least Squares regression fit (β).

For Equations (2) and (3), n is the number of samples in the calibration set, x is the laboratory measured BOD$_5$ concentration (mg L^{-1}), $\hat{x}$ is the calibrated *Proteus* BOD$_5$ equivalent (mg L^{-1}), and $\bar{x}$ is the mean value of all laboratory BOD$_5$ measurements in the given calibration dataset.

The mean of the results from the laboratories were combined with the raw sensor readings from the Proteus (TRYP$_{20}$, fDOM$_{20}$, turbidity, and EC) for the corresponding time interval. For a specific monitoring location or application (e.g. wastewater PST or FE, river water) the manufacturer provides a BOD$_5$ 'standard calibration' for the sonde based on a patented algorithm depending upon the combination of individual sensors used. A site-specific calibration is advised to improve the accuracy. This involves the collection of discrete (parallel) samples across the likely concentration range and composition variance (diel demand cycle) which are subsequently analysed in the lab for the parameter of interest (in this case BOD$_5$).

Using the collected laboratory data a model selection process was undertaken to optimise the *in-situ* parameter set (i.e. *Proteus* sensor combination) for BOD$_5$ estimation. Given that monitoring occurred across multiple treatment and process stages, models were developed based on all the data and specified subsets. This included: (i) a model for all treatment works and process stages combined ($n = 1$), (ii) Models that were process stage specific but combined over different sites ($n = 2$, PST or FE) and, (iii) site and process stages specific models ($n = 8$). In addition we explored the improvement in calibration based on the mean of the three labs rather than BOD$_5$ results from a single lab ($n = 11 \times 4$). The starting model was based on the manufacturers' patent protected algorithm:

$$\text{Pro}_{BOD} = f(\text{TRYP}_{20}) + f(\text{fDOM}_{20}) + f(\text{TRUB}) + f(\text{EC}) \tag{4}$$

However, to avoid overfitting the models to the specific data set we opted for a simple linear regression approach with no higher order polynomials or interactions between variables (Kuhn & Johnson 2013). Prior to model selection and validation all predictor variables were normalised using the Box-Cox transform and scaled (i.e. converted to z-scores). The optimal model for each scenario outlined above was identified using linear regression with backwards stepwise selection (AICc – Akaike Information Criterion modified for small sample sizes) and variable importance was calculated. Due to the relatively small sample size model performance was not assessed based on a training – validation partition of the data as this would lack the necessary power to facilitate valid judgments (Molinaro *et al.* 2005). Instead, a 10-fold cross-validation approach was adopted as this has been shown to produce appropriate estimates of model performance (Kuhn & Johnson 2013). Following Lepot *et al.* (2016) an appropriate suite of model

Uncorrected Proof

6   K. Khamis *et al.* | Calibration of an in-situ sensor for wastewater monitoring   **Water Science & Technology** | in press | 2021

fit metrics were used to evaluate performance across all sites and locations; specifically the mean coefficient of determination (R_{cv}^2) and the root mean square error based on the cross validation ($RMSE_{cv}$).

We assessed the robustness of the *Proteus* calibration for a given WwTP and treatment stage by resampling the data (without replacement; $n = 11$–29) and assessing the goodness of fit with the observed data based on the reduced calibration set. In addition the impact of the number of variables used to calibrate the *Proteus* BOD_5 had on goodness of fit was assessed. To do this linear models involving all possible combinations of $TRYP_{20}$, $fDOM_{20}$, Turbidity and EC were assessed with 10-fold cross validation and R_{cv}^2 retained. All data analysis was undertaken using R version 3.3.3 with model selection undertaken using the *Caret* package based on information criteria (Kuhn 2008). Model residuals were assessed using visual tools outlined in (Zuur *et al.* 2010) and no violations of assumptions were identified (e.g. normality or heteroscedasticity).

RESULTS AND DISCUSSION

Accuracy of laboratory BOD measurements

Of the 450 samples analysed for BOD_5, 36 were removed following outlier screening due to either high deviation from the group mean or trending in the opposite direction. Outliers were not evenly distributed between the three labs with a single laboratory accounting for 33 of the samples removed (Lab B). This could be due to differences in technical staff experience, the method used for dissolved oxygen measurement or variability in the inocula used (Wilcock *et al.* 1981; Fitzmaurice & Gray 1989). There was, however, general agreement between the measurements of BOD_5 from the different laboratories used in this study, with strong positive pairwise correlations between all combinations when considering the full dataset ($r > 0.95, p < 0.001$). When breaking down the relationship into individual WwTPs and monitoring locations, greater variability between labs was apparent for FE samples (Figure 1). Deviations in the slopes of individual labs vs. the mean of all labs were pronounced for FE (min – max β: -0.57–2.65), particularly for Sites A and C (Figure 1). While slopes for PST monitoring locations showed little deviation from the 1:1 line (min – max β: 0.96–1.02; Table 2). The RMSE was generally lower for FE than PST and goodness of fit metrics were also lower (r and R^2; Table 2). However, the mean coefficient of variation (CV %) for FE ($35.1 \pm 18.0\%$) compared to PST ($16.2 \pm 11.8\%$) highlights that between laboratory variability was

significantly increased for FE, exceeding the 20% variability reported for internal laboratory comparisons (Jouanneau *et al.* 2014). This measurement uncertainty highlights issues with precision and repeatability of the BOD_5 test at low concentrations (Khan *et al.* 1998) and is likely due to variability in microbial populations used for inoculation (Jouanneau *et al.* 2014). The variance in BOD_5 results, particularly for FE, highlights the need for replicate measurements when attempting to calibrate online monitoring systems against an uncertain reference parameter (Joannis *et al.* 2008).

In-situ parameter variability

When considering the data globally (i.e. all treatments works and monitoring locations) correlations between the parameters were all modest ($r < 0.7$; Figure 2), however, when data were subset by site stronger relationships between *in-situ* parameters were apparent. Correlation coefficients were highest between $TRYP_{20}$, $CDOM_{20}$ and EC and particularly so for Site A, where only FE was monitored (Figure 2). There was significant variability between sites and treatment stage for all monitored variables (Table 1). Single sensor variable correlations with BOD_5 were generally low, with the maximum observed at Site C for turbidity ($r = 0.74$). The low correlations between fluorescence and BOD_5 at the site scale reflect the variability in the relationship between BOD_5 and fluorescence at different treatment stages. This is a function of the greater reduction in BOD_5 across the wastewater treatment process relative to reductions in fluorescence (Christian *et al.* 2017). For example, the reduction in BOD_5 after primary clarification can be up to an order of magnitude greater than that observed for fluorescence (Christian *et al.* 2017; Li *et al.* 2020) and can vary significantly between treatment works and treatment processes (Cohen *et al.* 2014).

