Supplementary information for:

Assessing the potential of fluorescence spectroscopy to monitor contaminants in source waters and water reuse systems

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Supplemental Methods

Experimental setup and procedures for contaminant spiking

Contaminant additions were performed in the laboratory to assess the effect of increasing concentrations on DOM fluorescence. Known concentrations of caffeine, lopinavir, isoxathion and ibuprofen were each dissolved into 200ml of ultra-pure water to make a stock solution for each contaminant, while no stock solutions were made for diesel and gasoline. Varying amounts from each stock solution were then titrated into each water source starting with the lowest volume and subsequently increasing the concentration. Each contaminant was titrated against 4 liters of water of each type. At each step of titration, a 10 ml sample was collected in sterile and acid washed conical tubes and stored in the refrigerator for 3D fluorescence analysis. At each step of titration, measurements by a C3 insitu fluorometer were taken while constant mixing was done. Before using the fluorometer, it was calibrated with a known standard under controlled conditions in laboratory as recommended by the manufacturer. All standard solutions for calibration were prepared using deionized water. However, the C3 fluorometer measurements were taken in an uncalibrated raw fluorescence mode because we wanted a comparison with some of the previous studies like Watras et al., (2011) whose readings were also taken in Relative fluorescence units (RFU), so all units were in RFU. All the measurements for CDOM and tryptophan were done in the dark to minimize the interruption of other sources of light. Each sample was kept in contact with the sensor during 2-5 minutes to obtain readings. Between each solution, the sensor, and the beaker was extensively rinsed with ultrapure water and for the case of gasoline and diesel, the sensor was rinsed with hydrochloric acid solution (5% v/v) followed by hydrogen peroxide. There was continuous mixing to prevent creation of dead zones. All insitu measurement were done in the darkness to prevent any interruption of other light sources. A Minimum of duplicate values were

taken for all measurements on a 3D benchtop while in case of C3 fluorometer, an average value for reading taken (more than 200 readings) for each experiment and all experiments were carried out in duplicate.

PARAFAC Modeling

In order to identify individual fluorescent components in our EEMs, we applied PARAFAC modeling in MATLAB using the "drEEM Toolbox" (ver 0.1.0) following the recommendations and procedures of (Murphy et al. 2013). Regions of the spectrum influenced by Rayleigh scatter peaks were removed and a total of 102 samples from all experiments with all the six contaminants were used. Regions that are influenced by Rayleigh and scatter masking were removed. An outlier identification test was performed for 3 to 9 models run with non-negativity constraints and five samples emerged as outliers and were removed. Series of criteria were used which in order to identify a number of fluorescent components. These criteria included examination the shape of spectral loadings, the leverage analysis, the residual analysis and the split-half analysis. The 5, 6 and 7 component models had residual EEMs that contained mostly instrumental noise with minor systematic signals observed in the residual EEMs. Random initialization modeling gave core consistency values of 15.14%, 0.21%, and 0.70 % for a 5, 6 and 7 component models respectively. A PARAFAC model with 5 components was validated.

Supplemental Figures

Supplemental Figures

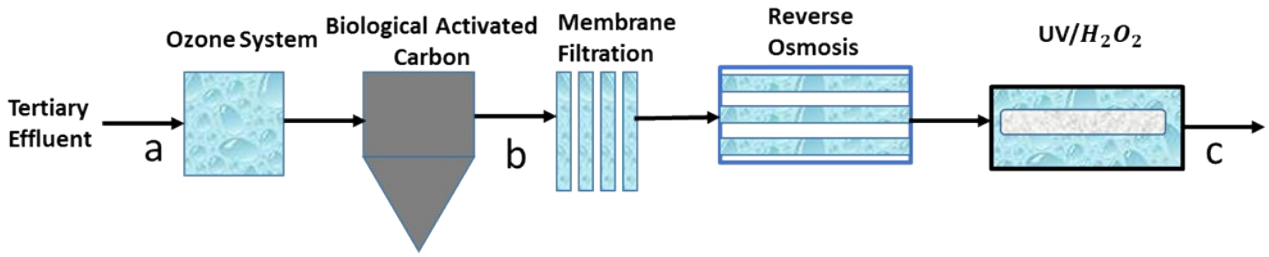

Figure S1. Treatment train of the Advanced Water Purification Facility in San Diego, CA. Grab water samples were collected at the orange marked points. (a) Pre-ozone water, (b) Post-ozone, (c) UV-AOP water

