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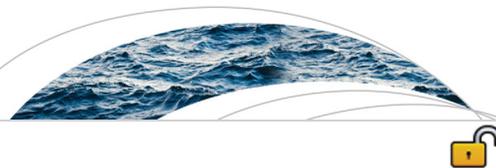
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RESEARCH ARTICLE

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Phillip J. Blauen and Nicolai Brekenfeld contributed equally to this work.

Key Points:

- Multitracer field fluorometry is challenging because variations in environmental conditions can cause substantial errors in measurements
- We found strong effects of temperature, turbidity, and concentration on measurements of conservative and reactive fluorescent tracers
- Correction and calibration methods are presented to improve the accuracy of fluorescence measurements during stream tracer tests

Supporting Information:

- Supporting Information S1

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Multitracer Field Fluorometry: Accounting for Temperature and Turbidity Variability During Stream Tracer Tests

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Abstract The use of multitracer field fluorometry is increasing in the hydrological sciences. However, obtaining high-quality fluorescence measurements is challenging given the variability in environmental conditions within stream ecosystems. Here, we conducted a series of stream tracer tests to examine the degree to which multitracer field fluorometry produces reliable estimates of tracer concentrations under realistic field conditions. Using frequently applied examples of conservative (Uranine) and reactive (Resazurin-Resorufin) fluorescent tracers, we show that in situ measurements of tracer breakthrough curves can deviate markedly from corresponding samples analyzed under laboratory conditions. To investigate the effects of key environmental variables on fluorescence measurements, we characterized the response of field fluorometer measurements to changes in temperature, turbidity, and tracer concentration. Results showed pronounced negative log-linear effects of temperature on fluorescence measurements for all tracers, with stronger effects observed typically at lower tracer concentrations. We also observed linear effects of turbidity on fluorescence measurements that varied predictably with tracer concentration. Based on our findings, we present methods to correct field fluorometer measurements for variation in these parameters. Our results show how changing environmental conditions can introduce substantial uncertainties in the analysis of fluorescent tracer breakthrough curves, and highlight the importance of accounting for these changes to prevent incorrect inferences being drawn regarding the physical and biogeochemical processes underpinning observed patterns.

1. Introduction

Fluorescent dyes have been used extensively as artificial tracers to investigate hydrological processes in both surface water and groundwater environments (Flury & Wai, 2003; Leibundgut et al., 2009). Typical applications include determining pathways and residence times of water in aquifers (Massei et al., 2006), identifying subglacial drainage networks (Chandler et al., 2013), characterizing flow velocities and storage processes (Hensley & Cohen, 2012; Schmadel et al., 2016), and tracking contaminant transport (Bottrell et al., 2010; Malaguerra et al., 2013). Commonly used fluorescent dyes (e.g., Uranine, Rhodamine WT, Eosine) are highly soluble in water, nontoxic, relatively inexpensive, and are readily detectable at concentrations as low as parts per trillion (Flury & Wai, 2003; Smart & Laidlaw, 1977); attributes that make them highly suitable for application as hydrological tracers.

Traditionally, fluorescent dyes employed in hydrological studies have been selected for their quasi-conservative chemical properties, although recently reactive, or “smart,” fluorescent dye tracers such as the Resazurin-Resorufin system (supporting information Text S1) have been developed (Gramling et al., 2002; Haggerty et al., 2008). The detection of fluorescent tracers commonly involves acquiring field samples and performing subsequent laboratory analysis by spectrofluorometry. However, portable field fluorometers have been developed for real-time tracer detection at higher temporal resolutions than possible by manual, or discrete automated, sampling approaches. Field fluorometers have traditionally been restricted to detecting one or two conservative dye tracers (Gooseff et al., 2008; Kunkel & Radke, 2011). However, technological advances in LEDs and spectral filters have enabled the development of field fluorometers capable of detecting three or more dye tracers simultaneously (Schnegg & Flynn, 2002).

The relatively low cost and ease of use of multitracer field fluorometers has resulted in their rapid uptake by the hydrological community (Lemke et al., 2013a, 2014; Schmadel et al., 2016). However, measurements by

field fluorimeters are susceptible to interference from environmental factors, including temperature, pH, turbidity, and background organic matter fluorescence (Flett et al., 2017; Flury & Wai, 2003; Khamis et al., 2015), which can exhibit greater variability in naturally dynamic in situ conditions than found in controlled laboratory environments. Consequently, on-site fluorimeter calibrations have been recommended to minimize measurement errors (Khamis et al., 2015; Lemke et al., 2013b). To date, however, application of multitracer field fluorimeters has been limited largely to ideal environmental conditions that are not representative of many rivers; due in part to their relatively recent introduction to hydrological field applications, but also due to the challenges involved in separating multiple overlapping tracer signals relative to single-tracer fluorometry.