In-situ BOD calibration

A global calibration model for all sites and treatment locations combined yielded a high $RMSE_{cv}$ (41.3 ± 8.41 mg L^{-1}) and relatively low R_{cv}^2 (0.55 ± 0.15), with significant error at lower concentration (i.e. FE monitoring locations; see Figure S1 supplementary material). When including site and location (FE or PST) as additional covariates the variance explained increased, hence, a location specific calibration was explored. For PST an adequate global calibration was achieved by pooling the samples from Sites B and C. A linear relationship between calibrated Proteus BOD and mean BOD_5 was apparent ($R_{cv}^2 = 0.67 \pm 0.20$;
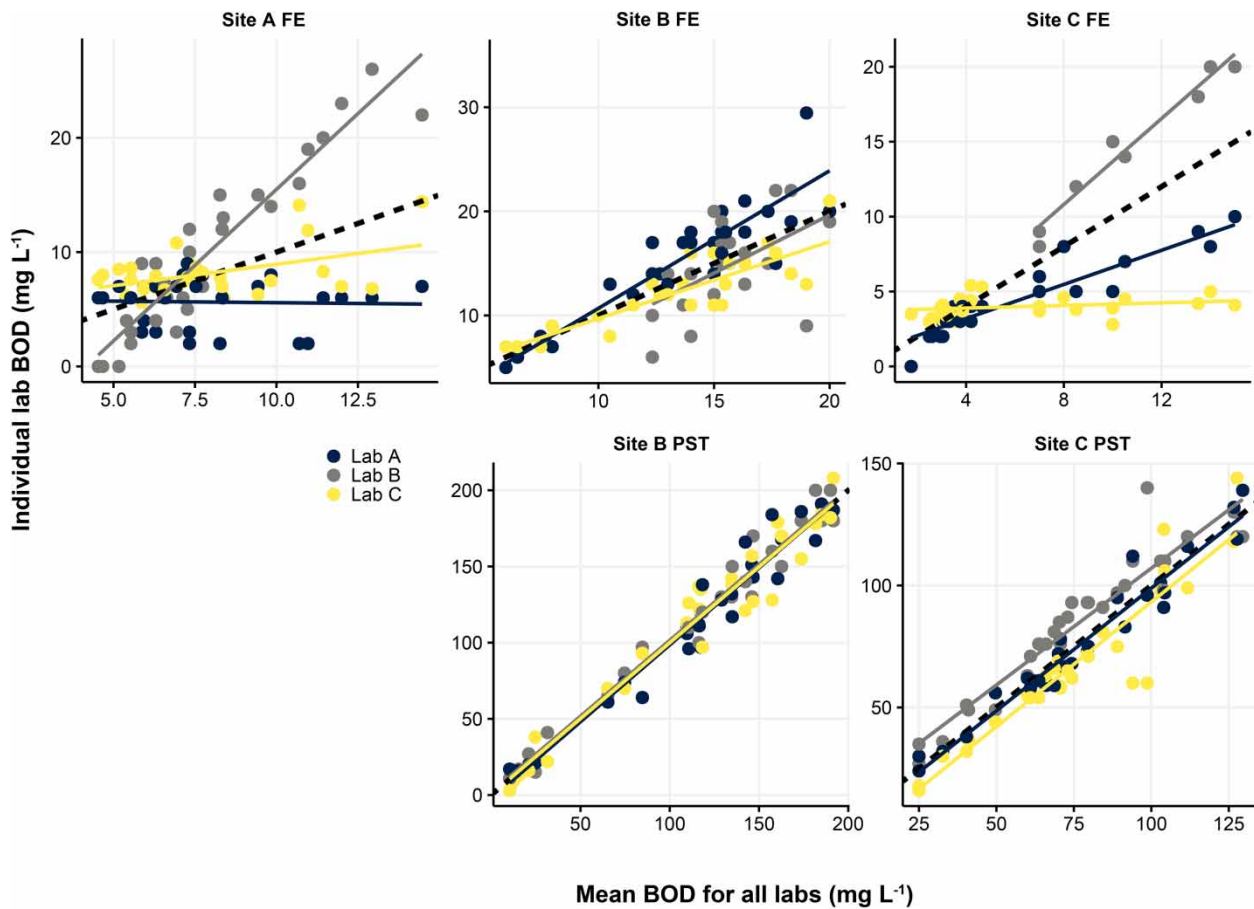
Figure 1 │ Relationship between BOD measurements from individual laboratories and the mean of all laboratory measurements. The dashed black line represents the 1:1 line and colours denote the line of best fit for individual labs (Ordinary Least Squares). Note sites are ordered by increasing size (left-right = smallest-largest).

Table 2 │ Goodness of fit metric for the relationship between individual laboratory BOD data and the mean of the three laboratory measurements.

Fit metric		Site A FE	Site B FE	Site C FE	Site B PST	Site C PST
Lab A	RMSE	3.95	3.69	2.4	11.8	6.7
	CV %	50.13	27.95	30.46	10.83	8.68
	r	0.1	0.87	0.92	0.98	0.97
	R^2	0.01	0.75	0.84	0.96	0.95
	β	-0.02	1.32	-0.57	1.01	1
Lab B	RMSE	5.19	3.82	4.23	9.54	12.18
	CV %	65.86	28.94	53.68	8.75	15.78
	r	0.94	0.54	0.98	0.99	0.95
	R^2	0.88	0.3	0.95	0.98	0.9
	β	2.65	1.12	1.43	0.99	0.96
Lab C	RMSE	2.43	2.38	0.76	13.03	13.12
	CV %	30.83	18.03	9.64	11.95	16.99
	r	0.46	0.85	0.68	0.98	0.93
	R^2	0.21	0.72	0.47	0.95	0.87
	β	0.38	0.79	0.04	0.99	1.02

RMSE = root mean square error, CV % = coefficient of variation (see equation [4] for calculation method), r = Pearson correlation coefficient, R^2 = coefficient of determination and β = slope of the fitted regression model (individual lab vs lab mean).

Figure 3) with the best model consisting of all the input variables (Figure 4). Turbidity and $TRYP_{20}$ were the most important variables in the model (i.e. explained the most variance) which is not surprising given turbidity provides a robust indication of volatile solids and $TRYP_{20}$ an indication of soluble BOD (Nguyen *et al.* 2014; Goffin *et al.* 2018). The $RMSE_{cv}$ was relatively high (35.8 ± 18.7 mg L^{-1}) and is likely a function of uncertainty inherent in the laboratory measurement of BOD_5 and differences in influent between the two sites (Li *et al.* 2020). However, when considering this as a coefficient of variation (CV = 29.8%) the deviation is comparable to that reported for a fast respirometer based online system (Iranpour & Zermeno 2008). Yet the respirometer has a lag of hours before results are available compared to the instantaneous measurement for the *Proteus*.

When pooling FE measurements from Sites A, B and C the best calibration model involved $fDOM_{20}$, EC and $TRYP_{20}$ (Figure 4). Interestingly fDOM was the most important variable in the model which is likely due to the higher
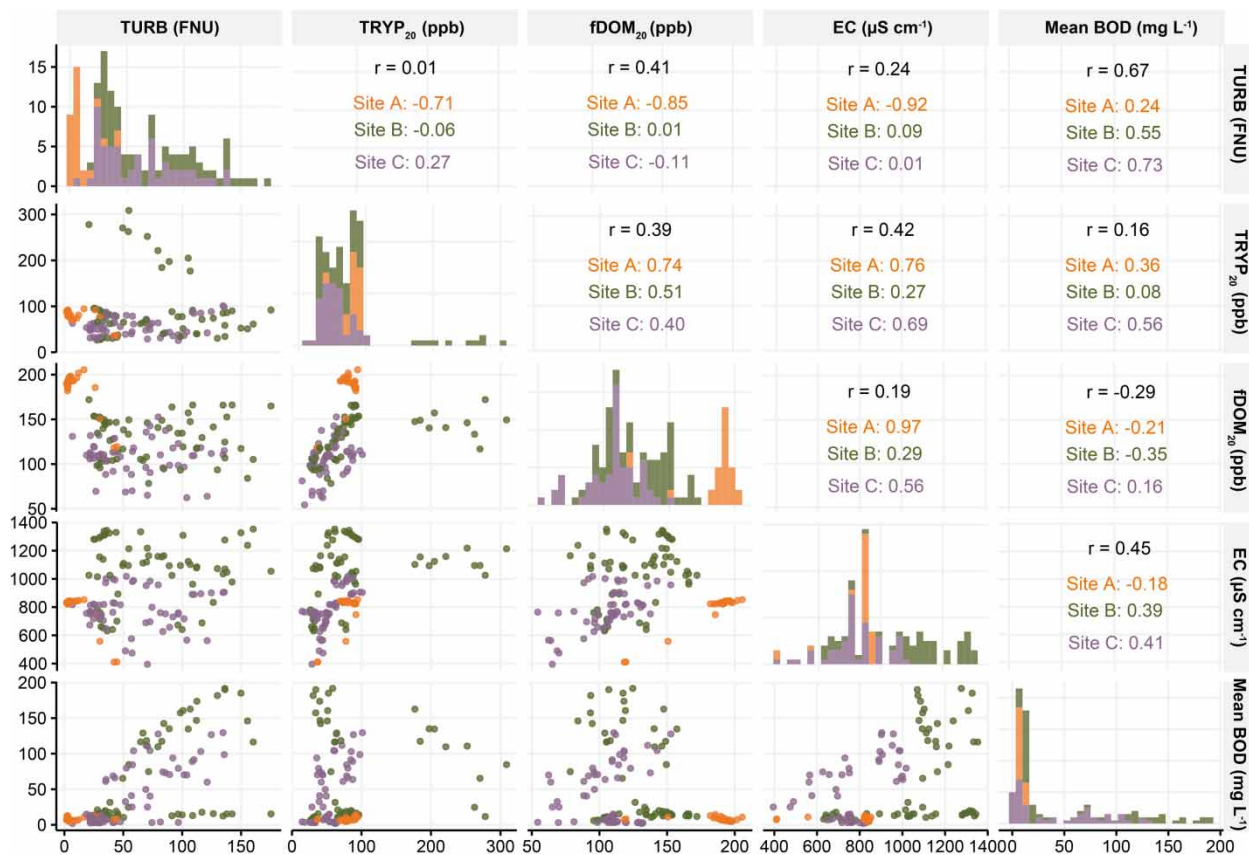
Figure 2 | Scatter plot matrix of *in-situ* measurements and laboratory derived BOD measurements. Upper panels are Spearman's correlation coefficients with colour denoting the site specific correlations and global correlation for all data displayed in black. Diagonal is a histogram based on values recorded for the specified variables. TURB = turbidity. fDOM$_{20}$ = temperature corrected humic-like fluorescence, TRYP$_{20}$ = temperature corrected tryptophan-like fluorescence and EC = electrical conductivity.
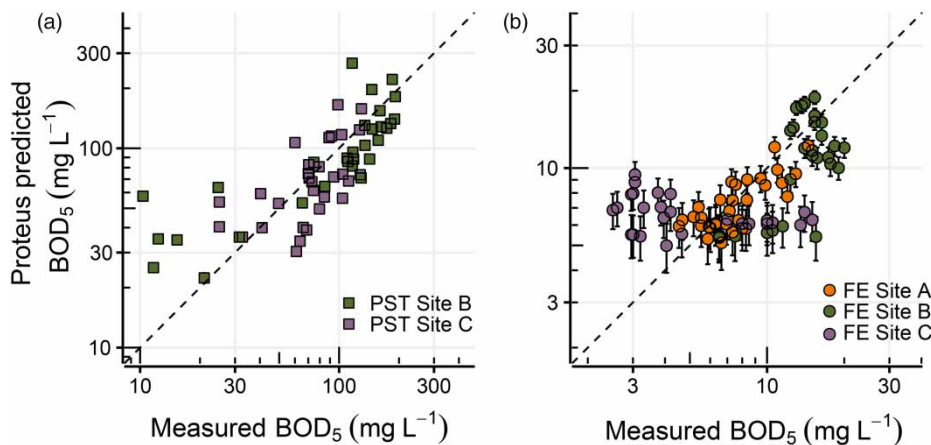
Figure 3 | Relationship between laboratory BOD and calibrated Proteus BOD measurements for (a) PST (post primary settlement) samples and (b) FE (final effluent) samples. Colour denotes the monitoring site (model structure is detailed in the text). Error bars represent ± SD based on 10-fold cross validation with 100 repeats.