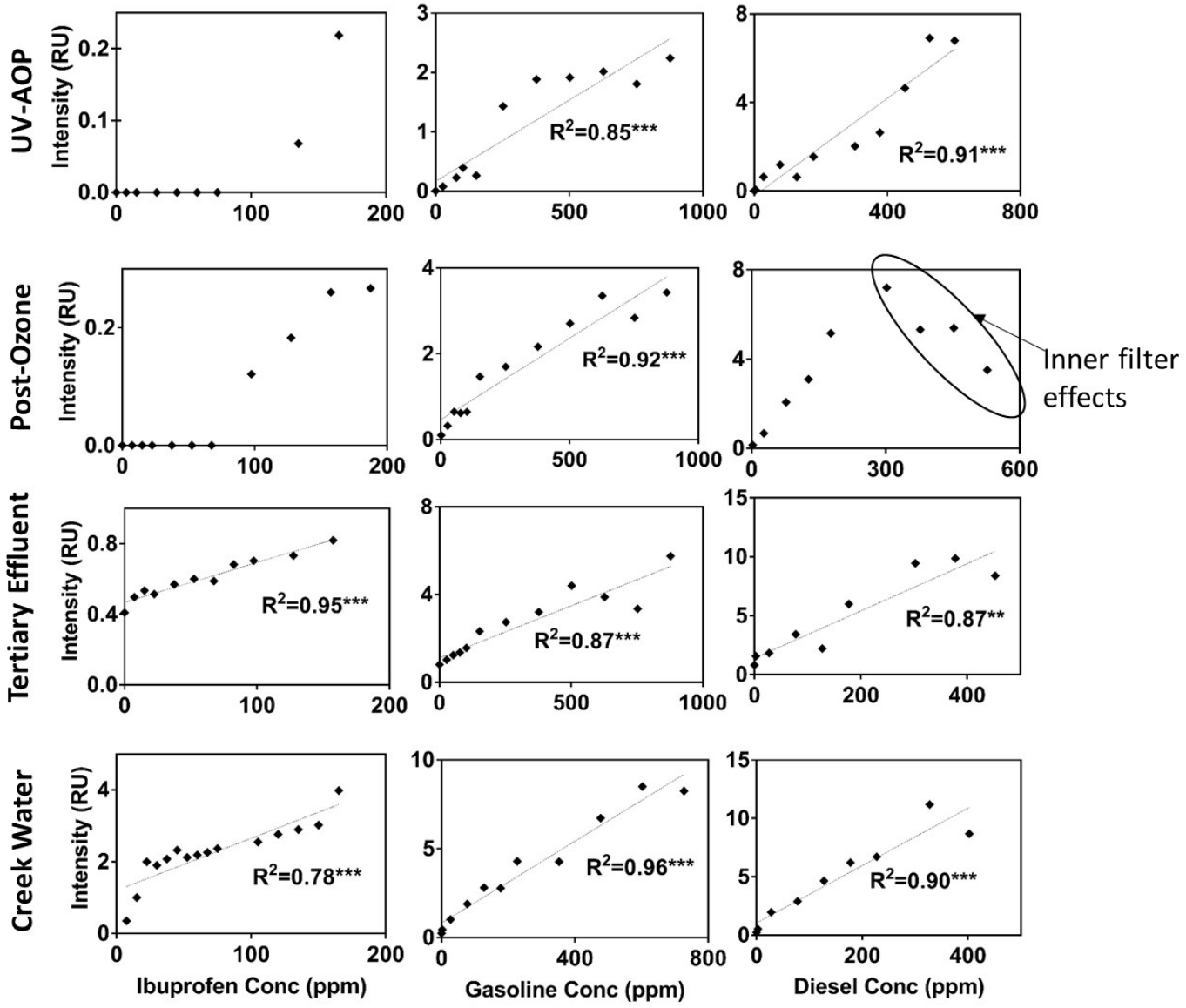

Figure S2. Examples of correlation analyses between contaminant concentration and tyrosine-like Peak B measured on the benchtop fluorometer. Significant relationships are shown (* $p<0.05$, ** $p<0.005$, *** $p<0.0001$).