In this paper, we present results from a series of tracer experiments, using the fluorescent dyes Uranine and Resazurin, to examine the degree to which multitracer field fluorimeters produce reliable estimates of conservative and reactive solute transport under realistic field conditions. The main objectives are to: (1) examine the degree to which multitracer field fluorometry produces reliable estimates of conservative and reactive solute transport under realistic field conditions; (2) highlight how variability in environmental field conditions can introduce uncertainties in the analysis of fluorescent tracer breakthrough curves; and (3) present methods to correct data measured by multitracer field fluorimeters for selected environmental variables.

The experiments of this study are designed to benchmark the suitability of in situ fluorometric analysis of conservative and reactive tracer breakthrough curves, using Uranine and Resazurin as frequently applied examples of hydrological tracers.

2. Methods

2.1. Study Site and Multitracer Field Injections

The experiments of this study were performed at the Mill Brook, at the facilities of the Birmingham Institute of Forest Research (www.birmingham.ac.uk/bifor), Staffordshire, UK in October 2016. Tracer injections of Uranine and Resazurin were conducted with GGUN FL30 (Albillia Sarl, Switzerland) on-line fluorimeters to detect breakthrough curves of Uranine, Resazurin, and Resorufin along a 1 km stream reach. Discrete water samples were also collected for laboratory analysis of tracer concentrations. For full details of the study site and field experiments see Blaen et al. (2017) and supporting information Text S2 and Table S1.

2.2. Determination of External Effects on Field Fluorimeter Tracer Signals

Detection of fluorimeter tracer signals can be affected by light attenuation in the water column (e.g., due to dissolved solutes or suspended particles) and by the effects of environmental conditions on the fluorescing material (Downing et al., 2012). We conducted a series of laboratory experiments to assess the effects of temperature and turbidity on field fluorimeter tracer measurements, based on previous studies that have highlighted strong impacts of both parameters on fluorescence measurements (Khamis et al., 2015; Leibundgut et al., 2009). We also investigated qualitatively the effects of tracer concentration. pH is recognized as an important determinant of fluorescence intensity for Uranine, Resazurin, and Resorufin at values below 7.5 (Lemke et al., 2013b). In our study, stream pH values were consistently above 8 and exhibited minimal temporal variation, therefore we did not investigate pH effects here. However, we note that under different environmental conditions, pH may play a more important role in influencing fluorescence measurements.

2.2.1. Temperature Effects

Instrument-specific temperature effects were determined over a range of 5 to 25°C using solutions of Uranine, Resazurin and Resorufin made with deionized water (18.2 MΩ). The central chamber of each fluorimeter was filled with tracer solution at ambient room temperature. Fluorimeters were then placed in a refrigerator and cooled to 5°C over ~6 h. Measurements were performed repeatedly using tracer concentrations of 7, 21 and 70 ppb for Uranine, 10, 30 and 100 ppb for Resorufin, and 30, 100 and 300 ppb for Resazurin, which are representative of concentrations measured during field tracer tests. To confirm that observed effects were truly temperature-dependent and not attributable to photodegradation, additional runs were performed over the same time period at constant temperature.

2.2.2. Turbidity Effects

Instrument-specific turbidity effects were determined for Uranine, Resazurin, and Resorufin. Fluorimeter tracer signals were measured over a range of turbidity from 0 to ~55 NTU, which is representative of those

experienced commonly during stream tracer tests (e.g., Kunkel & Radke, 2011). Solutions were prepared over a range of tracer concentrations (the same as for the temperature analysis and additionally 0 ppb). For each solution, the effects of turbidity on fluorescence measurements were analyzed at six (Uranine, Resorufin) and eight (Resazurin) different turbidity levels using Fuller’s Earth (Thermo Fisher Scientific, UK). In order to eliminate the impact of particles on pH, all tracer solutions were buffered to a pH of ~8.5, with a solution to buffer ratio of 100:1. The buffer was made by mixing equal volumes of 1M NaH₂PO₄·H₂O and 1M NaOH (Haggerty et al., 2008). Field fluorimeters were connected in series with silicone tubing and a Solinst (Georgetown, Canada) 410 peristaltic pump was used to pass the tracer solutions continuously through the instrument measuring chambers.

