
Analysis of in-vivo chlorophyll-a and optical brighteners using a Trilogy fluorometer

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Abstract

Turner Designs Trilogy fluorometer was used to measure in-vivo chlorophyll-a and optical brighteners in relative fluorescence units. Paired quantitative chlorophyll-a analyses were performed by EPA methods 445 and 446 at a NELAC-accredited laboratory. A calibration curve for estimating measured chlorophyll-a from relative fluorescence is presented, with an r^2 value of 0.92. Optical brighteners, which may be surrogate for wastewater contamination due to their presence in laundry detergents, were monitored upstream and downstream of domestic wastewater treatment plant outfalls. Optical brighteners did not follow expected patterns and do not appear to be useful in source water identification for domestic wastewater in Austin streams.

Introduction

Chlorophyll-a is an indicator of photosynthetic potential and algal biomass (Flemer 1969). Chlorophyll-a analysis is a vital component to water quality monitoring when evaluating the impact of nutrient enrichment on aquatic systems (Harding and Perry 1997). Quantitative analysis for chlorophyll-a at a contract laboratory is expensive relative to the cost of conventional water quality analytes such as nutrients. Frequent sampling may be needed to discover and evaluate algae blooms, which may be highly variable in duration and occurrence based on climatic conditions (COA 1992). In-house use of a bench top Turner Designs Trilogy laboratory fluorometer could enable rapid chlorophyll-a analysis at substantially reduced costs. The in-vivo chlorophyll-a method (Lorenzen 1966) yields results in relative fluorescence units, but a calibration curve could be established using paired quantitative analytical results and assessment of natural background water fluorescence to provide concentration data.

Optical brighteners (OB) are dyes added to detergents to make fabrics appear whiter by absorbing light in the ultraviolet spectrum and re-emitting it in the blue region. OBs may be a surrogate indicator for raw or treated wastewater contamination of streams with some laundry component and have been found to be useful in identifying water sources of environmental samples (Aley 1991). OBs may be removed from water by adsorption to soils or organic matter, and will decay by photolysis (Fay et al 1995). Surfactants associated with these detergents can be toxic, particularly to fish, in the aquatic environment although the concentration and type of surfactants varies widely across cleaning products (Knepper et al 2003).

Methods

Ambient water samples were collected in amber plastic bottles or whirlpack bags and delivered on ice in dark containers to the laboratory. Samples were agitated, and then 4 mL were extracted using disposable plastic syringes. Methylacrylate plastic cuvettes (13 x 13 X 100 mm) were rinsed three times with sample water injected via the syringe prior to filling and inserting into the fluorometer. Fluorescence was then measured in relative fluorescence units (RFU) using the chlorophyll-a in-vivo module of the Turner Designs Trilogy fluorometer. Cuvettes were handled with gloves to avoid fouling and agitated to release trapped air bubbles. Background fluorescence was determined for each sample following the same procedure, but samples were first filtered thru a 0.45 μ m filter disk screwed onto the syringe.

Optical brighteners were measured in relative fluorescence units using the Trilogy fluorometer OB module at one point upstream and at two points downstream of domestic wastewater discharges on 5 creeks in November 2009 as part of a separate City of Austin study. No other permitted wastewater discharge facilities were located in the headwaters of the watershed above the furthest upstream sample point. Samples were collected in whirl pack bags and placed in a cooler on ice until analysis within 8 hours of collection. Wastewater flows were small in magnitude relative to ambient flows. Samples were collected and handled by consistent grab sampling methods. Methylacrylate plastic cuvettes (13 x 13 X 100 mm) were rinsed three times with sample water injected via a disposable 5 mL plastic syringe prior to filling and inserting into the fluorometer for relative fluorescence measurement.

Results

Type I deionized water blanks were analyzed for chlorophyll-a in the lab (n=3, mean=6.54 rfu, std=0.22). Background fluorescence was determined for each filtered sample, and in-vivo chlorophyll-a was determined by relative fluorescence (RFU) for each whole water (unfiltered) sample. The difference between the whole water and the filtered sample was compared to laboratory-determined chlorophyll-a in µg/L by EPA method 445 or 446. Based on data from 20 sample events at sites ranging including spring outfalls, lakes and creeks, there is good linear agreement between RFU and measured chlorophyll-a (Table 1, Figure 1). A linear least-squares regression of RFU (total minus filtered) and measured chlorophyll-a yields an r^2 value of 0.92 (n=20).