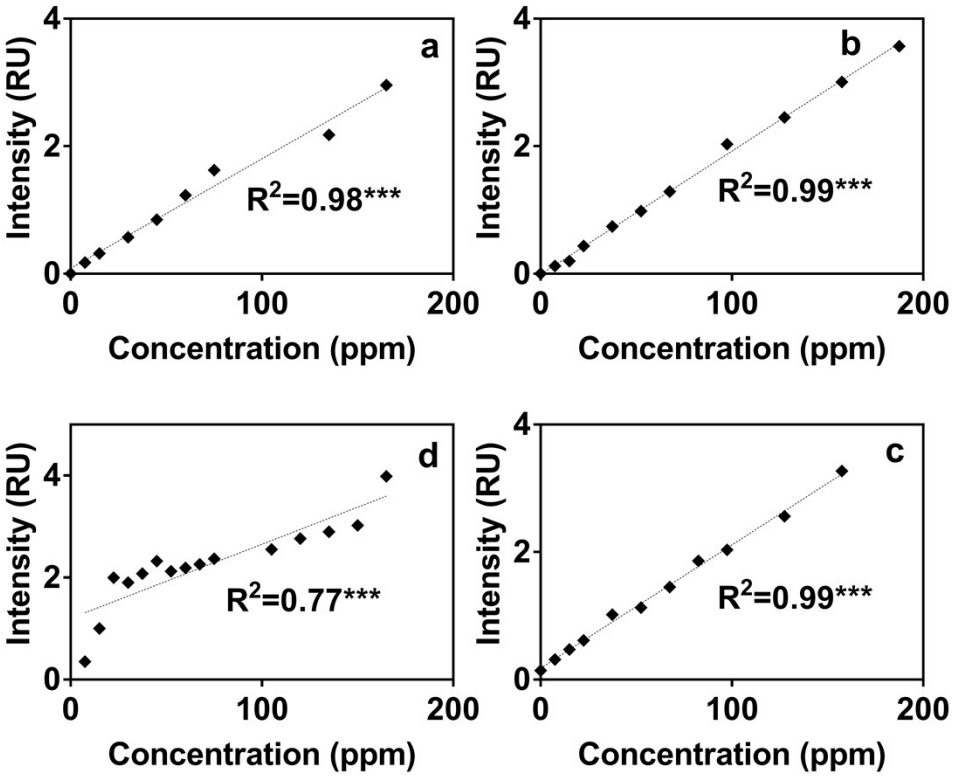

Figure S3. Scatterplots showing ibuprofen peak intensities (Peak I) and ibuprofen concentrations in different water types ((a) UV-AOP water, (b) post-ozone water, (c) creek water and (d) tertiary effluent as measured using the benchtop fluorometer. All correlations were significant ($p<0.05$, ** $p<0.005$, *** $p<0.0001$).