2.3. Data Analysis

2.3.1. Correction of Tracer Signals for Temperature and Turbidity Effects

Overlaps in excitation and emission spectra of Uranine, Resazurin, and Resorufin resulted in signals recorded by the three detectors of the field fluorimeters being a mixture of emission lights from all three tracers. To resolve this, tracer separation is usually achieved by solving three linear equations, resulting in the tracer concentrations C_j (ppb), with the calibration coefficients k_j^i (mV/ppb) and the intensity signals U_i (mV) as input parameters, where $i, j = 1,2,3$ represent the three different detectors (i) of the fluorometer and the three tracers (j), respectively (Schnegg, 2002). Based on this method, the calculated concentrations of the tracers (j) are only correct if the temperature, turbidity, and pH conditions (to name only a few) are the same during the calibration and subsequent field measurements.

Temperature and turbidity were not constant during our experiment. To correct for the effect of temperature, tracer, and detector-specific temperature (p_j^i [mV/mV]), correction coefficients were incorporated into the three sets of linear equations, which can be written in matrix form as:

$$\begin{bmatrix} k_1^1 & k_2^1 & k_3^1 \\ k_1^2 & k_2^2 & k_3^2 \\ k_1^3 & k_2^3 & k_3^3 \end{bmatrix} \circ \begin{bmatrix} p_1^1 & p_2^1 & p_3^1 \\ p_1^2 & p_2^2 & p_3^2 \\ p_1^3 & p_2^3 & p_3^3 \end{bmatrix} \cdot \begin{bmatrix} C_1 \\ C_2 \\ C_3 \end{bmatrix} = \begin{bmatrix} U_1 \\ U_2 \\ U_3 \end{bmatrix} \quad (1)$$

where \circ denotes the Hadamard product (element-wise).

The temperature correction coefficients p_j^i for field measurements were calculated following Leibundgut et al. (2009) as:

$$p_j^i = e^{(h_j^i \times (t_m - t_c))} \quad (2)$$

where t_m (K) and t_c (K) are the instantaneous field measurement temperature and the mean temperature during the calibration period, respectively. h_j^i (K⁻¹) are tracer and detector-specific parameters, which were calculated as:

$$h_j^i = \left[\ln(U_{1j}^i) - \ln(U_{2j}^i) \right] \times (t_1 - t_2)^{-1} \quad (3)$$

where U_{1j}^i (mV) and U_{2j}^i (mV) are the tracer (i) and detector (j) specific signal intensities at two different temperatures t_1 (K) and t_2 (K), respectively, which represents the slope relating the natural logarithm of tracer intensity to temperature.

The limited number of concentration levels investigated here prevented concentration-specific temperature correction factors being calculated. The mean of the three h_j^i , from the three concentrations for each tracer and detector during the laboratory experiments, was, therefore, used to determine the corrections.

The slope of fluorescence intensity (positive, zero or negative; Figure 2) due to increased turbidity ($d(\text{intensity})/d(\text{turbidity})$) is for each turbidity value a linear function of the fluorescence intensity, independent of the tracer analyzed (Figure 2e; different symbols). The slopes of these linear functions are more negative at higher turbidity (Figure 2e; different colors), and are linearly related to turbidity. All relationships are fluorimeter and detector-specific parameters that can be determined empirically from laboratory experiments.

To correct the measured fluorescence intensity (i.e., a mix of all three tracers) for turbidity effects, the measured turbidity was used to calculate the intensity dependent intensity-turbidity slope ($d(\text{Intensity})/$

d(Turbidity)), based on experimentally derived parameters. Based on the measured intensity, this slope was used to first calculate, and then exclude, the effects of turbidity on the fluorescence intensity, before the concentrations of the three tracers were calculated with the matrix describe in equation (1).

3. Results

3.1. Comparison of Uncorrected Field Fluorometer Measurements With Laboratory Measurements

The shape and peak timing of tracer breakthrough curves (BTC) measured by in situ FL30 field fluorometers showed good agreement with grab samples analyzed using a Varian Cary Eclipse laboratory fluorometer (supporting information Figure S2). However, in situ concentrations were underestimated compared to laboratory samples for all tracers. Mean concentrations measured in situ were 32%, 20%, and 37% lower than those of laboratory measurements for Uranine, Resazurin, and Resorufin, respectively. Although absolute differences between in situ and laboratory measurements increased with concentration for all tracers, relative differences were independent of tracer concentration for Resazurin. In contrast, relative differences in both Uranine and Resorufin between field and laboratory measurements revealed negative linear relationships with tracer concentration. This trend was small, yet significant, for Uranine ($r = -0.17, p < 0.05$) and more pronounced for Resorufin ($r = -0.58, p < 0.001$). In addition, a small number of field measurements diverged markedly from laboratory results in terms of both BTC shape and concentration (e.g., Uranine at Site 4). Repeated tracer injections over the 3 day study period reproduced similar BTC patterns at each measurement site.