fDOM concentrations recorded for site A (see Table 1). A weak linear relationship between the calibrated Proteus measurement and mean BOD$_5$ was apparent ($R^2_{cv} = 0.37 \pm 0.21$) with deviation from the lab measurements

($RMSE_{cv} = 3.63 \pm 0.79$ mg L^{-1}; CV = 39.9%), particularly for Site C where there was consistent overestimation of BOD$_5$ concentration (Figure 3(b)). This variability could represent differences in treatment efficiency and processes

Uncorrected Proof

9 K. Khamis *et al.* │ Calibration of an in-situ sensor for wastewater monitoring   Water Science & Technology │ in press │ 2021
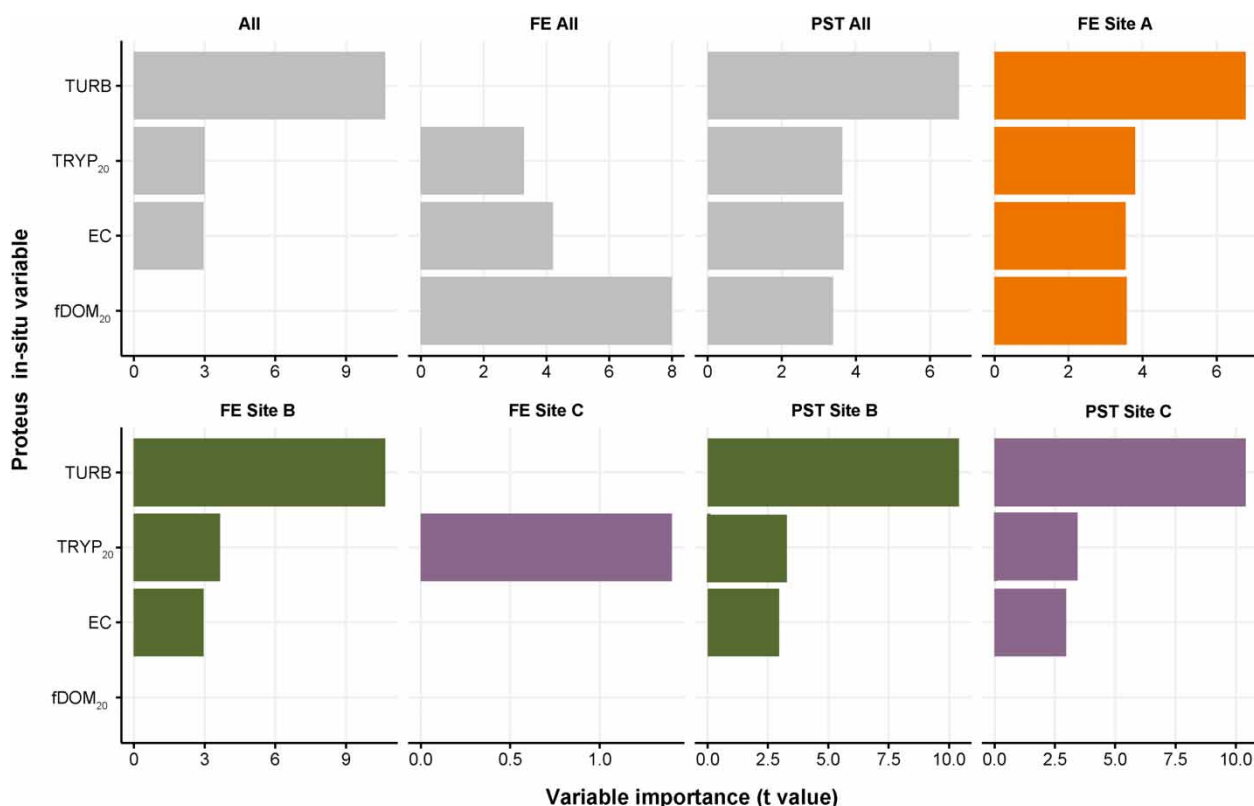
Figure 4 │ Variable importance (t. value) for the parameters retained in the best Proteus BOD calibration model. Colours denote site (as in Figure 4) with grey used for models where data were combined across multiple sites. TURB = Turbidity, EC = electrical conductivity, TRYP$_{20}$ = temperature corrected tryptophan-like fluorescence and fDOM$_{20}$ = temperature corrected humic-like fluorescence.

between the WwTPs (i.e. all sites had differing treatment processes). It could also be a function of differing amounts of recalcitrant vs labile organic compounds remaining in the FE leading to site difference in the relationship between fluorescence and BOD$_5$ (Dubber & Gray 2010).

Site specific calibration

Given that there was still significant uncertainty associated with the calibration models developed for the global and treatment stage specific datasets, we explored site-specific calibration procedures. This approach was anticipated to improve fit, precision and long-term stability as the coefficients were calculated for a specific wastewater matrix (Rieger *et al.* 2006). For the five site-treatment stage combinations, improvements in fit were observed between sensor and lab data when compared to the global and treatment specific calibrations (All R$^2_{cv}$ >0.60; Figure 5). The fits were comparable to those obtained for local calibrations at various WwTPs and sewage monitoring locations using UV absorbance sensors, with full spectral scans and partial least square models (Lepot *et al.*

2016) and using Excitation Emission Matrix spectroscopy with peak picking (Goffin *et al.* 2018).

There were differences in goodness of fit when comparing models that were based on BOD$_5$ data from individual labs (Table 3). This was most pronounced for FE with the lab mean consistently providing the better fit, except for Site C. Compared to the global FE calibration, the CV was ~50% lower at Sites A and B but was still high for Site C (CV = 61.6%). The elevated CV for Site C FE may have been due to the turbulent flow conditions at the only suitable installation location. Subsequently, there was likely interference with optical measurements due to bubbles and foam. An alternative installation configuration to overcome this issue would be a to use a pump through flow cell, however fouling is then a major issue and comparison with other sites difficult (Bourgeois *et al.* 2001). In addition, for Site C FE, there were issues with laboratory measurements from Lab C (i.e. had a substantially elevated RMSE$_{cv}$ and CV compared to models based on Labs A or B).