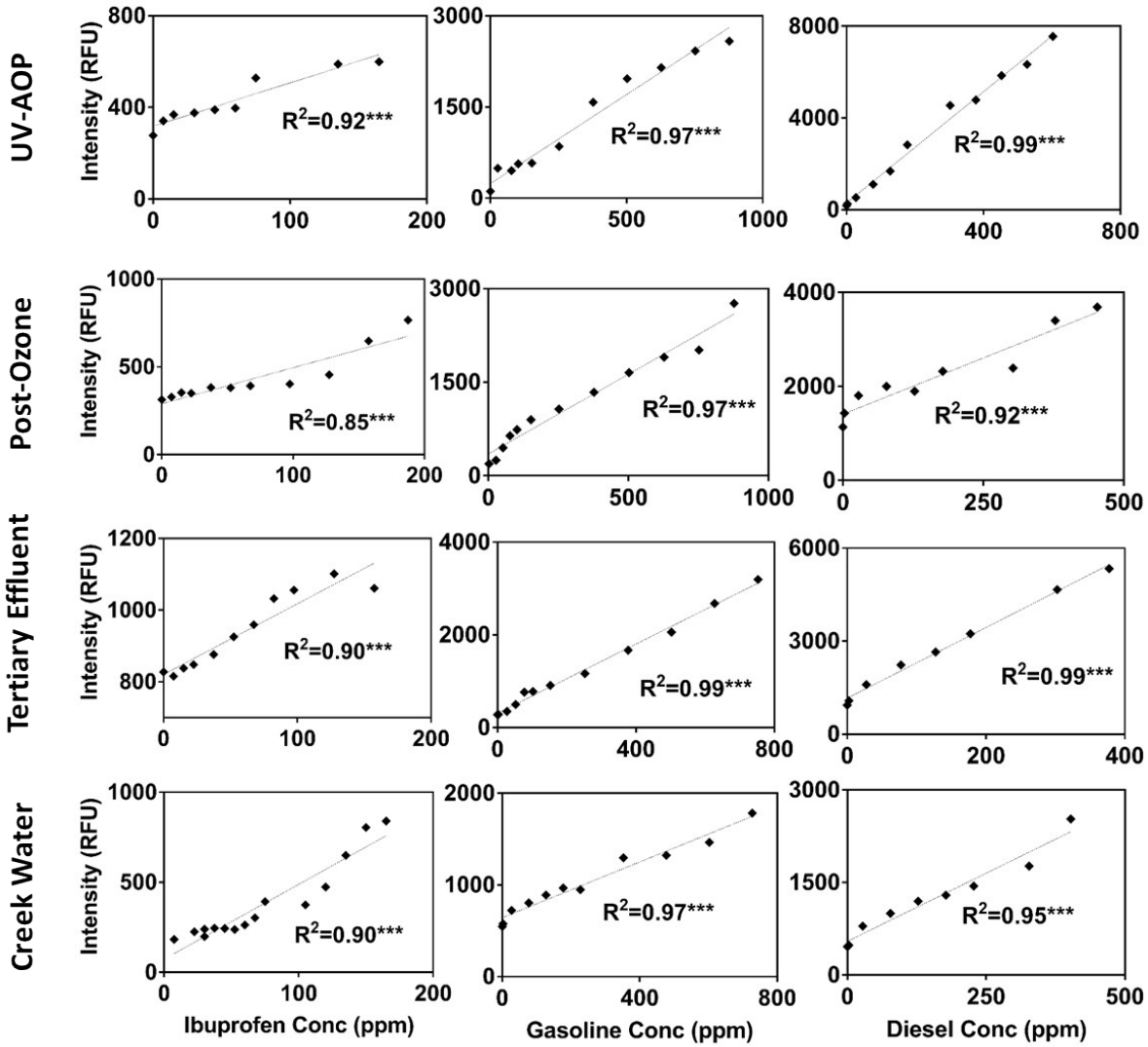

Figure S4. Scatterplots of contaminant concentrations for ibuprofen (left panel), gasoline (middle panel), and diesel (right panel) and tryptophan in different water types for a submersible fluorometer. All correlations were significant ($p<0.05$, ** $p<0.005$, *** $p<0.0001$).