3.2. Temperature Effects on Field Fluorometer Tracer Signals

Fluorescence intensities of all tracers decreased with increasing temperature (Figure 1). The response pattern was log-linear for Uranine and Resorufin for different tracer concentrations across the entire temperature range, but was more variable for Resazurin. In addition, the slope of the temperature-intensity relationships increased with tracer concentration for Uranine and Resorufin but exhibited no clear trend for Resazurin. These trends were broadly consistent across all field fluorometers, although the exact impact of temperature on specific tracer fluorescence intensities was unique to each device. Additional runs performed only at room temperature showed no significant trend in fluorescence intensities over the same time period.

3.3. Turbidity Effects on Field Fluorometer Tracer Signals

Strong linear responses in fluorescence intensities to changes in turbidity were observed for all tracers (Figures 2a–2c). These responses were intensity-dependent and exhibited decreases in the slope of the fluorescence intensity-turbidity relationship with increasing fluorescence intensity (Figures 2d and 2e). Responses were independent of the tracer analyzed (i.e., the absolute fluorescence quantum yield, rather than the tracer concentration, determined the slope of the relationship), thereby enabling the application of correction factors as detailed below.

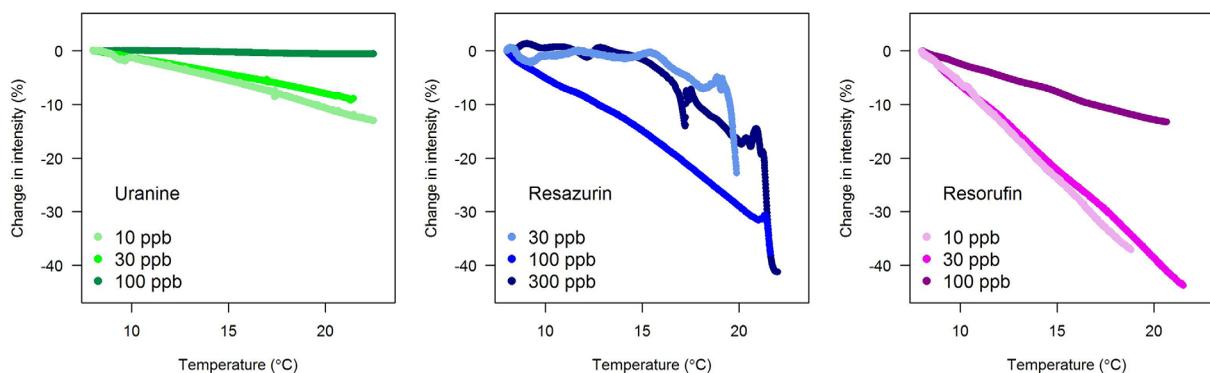


Figure 1. Temperature dependence of fluorescence intensities for Uranine, Resazurin, and Resorufin at different tracer concentrations. Data are shown from one FL30 field fluorometer. Similar patterns were observed across multiple devices.