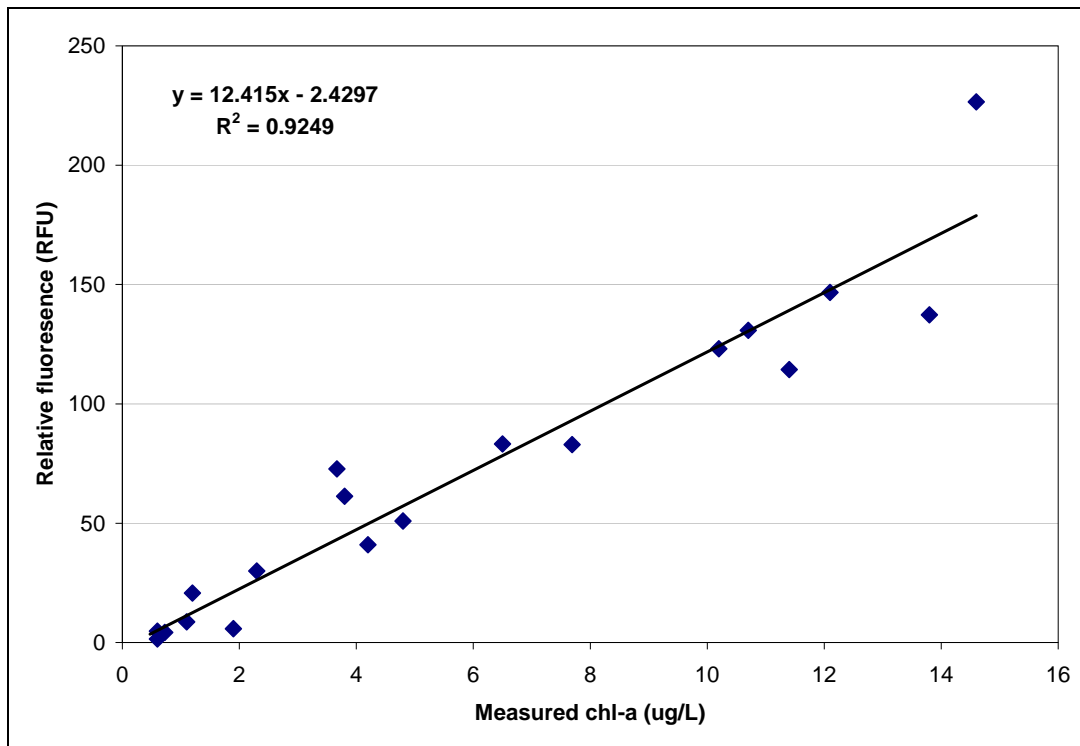


Figure 1. Least squares regression of relative fluorescence and measured chlorophyll-a for whole water samples (n=20).

Table 1. Summary of measured chlorophyll-a and relative fluorescence.

Date	Site	Medium	Measured chl-a (EPA 445 or 446)	Filtered RFU	Total RFU
09/09/09	Barton Spring	Ground Water	0.476	.	8.36
09/09/09	Barton Spring Pool @ Downstream Dam	Ground Water	1.9	8.86	14.66
09/09/09	Barton Springs Pool @ Shallow End	Ground Water	0.598	.	10.41
09/09/09	Barton Springs Pool site BSP-9	Ground Water	0.726	.	13.06
09/16/09	Town Lake @ 1st St (CC)	Surface Water	3.67	17.88	90.64
09/16/09	Town Lake @ Basin (AC)	Surface Water	14.6	25.73	252.26
09/24/09	Town Lake @ 1st St (CC)	Surface Water	7.69	19.53	102.47
10/01/09	Bear Creek @ Davis Pond	Surface Water	4.8	38.19	89.14
10/01/09	Bear Creek Upstream of Bear Creek Pass	Surface Water	4.2	40.18	81.12
10/07/09	Barton Creek @ Hwy 71 Below Little Barton	Surface Water	1.1	23.24	31.97
10/07/09	West Country Club Creek @ East Oltorf St	Surface Water	0.6	35.34	40.1
11/19/09	Town Lake @ 1st St (CC)	Surface Water	1.2	14.24	35.04
11/19/09	Town Lake @ Basin (AC)	Surface Water	13.8	16.34	153.65
11/19/09	Town Lake @ Red Bud Isle (EC)	Surface Water	6.5	15.45	98.67
01/27/10	Town Lake @ 1st St (CC)	Surface Water	2.3	12.77	42.77
01/27/10	Town Lake @ Basin (AC)	Surface Water	12.1	16.26	162.95
01/27/10	Town Lake @ Red Bud Isle (EC)	Surface Water	3.8	13.32	74.61
03/18/10	Lake Long @ Dam (LWL3)	Surface Water	10.7	19.87	150.67
03/18/10	Lake Long mid-lake of Eastern Arm (LWL4)	Surface Water	11.4	19.85	134.3
03/18/10	Lake Long mid-lake of Western Arm (LWL5)	Surface Water	10.2	20.46	143.57
10/21/09	Barton Spring Pool @ Downstream Dam	Benthic Cover	2270	767.51	5014.68
10/21/09	Barton Springs Pool @ Shallow End	Benthic Cover	5040	1667.27	22274.28
10/21/09	Barton Springs Pool site BSP-9	Benthic Cover	1230	1303.22	10336.71

Although based on repeated measures from only one water body, background fluorescence appears highly variable between samples when analyzed as an absolute value or a percentage of the total RFU reading (Table 2). Therefore, it is not reasonable to assume a constant background value. Also, the filtered measurement should be conducted with every sampling event so that the difference (total minus background) can be used in the calibration.