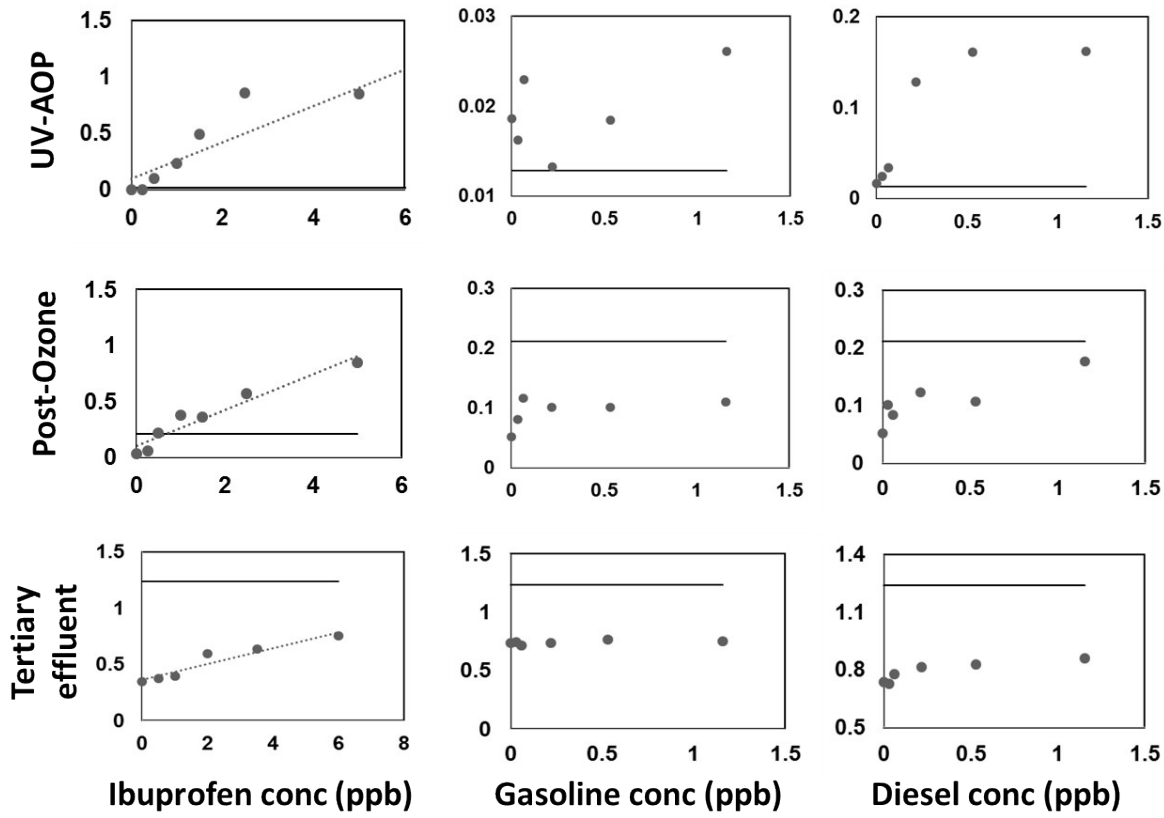

Figure S5. Scatterplots showing contaminant concentrations and peak intensities for ibuprofen (left panel), gasoline (middle panel), and diesel (right panel) in different water types at very low concentrations for a 3D benchtop fluorometer. Peak B intensities were used for Gasoline and Diesel while peak I intensities were use for Ibuprofen. The solid line represents the IDLs for each water source.