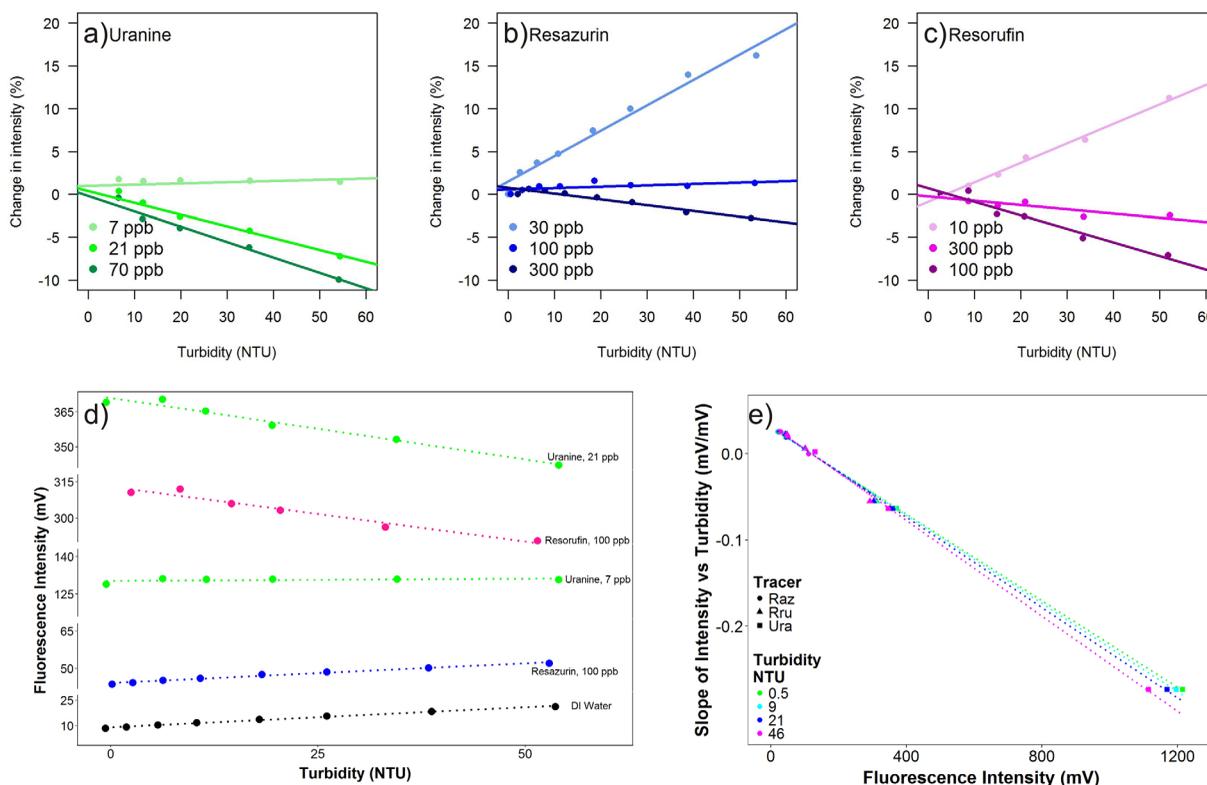


Figure 2. Turbidity dependence of fluorescence intensities for (a) Uranine, (b) Resazurin, and (c) Resorufin at different tracer concentrations. Changes in the slope of the fluorescence intensity-turbidity relationship are shown in (d), which are consistent for all tracers at different turbidity levels (e). Data are shown from one FL30 field fluorometer. Similar patterns were observed across multiple devices.

3.4. Application of Temperature and Turbidity Correction Factors

Correction methods were applied to laboratory data to demonstrate the ability of the methods to correct for the impacts of temperature and turbidity. Temperature corrections improved the fit of all fluorescent tracers, particularly for Uranine and Resazurin (Figure 3). In contrast, temperature corrections for Resorufin were less well defined, although these still represented a noticeable improvement over the uncorrected measurements. Turbidity corrections were highly effective for improving the fit of all fluorescent tracers (Figure 4). A representative example of adjusted field fluorometer BTCs after correction for the effects of temperature and turbidity is shown for Site 3 in Figure 5. Correction factors had negligible effects on