For PST, the site-specific calibration led to a less pronounced improvement in goodness of fit relative to the
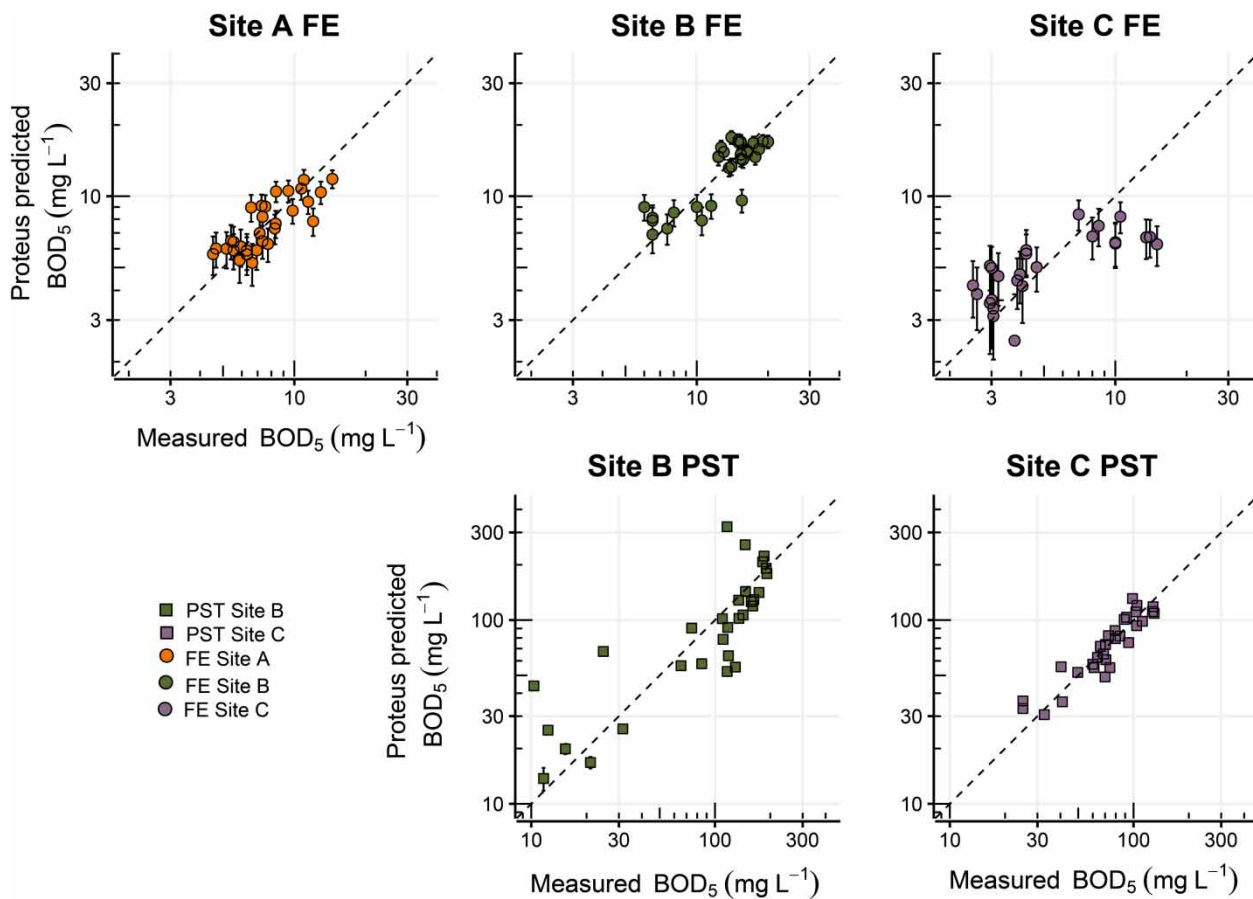
Uncorrected Proof

10   K. Khamis et al. | Calibration of an in-situ sensor for wastewater monitoring   **Water Science & Technology** | in press | 2021

Figure 5 | Relationship between laboratory BOD and calibrated *Proteus* BOD measurements for each monitoring location based on either samples from a single laboratory or the mean of all the laboratory measurements. Colour denotes the treatment works and shape the stage in the treatment process. The calibration model used is described in the text. Error bars represent ±SD based on 10-fold cross validation with 100 repeats. Note sites are ordered by increasing size (left-right = smallest-largest).

global PST calibration, for example a 25.9% increase in RMSE was apparent for Site B while a 64.0% reduction was observed for Site C. The calibration models based on BOD_5 measurements from a single lab were similar in terms of fit (Table 3), further highlighting that the BOD_5 test is only repeatable at high concentrations or when adequate microbial communities are present in the sample to offset variations in inocula (Jouanneau et al. 2014).

When considering the variables retained in the best model for a given site-treatment location, turbidity and tryptophan-like fluorescence were most frequently included (Figure 4). This is not surprising given the body of work highlighting correlations between tryptophan-like fluorescence and BOD_5 (Reynolds & Ahmad 1997; Carstea et al. 2018) and also links between turbidity and the particulate BOD load which often represents 60–80% of total BOD in wastewater (Bukhari 2008). At Site C, EC was also included in the best calibration models, while all four sensors, including fDOM, were used in the best model for Site

A FE (Figure 4). These exceptions are interesting as the correlation between fDOM (humic-like fluorescence) and BOD_5 in WwTPs is generally less strong than for tryptophan-like fluorescence (Yang et al. 2014), but recent work has highlighted this fluorescence peak is less prone to quenching associated errors – particularly those associated with high nitrate/nitrite concentrations (Sgroi et al. 2020b). The inputs of tankered waste and mixed liquors at Sites B and C would likely lead to large swings in nitrate which could explain why EC (a potential surrogate of effluent strength; Suresh et al. 2009) is a usual addition to the parameter suite for BOD_5 quantification.

Resampling of the data indicated that the calibrations were robust for all sites with an asymptote in model fit reached at ~20 samples for all sites (Figure 6). However, it is important to note the increased noise in the FE samples which suggests more samples may be required for calibration compared to PST monitoring locations. Interestingly, this asymptote at 20 samples is comparable to a study of

Uncorrected Proof

11   K. Khamis *et al.* │ Calibration of an in-situ sensor for wastewater monitoring   Water Science & Technology │ in press │ 2021

Table 3 │ Mean goodness of fit (R^2_{cv}), $RMSE_{cv}$ and coefficient of variation for Proteus BOD_5 prediction based on BOD results reported from a single lab or a mean of all laboratories

Location	Site(s)	GOF Metric	Lab A	Lab B	Lab C	Lab mean
All		R^2_{cv}	0.54 ± 0.15	0.58 ± 0.14	0.56 ± 0.12	0.55 ± 0.15
		$RMSE_{cv}$	39.9 ± 8.51	38.9 ± 6.7	40.4 ± 8.43	41.3 ± 8.41
		CV %	100.1 ± 20.1	74.1 ± 16.9	94.7 ± 22.7	94.7 ± 20.3
PST	All	R^2_{cv}	0.63 ± 0.22	0.6 ± 0.25	0.71 ± 0.2	0.67 ± 0.20
		$RMSE_{cv}$	42.8 ± 22.5	37.8 ± 17.1	38.9 ± 24.2	35.8 ± 18.7
		CV %	36.0 ± 21.2	31.5 ± 19.1	32.4 ± 23.6	29.8 ± 21.9
FE	All	R^2_{cv}	0.56 ± 0.18	0.36 ± 0.23	0.34 ± 0.21	0.37 ± 0.21
		$RMSE_{cv}$	3.17 ± 0.84	8.59 ± 2.83	4.51 ± 1.60	3.63 ± 0.79
		CV %	38.3 ± 10.2	70.9 ± 23.9	53.2 ± 19.1	39.9 ± 9.5
FE	Site A	R^2_{cv}	0.72 ± 0.34	0.6 ± 0.35	0.7 ± 0.34	0.84 ± 0.24
		$RMSE_{cv}$	1.35 ± 0.76	10.5 ± 9.14	1.55 ± 0.84	1.54 ± 0.64
		CV %	18.8 ± 23.4	99.99 ± 89.5	25.8 ± 14.3	18.8 ± 8.2
FE	Site B	R^2_{cv}	0.76 ± 0.31	0.69 ± 0.31	0.79 ± 0.26	0.84 ± 0.25
		$RMSE_{cv}$	2.29 ± 0.98	4.39 ± 1.90	3.89 ± 2.44	2.38 ± 0.96
		CV %	18.4 ± 8.4	30.8 ± 18.2	24.9 ± 15.4	17.5 ± 7.5
FE	Site C	R^2_{cv}	0.61 ± 0.4	0.69 ± 0.36	0.65 ± 0.36	0.76 ± 0.31
		$RMSE_{cv}$	0.75 ± 0.41	1.22 ± 0.78	3.99 ± 4.78	3.57 ± 9.4
		CV %	8.0 ± 5.1	18.5 ± 9.3	96.3 ± 115.1	61.6 ± 161.9
PST	Site B	R^2_{cv}	0.78 ± 0.27	0.85 ± 0.22	0.71 ± 0.33	0.89 ± 0.19
		$RMSE_{cv}$	55.0 ± 48.7	41.5 ± 27.7	53.1 ± 43.2	45.1 ± 35.5
		CV %	48.7 ± 41.9	38.6 ± 25.2	42.6 ± 26.5	40.0 ± 28.0
PST	Site C	R^2_{cv}	0.85 ± 0.25	0.89 ± 0.18	0.86 ± 0.21	0.88 ± 0.19
		$RMSE_{cv}$	16.4 ± 11.1	12.3 ± 5.05	16.4 ± 7.40	12.8 ± 5.19
		CV %	23.7 ± 17.2	14.7 ± 5.9	20.8 ± 8.9	16.4 ± 6.9