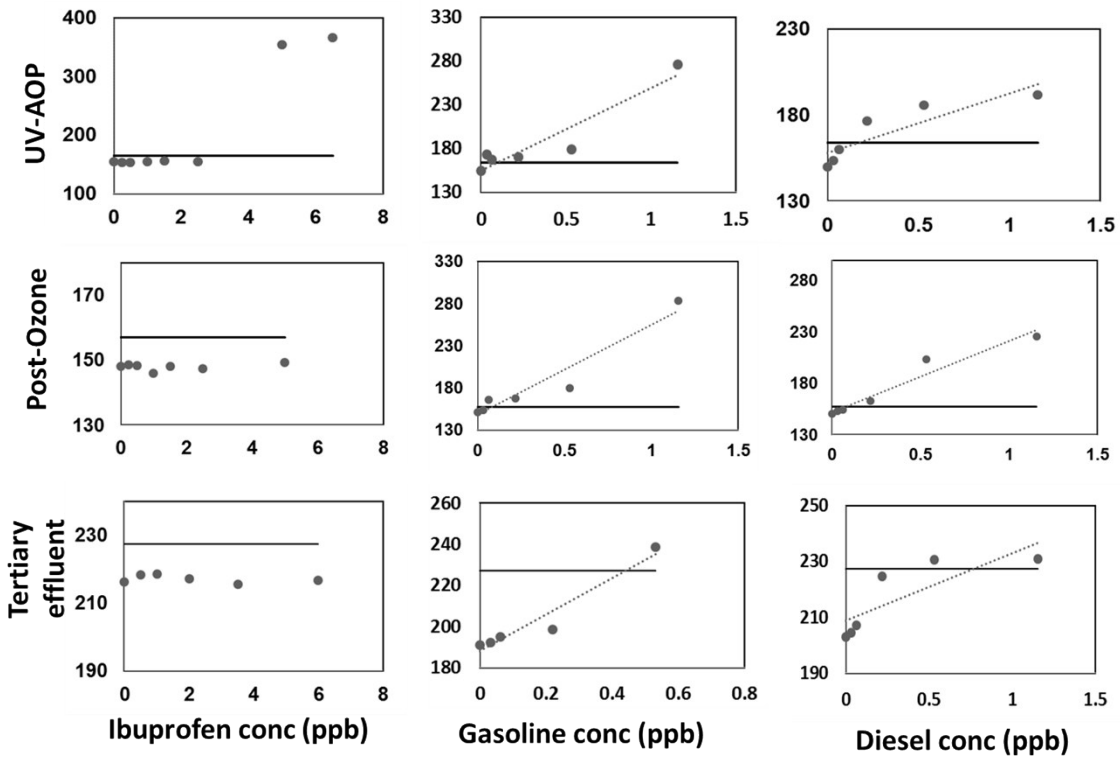

Figure S6. Examples of correlation analyses between contaminant concentrations for ibuprofen (left panel), gasoline (middle panel), and diesel (right panel) and Region T intensities in different water types at very low concentrations for a Submersible fluorometer. The solid line represents the IDLs for each water source.

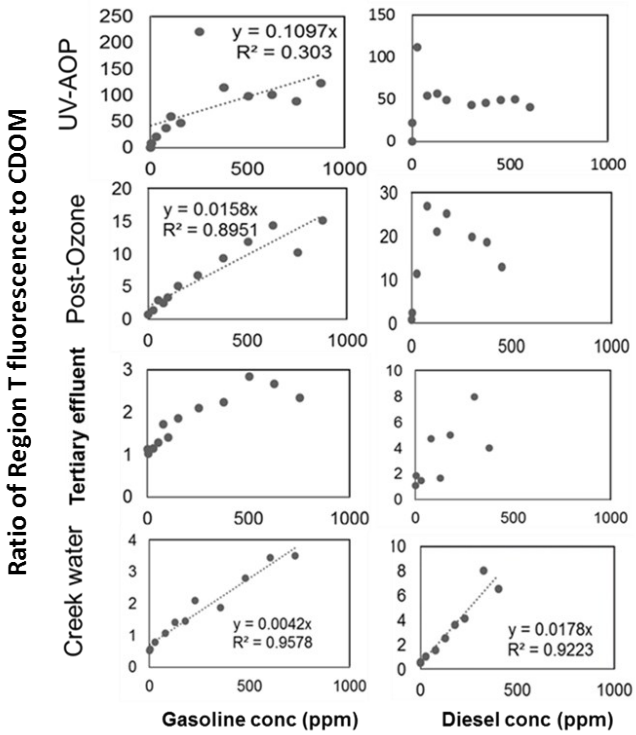

Figure S7. Regression analysis of ratio of tryptophan to CDOM peak intensities with contaminant concentration from a 3D Bench top fluorometer.