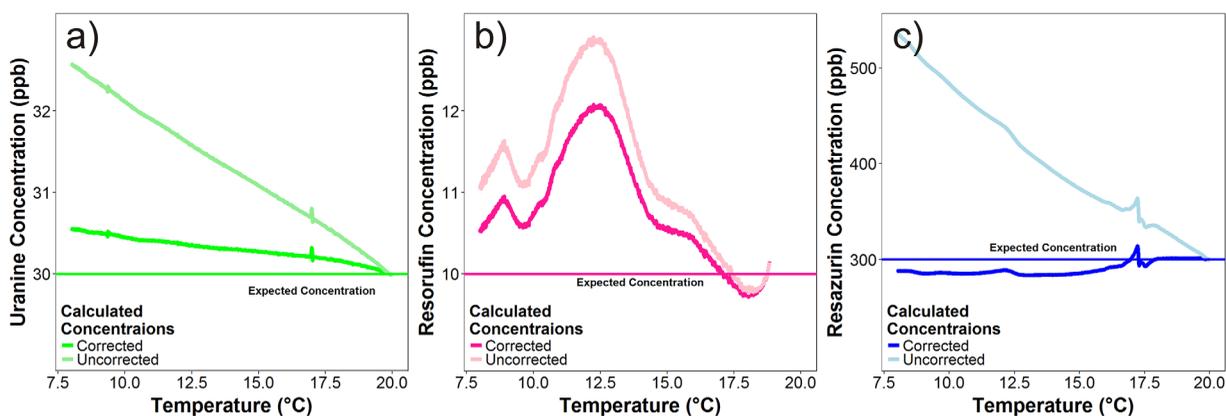


Figure 3. Comparisons of calculated tracer concentrations from laboratory measurements with expected concentrations for (a) Uranine, (b) Resorufin, and (c) Resazurin before and after correcting for changes in temperature.

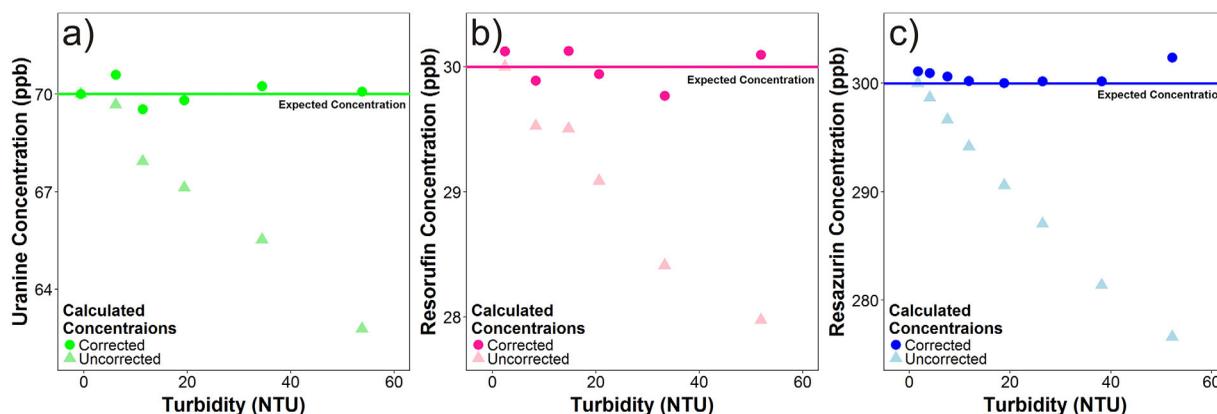


Figure 4. Comparisons of calculated tracer concentrations from laboratory experiments for (a) Uranine, (b) Resorufin, and (c) Resazurin before and after correcting for changes in turbidity.

Uranine BTCs. For Resazurin, temperature and turbidity corrections increased BTC concentrations at all measurement sites and improved the fit between field and laboratory measurements. In contrast, the application of temperature and turbidity correction factors to Resorufin BTCs reduced tracer concentrations slightly, thereby increasing the discrepancy between field and laboratory measurements (Figure 5).

3.5. Comparison of Field and Laboratory Fluorometers

In a temperature-controlled laboratory environment, filtered grab samples analyzed on a single FL30 field fluorometer showed good agreement with those analyzed on a laboratory Varian Cary Eclipse fluorometer (supporting information Figure S3). Filtered samples of Uranine and Resorufin were more closely related to laboratory measurements than their unfiltered counterparts, while Resazurin samples showed little difference. The FL30 field fluorometer underestimated tracer concentrations relative to the laboratory fluorometer. However, this difference was less pronounced for filtered grab samples than measurements made in situ.

4. Discussion

The recent increase in the application of multitracer field fluorometers within the hydrological sciences community can be attributed to their advantages over more traditional measurement methods, allowing for a more detailed analysis of highly dynamic system behavior and hydrological events (Schmadel et al., 2016). However, the results of this study highlight the potentially large discrepancies in fluorescent tracer concentrations that remain between in situ measurements and samples analyzed under laboratory conditions, despite field calibrations being performed immediately prior to deployment. The similarity of repeated tracer injections over 3 days suggested that these errors were consistent through time, although it is not possible to determine whether errors were device or site-specific because the field fluorometers remained in the same location for the duration of the study period. Irrespective of this, errors in BTCs measured by field fluorometers have the potential to affect calculations of solute transport dynamics and rates of ecosystem respiration. Consequently, applying correction factors to reduce the effect of such discrepancies is critical to prevent incorrect inferences being drawn regarding the physical and biogeochemical processes underpinning such patterns.