calibration requirements for spectral analysers measuring chemical oxygen demand on combined sewage overflows (Caradot *et al.* 2015).

A second modelling process to assess the impact of the number of variables used to build the calibration model highlighted that increasing this number generally increased the fit, with the exception of Site B PST and Site C FE (Figure 7). However, when more than two sensors inputs were included in the calibration function the improvement in fit (here considered in terms of R^2 values) was minimal (Figure 7). Given that treatment works generally display similar final effluent composition regardless of the treatment process (Quaranta *et al.* 2012), the different calibration requirements for final effluent sites is surprising (see Figure 4) and is likely a function of increased variability in influent flow volume and composition at smaller treatment works (Boller 1997). This can potentially lead to the loss of biomass, particularly following low flow conditions, which

in turn impacts treatment processes by changing the matrix and the relationship between sensor parameters and BOD_5 concentration (Kegebein *et al.* 2007). Hence, all four sensor inputs retained in the best model for Site A (p.e. = 360).

Recommendations and future directions

This study has identified some important points regarding calibration of *in-situ* sensors for monitoring organic load in wastewater. First, the need for a site-specific calibration is more pronounced for FE BOD_5 monitoring applications when compared to PST and doing so improves the calibration accuracy – similar to patterns observed for other spectral sensors for COD monitoring (Lepot *et al.* 2016). Second, the challenges associated with calibration against a parameter which is associated with considerable error (Bridgeman *et al.* 2013), were a particular problem for FE
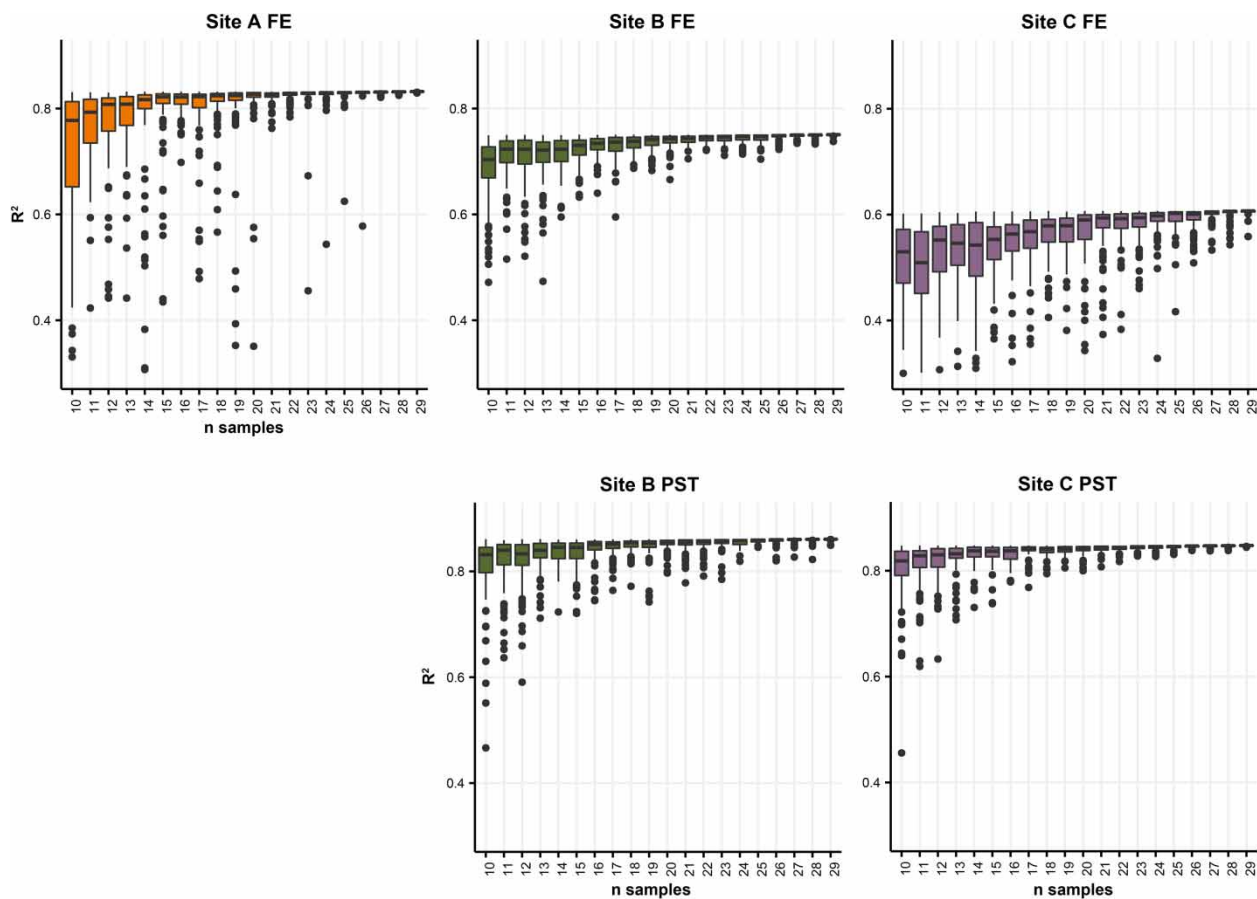
Figure 6 | R^2 values based on model coefficients obtained by resampling (100 draws without replacement) the laboratory data ($n = 10$–30) and corresponding sensor measurements. For each permutation, the coefficients were used to calculate the calibrated BOD_5 value based on all the data for each site-treatment stage (i.e. 30 samples). Note sites are ordered by increasing size (left-right = smallest-largest).

monitoring (Table 2). The poor agreement between lab measurements and subsequently high uncertainty in BOD_5 concentration can be overcome to some degree with careful initial calibration (i.e. mean BOD measurement based on multiple labs and/ or repeat measurements; Table 3). Third, installation issues can lead to greater uncertainty in the calibration, particularly when the only available monitoring locations are not ideal for sensor deployment. For example in this study access to the FE at Site C was limited to a single location with highly turbulent conditions. Hence, this was not ideal for optical sensor measurements as bubbles can cause spurious readings and may be the reason for the low correlation at this site. This can also be an issue at smaller WwTPs where the flow depth may not be sufficient for submersible sensor deployment. In both these scenarios a flow cell deployment may be feasible where water is pumped from the FE to the sensor deployed bankside. This type of installation was beyond the scope of this study, particularly as this would have added an extra

factor to our analysis (deployment method), but it is clear further work is needed to assess the maintenance requirements and comparability of flow cell relative to submersible deployments.