Table 2. Summary of repeated measures of background fluorescence.

Site	N	Filtered % of Total		Filtered RFU	
		Mean	Std	Mean	Std
Town Lake @ 1st St (CC)	4	27.32	10.16	16.11	3.13
Town Lake @ Basin (AC)	3	10.27	0.33	19.44	5.44
Town Lake @ Red Bud Isle (EC)	2	16.76	1.55	14.39	1.51

Benthic periphyton scrapings, generated by scraping a known area of the surface of a rock removed from a creek with a wire brush and collecting the rinse water, yield substantially higher estimates of background relative fluorescence than whole water samples. The abrasion of the rocks may be rupturing photosynthetic cell walls releasing pigment, or may be releasing minerals from the rocks themselves. More sampling is necessary to fully evaluate the use of the in-vivo method for periphyton scrapings.

Optical brighteners did not follow expected patterns of increase downstream of domestic wastewater outfalls (figure 2), but ambient samples did yield higher values than a type I deionized water sample (1852.4 RFU). In only one (Mustang Creek) of four watersheds was an expected pattern of some increase downstream of the wastewater outfall observed. One sample (upstream at South Fork San Gabriel) was damaged during transportation and could not be analyzed. Mustang Creek was also the most turbid creek sampled, and did yield the highest RFU values. However, the OB concentration appeared to increase with increasing distance downstream of the outfall.

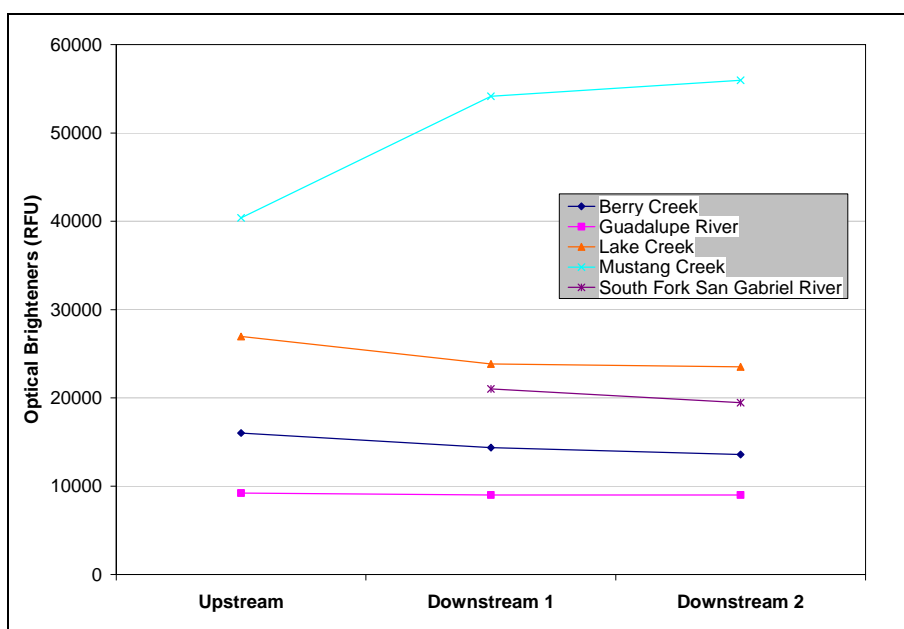


Figure 2. Optical brighteners in relation to wastewater outfalls from 5 creeks in November 2009.

Conclusions

Relative fluorescence measurement with the fluorometer may be used with the following calibration curve to yield reasonable estimates of measured chlorophyll-a in whole water samples, when project quality control specifications do not require measurement at an accredited laboratory:

$$\text{Chlorophyll-a } (\mu\text{g/L}) = (\text{RFU} + 2.4297) / 12.415$$

Because of variability in background fluorescence, each sample should be measured for both filtered and total RFU. The RFU method should not be used to estimate chlorophyll-a for periphytic algae sampling via scrapings until additional verification data is collected and analyzed.

Optical brighteners do not consistently follow expected trends downstream of wastewater outfalls. Use of the Trilogy fluorometer OB module as a surrogate for wastewater contamination of streams is not supported by this evaluation. OBs by this analytical method do not appear to be useful in source water identification.

References

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