Laboratory tests demonstrated the strong influence of both temperature and turbidity on fluorescence intensities as measured by field fluorometers, highlighting the need to account for changes in these parameters during stream tracer experiments. The fluorescence intensity of all tracers decreased linearly under increased temperature, as would be expected for fluorescent solutions because collisional quenching increases with temperature (Baker, 2005). Similar findings for Uranine have been reported previously (Leibundgut et al., 2009) but the observed effects of temperature on Resazurin and Resorufin fluorescence intensities contradict those found by other studies. For example, Haggerty et al. (2008) suggested that temperature has a negligible effect on the fluorescence intensity of Resazurin, while our results show the opposite, although we note that our experimental temperature range (5–25°C) is wider and more representative

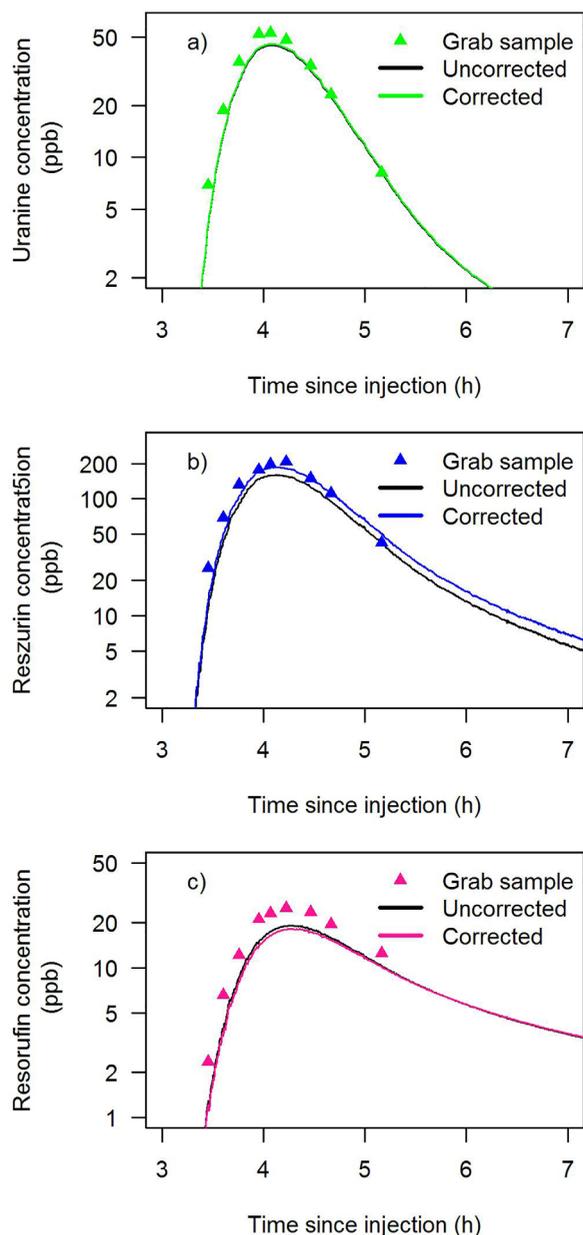


Figure 5. Breakthrough curves of (a) Uranine, (b) Resazurin, and (c) Resorufin at site 3 (see supporting information Figure S1) measured by an FL30 field fluorometer before and after correction for temperature and turbidity. Also shown are grab samples analyzed on a laboratory fluorometer. Similar patterns were observed at other sites.

to the measured signal U_j (equation (1)) is lower than that of Raz, compared to the uncorrected signal. While changes between uncorrected and corrected BTCs may appear small, they can make a substantial difference to estimates of metabolic activity within the stream reach. As a representative example, volume-averaged Resazurin-Resorufin transformation rate coefficients, a proxy for aerobic ecosystem respiration, between Sites 2 and 3 were 7.1% higher using corrected BTCs relative to uncorrected data (see supporting information Text S3 for calculation details).