Online high frequency monitoring of BOD (e.g. sub-hourly; Figure 8) can provide new information on treatment process dynamics and efficiency, demand cycles, and ensure compliance to environmental discharge permits (Yuan *et al.* 2019). Furthermore, data can be used to calibrate and improve wastewater models (Martin & Vanrolleghem 2014) and provide information on how treatment processes respond to changing climate extremes thus guide mitigation and adaptation strategies (Zouboulis & Tolkou 2015; Kirchhoff & Watson 2019). Unlike single measurement parameter systems, such as nitrate or dissolved oxygen, multi-parameter monitoring systems (e.g. *Proteus*) can generate new knowledge beyond that of the initial process interest and inform more widely on WwTPs operation (Corominas *et al.* 2018).
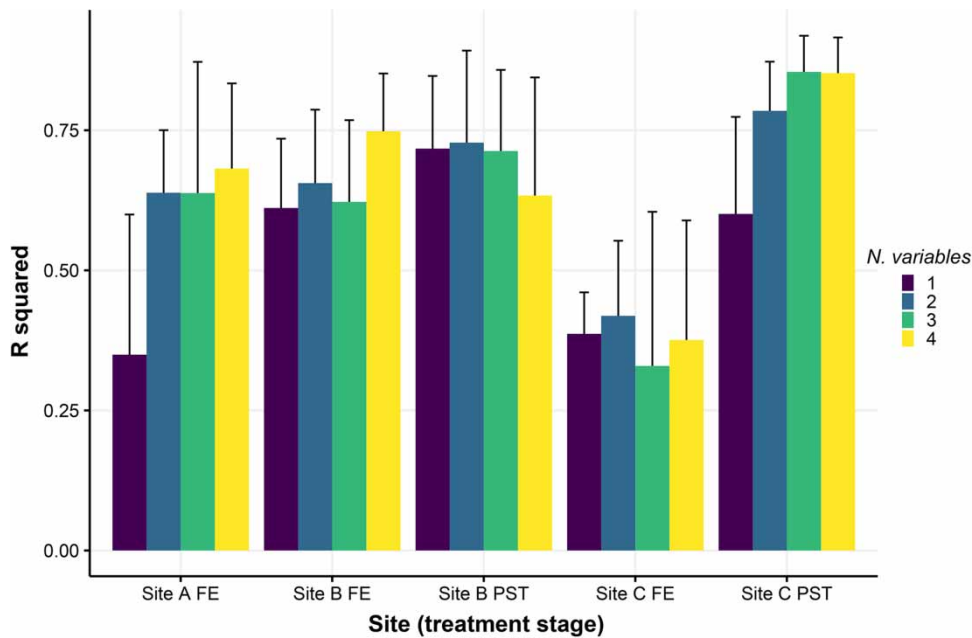
Figure 7 │ Goodness of fit (R^2) for the best model as a function of the number of variables (sensor inputs) used. Error bars represent 1 SD based on 10-fold cross validation with 100 repeats.
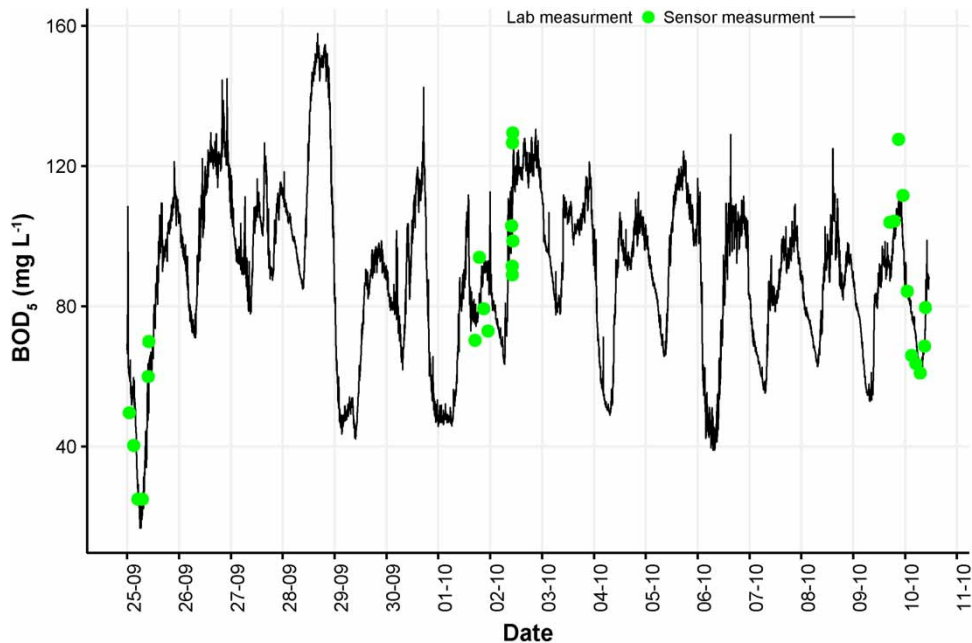

Figure 8 │ Calibrated *in-situ* BOD_5 measurements based on a site-specific calibration (mean BOD_5 of the three labs) for PST at Site C (autumn 2019). The green dots represent the laboratory measurements of BOD and the black line the fitted model. Dates are in the format DD-MM.

It is important to consider factors that can influence fluorescence based sensor measurements, particularly temperature, turbidity, inner filtering and nitrate/nitrate interference (Sgroi *et al.* 2020b). While the monitoring platform here tested included temperature and turbidity sensors future modifications to include nitrate and absorbance

sensors may improve measurement accuracy. In addition not all organic compounds are chromophoric (e.g. carbohydrates and saturated hydrocarbons; Vanrolleghem & Lee 2003). We suggest, future work should focus on sensor performance across WwTPs with varied influent (i.e. trade, industrial-dominated and municipal), treatment processes

Uncorrected Proof

14   K. Khamis *et al.* │ Calibration of an in-situ sensor for wastewater monitoring   Water Science & Technology │ in press │ 2021

(i.e. impact of disinfection processes on sensor measurements; (Sgroi *et al.* 2020a) and the response to 'shock' loads from industrial sources as these are likely to have a higher contribution of non-chromophoric compounds. Finally, a wider assessment of maintenance requirements is needed as deployments in this study lasted ~14 days (Schneider *et al.* 2019). Despite this relatively short deployment it was clear that maintenance requirements for FE locations were less than for PST deployments. For the later the wiper required regular inspection and cleaning (i.e. every couple of days) to ensure fouling did not occur.

CONCLUSIONS

This assessment of the performance of an *in-situ* optical monitoring platform highlights the potential to use multi-parameter instruments as reliable BOD_5 surrogates across a range of WwTPs (size and type of works) and treatment stages. We have addressed the objectives outlined in the introduction, and have generated new insight into the calibration of fluorescence based multi-parameter probes, specifically:

i.   The uncertainty associated with laboratory measurement of BOD_5 is greater for final effluent (FE) than settled sewage (PST), hence, local calibration and replicated FE sampling may be required to gain reliable datasets for process instrument calibration;

ii. It is possible to create robust site and process stage specific calibrations. However, a global FE calibration had relatively low predictive power compared to PST. This suggests sensors can be moved between PST sites/WwTPs and represents a desirable attribute of an online process sensor as calibration can be labour intensive and costly;

iii. For a robust calibration a minimum of 20 laboratory samples, spanning at least two diel demand cycles, is suggested. In addition, it appears a larger suite of monitoring variables are required for FE (i.e. TRYP, fDOM, Turbidity and EC) relative to PST (i.e. TRYP and Turbidity).

AUTHOR CONTRIBUTIONS

There are no conflicts of interest to declare. KK, CB, RS and DMH designed the study. HJG, GB and RS undertook fieldwork and sample collection. KK and CB undertook all data analysis independently and the manuscript was written by KK with input from CB and DMH. All authors provided editorial comments on the final draft of the manuscript.

ACKNOWLEDGEMENTS

DATA AVAILABILITY STATEMENT

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

REFERENCES

Boller, M. 1997 Small wastewater treatment plants – a challenge to wastewater engineers. *Water Science and Technology: A Journal of the International Association on Water Pollution Research* **35** (6), 1–12.

Bourgeois, W., Burgess, J. E. & Stuetz, R. M. 2001 On-line monitoring of wastewater quality: a review. *Journal of Chemical Technology and Biotechnology* **76**, 337–348.

Bridgeman, J., Baker, A., Carliell-Marquet, C. & Carstea, E. 2013 Determination of changes in wastewater quality through a treatment works using fluorescence spectroscopy. *Environmental Technology* **34** (21–24), 3069–3077. doi:10.1080/09593330.2013.803131.

Brito, R. S., Pinheiro, H. M., Ferreira, F., Matos, J. S. & Lourenço, N. D. 2014 In situ UV-Vis spectroscopy to estimate COD and TSS in wastewater drainage systems. *Urban Water Journal* **11** (4), 261–273. doi:10.1080/1573062X.2013.783087.

Bukhari, A. A. 2008 Investigation of the electro-coagulation treatment process for the removal of total suspended solids and turbidity from municipal wastewater. *Bioresource Technology* **99** (5), 914–921. doi:10.1016/j.biortech.2007.03.015.

Caradot, N., Sonnenberg, H., Rouault, P., Gruber, G., Hofer, T., Torres, A., Pesci, M. & Bertrand-Krajewski, J.-L. 2015 Influence of local calibration on the quality of online wet weather discharge monitoring: feedback from five international case studies. *Water Science and Technology* **71** (1), 45–51. doi:10.2166/wst.2014.465.

Carstea, E. M., Bridgeman, J., Baker, A. & Reynolds, D. M. 2016 Fluorescence spectroscopy for wastewater monitoring: a

review. *Water Research* **95**, 205–219. doi:10.1016/j.watres. 2016.03.021.

Carstea, E. M., Zakharova, Y. S. & Bridgeman, J. 2018 Online fluorescence monitoring of effluent organic matter in wastewater treatment plants. *Journal of Environmental Engineering* **144** (5), 04018021. doi:10.1061/(ASCE)EE.1943-7870.0001360.

Carstea, E. M., Popa, C. L., Baker, A. & Bridgeman, J. 2020 In situ fluorescence measurements of dissolved organic matter: a review. *The Science of the Total Environment* **699**, 134361. doi:10.1016/j.scitotenv.2019.134361.

Choi, Y. Y., Baek, S. R., Kim, J. I., Choi, J. W., Hur, J., Lee, T. U., Park, C. J. & Lee, B. 2017 Characteristics and biodegradability of wastewater organic matter in municipal wastewater treatment plants collecting domestic wastewater and industrial discharge. *Water* **9** (6), 409. doi:10.3390/w9060409.

Chow, C. W. K., Liu, J., Li, J., Swain, N., Reid, K. & Saint, C. P. 2018 Development of smart data analytics tools to support wastewater treatment plant operation. *Chemometrics and Intelligent Laboratory Systems* **177**, 140–150. doi:10.1016/j.chemolab.2018.03.006.

Christian, E., Batista, J. R. & Gerrity, D. 2017 Use of COD, TOC, and fluorescence spectroscopy to estimate BOD in wastewater. *Water Environment Research: A Research Publication of the Water Environment Federation* **89** (2), 168–177. doi:10.2175/106143016X14504669768976.

Cohen, E., Levy, G. J. & Borisover, M. 2014 Fluorescent components of organic matter in wastewater: efficacy and selectivity of the water treatment. *Water Research* **55**, 323–334. doi:10.1016/j.watres.2014.02.040.

Corominas, L., Garrido-Baserba, M., Villez, K., Olsson, G., Cortés, U. & Poch, M. 2018 Transforming data into knowledge for improved wastewater treatment operation: a critical review of techniques. *Environmental Modelling & Software* **106**, 89–103. doi:10.1016/j.envsoft.2017.11.023.

Downing, B. D., Pellerin, B. A., Bergamaschi, B. A., Saraceno, J. F. & Kraus, T. E. C. 2012 Seeing the light: the effects of particles, dissolved materials, and temperature on in situ measurements of DOM fluorescence in rivers and streams. *Limnology and Oceanography, Methods* **10** (10), 767–775. doi:10.4319/lom.2012.10.767.

Dubber, D. & Gray, N. F. 2010 Replacement of chemical oxygen demand (COD) with total organic carbon (TOC) for monitoring wastewater treatment performance to minimize disposal of toxic analytical waste. *Journal of Environmental Science and Health. Part A* **45** (12), 1595–1600. doi:10.1080/10934529.2010.506116.

Ebrahimi, M., Gerber, E. L. & Rockaway, T. D. 2017 Temporal performance assessment of wastewater treatment plants by using multivariate statistical analysis. *Journal of Environmental Management* **193**, 234–246. doi:10.1016/j.jenvman.2017.02.027.

Fitzmaurice, G. D. & Gray, N. F. 1989 Evaluation of manufactured inocula for use in the BOD test. *Water Research* **23** (5), 655–657. doi:10.1016/0043-1354(89)90032-8.

Goffin, A., Guérin, S., Rocher, V. & Varrault, G. 2018 Towards a better control of the wastewater treatment process: excitation-emission matrix fluorescence spectroscopy of dissolved organic matter as a predictive tool of soluble BOD5 in influents of six Parisian wastewater treatment plants. *Environmental Science and Pollution Research International* **25** (9), 8765–8776. doi:10.1007/s11356-018-1205-1.

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 Research* **43** (4), 863–881. doi:10.1016/j.watres.2008.11.027.

Hudson, N., Baker, A., Ward, D., Reynolds, D. M., Brunsdon, C., Carliell-Marquet, C. & Browning, S. 2008 Can fluorescence spectrometry be used as a surrogate for the biochemical oxygen demand (BOD) test in water quality assessment? an example from South West England. *The Science of the Total Environment* **391** (1), 149–158. doi:10.1016/j.scitotenv.2007.10.054.

Ignatev, A. & Tuhkanen, T. 2019 Monitoring WWTP performance using size-exclusion chromatography with simultaneous UV and fluorescence detection to track recalcitrant wastewater fractions. *Chemosphere* **214**, 587–597. doi:10.1016/j.chemosphere.2018.09.099.

Imai, A., Fukushima, T., Matsushige, K., Kim, Y.-H. & Choi, K. 2002 Characterization of dissolved organic matter in effluents from wastewater treatment plants. *Water Research* **36** (4), 859–870. doi:10.1016/s0043-1354(01)00283-4.

Iranpour, R. & Zermeno, M. 2008 Online biochemical oxygen demand monitoring for wastewater process control: full-scale studies at Los Angeles Glendale wastewater plant, California. *Water Environment Research* **80** (4), 298–307. doi:10.2175/106143007(184672.

Joannis, C., Ruban, G., Gromaire, M.-C., Chebbo, G., Bertrand-Krajewski, J.-L., Joannis, C. & Ruban, G. 2008 Reproducibility and uncertainty of wastewater turbidity measurements. *Water Science And Technology* **57** (10), 1667–1673. doi:10.2166/wst.2008.292.

Jouanneau, S., Recoules, L., Durand, M. J., Boukabache, A., Picot, V., Primault, Y., Lakel, A., Sengelin, M., Barillon, B. & Thouand, G. 2014 Methods for assessing biochemical oxygen demand (BOD): a review. *Water Research* **49**, 62–82. doi:10.1016/j.watres.2013.10.066.

Kegebein, J., Hoffmann, E. & Hahn, H. H. 2007 Performance of very small wastewater treatment plants with pronounced load variations. *Water Science and Technology* **55** (7), 31–38. doi:10.2166/wst.2007.123.

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. *Environmental Science. Processes & Impacts* **17** (4), 740–752. doi:10.1039/c5em00030 k.

Khamis, K., Bradley, C., Stevens, R. & Hannah, D. M. 2017 Continuous field estimation of dissolved organic carbon concentration and biochemical oxygen demand using dual-wavelength fluorescence, turbidity and temperature: dual-wavelength fluorescence to estimate DOC and BOD. *Hydrological Processes* **31** (3), 540–555. doi:10.1002/hyp.11040.

Khan, E., Babcock, R. W., Suffet, I. H. & Stenstrom, M. K. 1998 Method development for measuring biodegradable organic

Uncorrected Proof

16   K. Khamis *et al.* │ Calibration of an in-situ sensor for wastewater monitoring   **Water Science & Technology** │ in press │ 2021

carbon in reclaimed and treated wastewaters. *Water Environment Research* **70** (5), 1025–1032. doi:10.2175/106143098(123354.

Kirchhoff, C. J. & Watson, P. L. 2019 Are wastewater systems adapting to climate change? *Journal of the American Water Resources Association* **55** (4), 869–880. doi:10.1111/1752-1688.12748.

Kuhn, M. 2008 Building predictive models in R using the caret package. *Journal of Statistical Software* **28** (5), 1–26.

Kuhn, M. & Johnson, K. 2013 *Applied Predictive Modeling*. Springer, New York.

Lepot, M., Aubin, J. B. & Bertrand-Krajewski, J. L. 2013 Accuracy of different sensors for the estimation of pollutant concentrations (total suspended solids, total and dissolved chemical oxygen demand) in wastewater and stormwater. *Water Science and Technology* **68** (2), 462–471. doi:10.2166/wst.2013.276.