While it is evident that changes in temperature and turbidity can influence fluorescence measurements, it may be that our results were also susceptible to additional environmental factors, such as changes in

of most field conditions than the 23–31°C range used by Haggerty et al. (2008). Similarly, Lemke et al. (2013b) reported a mildly positive effect of temperature on the fluorescence intensity of Resorufin for a single instrument, while our results indicate a strong negative relationship, which was consistent for multiple instruments, despite measurements being conducted across a similar temperature range to Lemke et al. (2013b). Our repeated measurements indicate that thermal quenching of fluorescence quantum yields occurs across a range of tracer concentrations. The strength of this effect appears stronger at lower concentrations for Uranine and Resorufin whereas the pattern for Resazurin is less clear owing to noise in the data set. Changes in turbidity also caused pronounced linear responses in fluorescence intensity for all measured tracers, which cannot be attributed solely to the linear effects of stray light as suggested by Schnegg and Flynn (2002). Amplification of fluorescence intensity with turbidity at lower concentrations has also been reported for certain fractions of organic carbon (Khamis et al., 2015) and may be attributable either to suspended particles scattering the excitation light towards the detector (Leeuw et al., 2013; Schnegg & Flynn, 2002) or to the fluorescence of organic material contained within the sediment. At high concentrations, quenching of fluorescent signals observed for all three tracers is likely due to a combination of light absorption and scattering by dissolved constituents and suspended particles, as reported previously for fluorescence measurements of organic matter (Downing et al., 2012; Saraceno et al., 2009).

Application of correction factors to field fluorometer BTC signals had the most impact on Resazurin and Resorufin concentrations and a smaller impact on Uranine concentrations. This is likely due to the relatively small effect of temperature on Uranine fluorescence intensity combined with the environmental conditions experienced during the experiments. Temperature varied by 3–4°C between the calibration period and subsequent tracer tests, which changed the tracer intensities by up to 15%. Turbidity values also varied by up to 10 NTU, but exerted less influence on measured fluorescence intensities relative to temperature changes. Note, however, that more dynamic flow conditions, such as storm-induced flood events, would most likely cause greater variability in temperature and turbidity (Flett et al., 2017; Khamis et al., 2015). The degree to which the correction factors improved the fit of the data, as measured against laboratory-analyzed grab samples, was inconsistent: Uranine showed little change, Resazurin concentrations fitted more closely with laboratory samples, but Resorufin concentrations showed larger discrepancies. This is most likely because the value of h_j^{ru} (equation (3)) is more negative than h_j^{az} , and consequently the value of p_j^{ru} (equation (2)) is lower than p_j^{az} ; therefore, the relative contribution of Rru

background dissolved organic matter composition or the site-specific particle size distribution of suspended sediments (Gregory, 2005), which were not accounted for. This may explain the mismatch between field and laboratory measurements. Consequently, our results highlight the necessity of collecting regular control samples throughout BTCs to validate field results. Ideally, control samples would be analyzed using a laboratory fluorometer. However, the expense and size of these instruments can impose limits on their availability. Our results show a single field fluorometer operating in benchtop mode can produce comparable results to a laboratory fluorometer at approximately 10–20% of the cost. It follows that such devices may be of use for validating BTCs using grab samples in situations where a laboratory fluorometer is unavailable, for example in remote field locations where logistical constraints prevent laboratory access.

Based on our results, we propose guidelines for future experiments using multitracer field fluorometers:

1. Calibrate instruments in the field under conditions that match those during the measurement window.
2. Unless targeting dynamic events specifically, choose a study period where background conditions are as stable as possible (e.g., base flow).
3. Characterize background environmental conditions (e.g., temperature, turbidity, pH, discharge) continuously throughout the experiment.
4. Acquire grab samples through BTCs at each location for subsequent analysis on a single instrument (ideally a laboratory fluorometer). The number of grab samples required is dependent on site-specific conditions (e.g., flow velocity) and the purpose of the test.
5. If conducting multiple concurrent tracer tests, ensure fluorometers are cleaned between tests to minimize sensor fouling.

5. Conclusions

Multitracer field fluorometers are used increasingly within the hydrological sciences to characterize stream transport properties and ecosystem reaction rates (González-Pinzón et al., 2016; Schmadel et al., 2016). However, obtaining high-quality fluorescence measurements is challenging given the variability in environmental conditions that exists within stream ecosystems (Abbott et al., 2016; Blaen et al., 2016; Krause et al., 2015). This study enhances our understanding of how field fluorometer measurements are affected by changes in temperature, turbidity, and tracer concentration, thus highlighting some of the potential sources of error that can occur under realistic field conditions. We conclude that multitracer field fluorometers can be extremely useful devices for characterizing tracer dynamics in situ, but suggest that field measurements should always be supplemented by grab samples to ensure their validity. Further work is also required to establish the degree to which other environmental factors influence field fluorometer measurements. Careful application of these guidelines will improve our capacity to use conservative and reactive fluorescent tracers to measure and understand the interactions between solute transport and retention dynamics and metabolic processes in stream ecosystems.

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