Lepot, M., Torres, A., Hofer, T., Caradot, N., Gruber, G., Aubin, J. B. & Bertrand Krajewski, J. L. 2016 Calibration of UV/Vis spectrophotometers: a review and comparison of different methods to estimate TSS and total and dissolved COD concentrations in sewers, WWTPs and rivers. *Water Research* **101**, 519–534. doi:10.1016/j.watres.2016.05.070.

Li, J., Wang, L., Geng, J., Li, S., Yu, Q., Xu, K. & Ren, H. 2020 Distribution and removal of fluorescent dissolved organic matter in 15 municipal wastewater treatment plants in China. *Chemosphere* 126375. doi:10.1016/j.chemosphere.2020.126375.

Martin, C. & Vanrolleghem, P. A. 2014 Analysing, completing, and generating influent data for WWTP modelling: a critical review. *Environmental Modelling & Software* **60**, 188–201. doi:10.1016/j.envsoft.2014.05.008.

Mesquita, D. P., Quintelas, C., Luís Amaral, A. & Ferreira, E. C. 2017 Monitoring biological wastewater treatment processes: recent advances in spectroscopy applications. *Reviews in Environmental Science and Bio/Technology* **16** (3), 395–424. doi:10.1007/s11157-017-9439-9.

Molinaro, A. M., Simon, R. & Pfeiffer, R. M. 2005 Prediction error estimation: a comparison of resampling methods. *Bioinformatics* **21** (15), 3301–3307. doi:10.1093/bioinformatics/bti499.

Nguyen, L. A. T., Ward, A. J. & Lewis, D. 2014 Utilisation of turbidity as an indicator for biochemical and chemical oxygen demand. *Journal of Water Process Engineering* **4**, 137–142. doi:10.1016/j.jwpe.2014.09.009.

Niku, S. & Schroeder, E. D. 1981 Factors affecting effluent variability from activated sludge processes. *Journal of the Water Pollution Control Federation* **53** (5), 546–559.

Quaranta, M. L., Mendes, M. D. & MacKay, A. A. 2012 Similarities in effluent organic matter characteristics from connecticut wastewater treatment plants. *Water Research* **46** (2), 284–294. doi:10.1016/j.watres.2011.10.010.

Raboni, M., Torretta, V. & Urbini, G. 2013 Influence of strong diurnal variations in sewage quality on the performance of biological denitrification in small community wastewater treatment plants (WWTPs). *Sustainability: Science Practice And Policy* **5** (9), 3679–3689. doi:10.3390/su5093679.

Reynolds, D. M. & Ahmad, S. R. 1997 Rapid and direct determination of wastewater BOD values using a fluorescence technique. *Water Research* **31** (8), 2012–2018. doi:10.1016/s0043-1354(97)00015-8.

Rieger, L., Langergraber, G., Thomann, M., Fleischmann, N. & Siegrist, H. 2004 Spectral *in-situ* analysis of NO2, NO3, COD, DOC and TSS in the effluent of a WWTP. *Water Science And Technology* **50** (11), 143–152.

Rieger, L., Thomann, M., Gujer, W. & Siegrist, H. 2005 Quantifying the uncertainty of on-line sensors at WWTPs during field operation. *Water Research* **39** (20), 5162–5174. doi:10.1016/j.watres.2005.09.040.

Rieger, L., Langergraber, G. & Siegrist, H. 2006 Uncertainties of spectral in situ measurements in wastewater using different calibration approaches. *Water Science and Technology* **53** (12), 187–197. doi:10.2166/wst.2006.421.

Schneider, M. Y., Carbajal, J. P., Furrer, V., Sterkele, B., Maurer, M. & Villez, K. 2019 Beyond signal quality: the value of unmaintained pH, dissolved oxygen, and oxidation-reduction potential sensors for remote performance monitoring of on-site sequencing batch reactors. *Water Research* **161**, 639–651. doi:10.1016/j.watres.2019.06.007.

Sgroi, M., Gagliano, E., Vagliasindi, F. G. A. & Roccaro, P. 2020a Absorbance and EEM fluorescence of wastewater: effects of filters, storage conditions, and chlorination. *Chemosphere* **243**, 125292. doi:10.1016/j.chemosphere.2019.125292.

Sgroi, M., Gagliano, E., Vagliasindi, F. G. A. & Roccaro, P. 2020b Inner filter effect, suspended solids and nitrite/nitrate interferences in fluorescence measurements of wastewater organic matter. *The Science Of The Total Environment* **711**, 134663. doi:10.1016/j.scitotenv.2019.134663.

Suresh, A., Choi, H. L., Oh, D. I. & Moon, O. K. 2009 Prediction of the nutrients value and biochemical characteristics of swine slurry by measurement of EC–electrical conductivity. *Bioresource Technology* **100** (20), 4683–4689. doi:10.1016/j.biortech.2009.05.006.

Ulliman, S. L., Korak, J. A., Linden, K. G. & Rosario-Ortiz, F. L. 2020 Methodology for selection of optical parameters as wastewater effluent organic matter surrogates. *Water Research* **170**, 115321. doi:10.1016/j.watres.2019.115321.

Vanrolleghem, P. A. & Lee, D. S. 2003 On-line monitoring equipment for wastewater treatment processes: state of the art. *Water Science And Technology* **47** (2), 1–34.

Wilcock, R. J., Stevenson, C. D. & Roberts, C. A. 1981 An interlaboratory study of dissolved oxygen in water. *Water Research* **15** (3), 321–325. doi:10.1016/0043-1354(81)90035-X.

Wu, L., Ning, D., Zhang, B., Li, Y., Zhang, P., Shan, X., Zhang, Q., Brown, M. R., Li, Z., Van Nostrand, J. D., Ling, F., Xiao, N., Zhang, Y., Vierheilig, J., Wells, G. F., Yang, Y., Deng, Y., Tu, Q., Wang, A., Global Water Microbiome ConsortiumZhang, T., He, Z., Keller, J., Nielsen, P. H., Alvarez, P. J. J., Criddle, C. S., Wagner, M., Tiedje, J. M., He, Q., Curtis, T. P., Stahl, D. A., Alvarez-Cohen, L., Rittmann, B. E., Wen, X. & Zhou, J. 2019 Global diversity and biogeography of bacterial communities in wastewater treatment plants. *Nature Microbiology* **4** (7), 1183–1195. doi:10.1038/s41564-019-0426-5.

Uncorrected Proof

17   K. Khamis *et al.* │ Calibration of an in-situ sensor for wastewater monitoring   **Water Science & Technology** │ in press │ 2021

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** (1), 1771–1786. doi:10.3390/s140101771.

Young, J. C., McDermott, G. N. & Jenkins, D. 1981 Alterations in the BOD procedure for the 15th edition of 'Standard Methods for the Examination of Water and Wastewater'. *Journal – Water Pollution Control Federation* 1253–1259.

Yu, H., Song, Y., Tu, X., Du, E., Liu, R. & Peng, J. 2013 Assessing removal efficiency of dissolved organic matter in wastewater treatment using fluorescence excitation emission matrices with parallel factor analysis and second derivative synchronous fluorescence. *Bioresource Technology* **144**, 595–601. doi:10.1016/j.biortech.2013.07.025.

Yuan, Z., Olsson, G., Cardell-Oliver, R., van Schagen, K., Marchi, A., Deletic, A., Urich, C., Rauch, W., Liu, Y. & Jiang, G. 2019 Sweating the assets: the role of instrumentation, control and automation in urban water systems. *Water Research* **155**, 381–402. doi:10.1016/j.watres.2019.02.034.

Zambrano-Bigiarini, M. 2014 *hydroGOF: Goodness-of-fit Functions for Comparison of Simulated and Observed Hydrological Time Series*. R Package Version 0. pp. 3–8.

Zouboulis, A. & Tolkou, A. 2015 Effect of Climate Change in Wastewater Treatment Plants: Reviewing the Problems and Solutions. In: *Managing Water Resources Under Climate Uncertainty: Examples From Asia, Europe, Latin America, and Australia* (S. Shrestha, A. K. Anal, P. A. Salam & M. van der Valk, eds). Springer International Publishing, Cham, pp. 197–220. doi:10.1007/978-3-319-10467-6_10.

Zuur, A. F., Ieno, E. N. & Elphick, C. S. 2010 A protocol for data exploration to avoid common statistical problems. *Methods In Ecology And Evolution* **1** (1), 3–14. doi:10.1111/j.2041-210X.2009.00001.x.