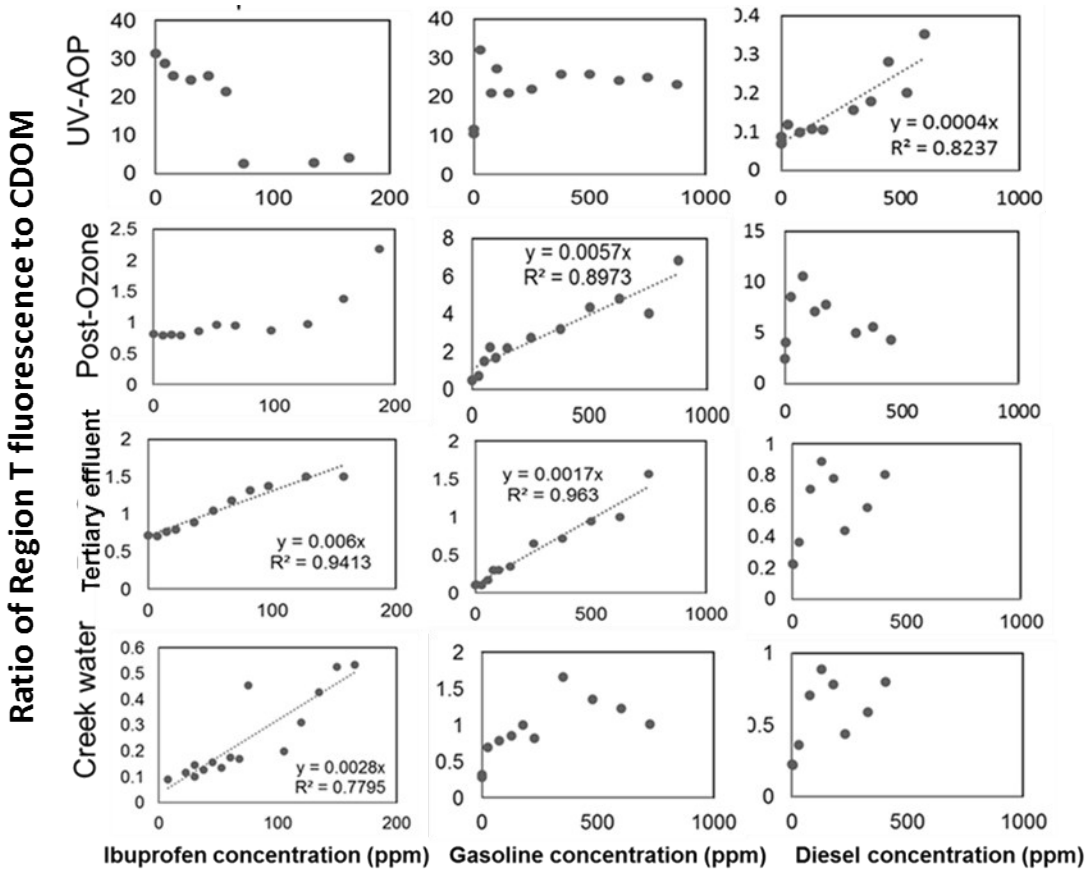

Figure S8. Regression analysis of ratio of tryptophan to CDOM peak intensities with contaminant concentration from a submersible fluorometer.

Supplemental Tables

Table S1. C3 submersible fluorometer physical and electrical specifications.

Parameter	Value
Weight	1.64 kg
Length	23 cm
Diameter	10 cm
Material	Delrin Plastic
Temperature	-2 to 50 °C.
Depth	0 to 600 meters
Interface	RS232 Interface
Minimum Sample Interval	1 Second
Minimum Power Supply	8 to 30 volts
Maximum Current Draw at 12 volts	
- operational	200 mA
- sleep mode	3 mA

Source: Turner Designs

Table S2. CDOM and TRP ex/em wavelengths for the submersible fluorometer and the Aqualog benchtop fluorometer.

Equipment	Peak	Excitation and emission wavelength
In-situ portable submersible fluorometer	CDOM	Ex 325±120, em470±60
	Region T	Ex285, em350±55
3D benchtop fluorometer	CDOM	Ex325, em470
	Region T	Ex285, em350

Table S3. Summary Regression analysis results for the five peak intensities and contaminant concentrations from 3D bench top fluorometer

Contaminant	Water source	n	PEAK A		PEAK B		PEAK T		PEAK C		PEAK M	
			R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value
Diesel	Tertiary effluent	9	0.82	<0.005	0.87	<0.005	0.87	<0.0005	0.50	<0.05	0.68	<0.05
	Post Ozone	9	0.26	>0.05	0.46	<0.05	0.31	>0.05	0.29	>0.05	0.20	>0.05
	UV-AOP	11	0.78	<0.005	0.91	<0.0001	0.90	<0.0001	0.79	<0.005	0.85	<0.0001
	Creek water	9	0.78	<0.05	0.90	<0.005	0.88	<0.005	0.11	>0.05	0.85	<0.005
Gasoline	Tertiary effluent	13	0.04	>0.05	0.87	<0.0001	0.87	<0.0001	0.01	>0.05	0.01	>0.05
	Post Ozone	12	0.94	<0.0001	0.92	<0.0001	0.89	<0.0001	0.41	<0.05	0.43	<0.05
	UV-AOP	12	0.74	<0.005	0.84	<0.0001	0.82	<0.0001	0.64	<0.05	0.79	<0.05
	Creek water	11	0.44	<0.05	0.96	<0.0001	0.96	<0.0001	0.61	<0.005	0.60	<0.05
Ibuprofen	Tertiary effluent	11	0.01	>0.05	0.94	<0.0001	0.01	>0.05	0.18	>0.05	0.40	<0.05
	Post Ozone	11	0.79	<0.005	0.90	<0.0001	0.09	>0.05	0.75	<0.05	0.54	<0.05
	UV-AOP	9	0.75	<0.05	0.70	<0.05	na	na	0.84	<0.005	0.40	>0.05
	Creek water	15	0.14	>0.05	0.76	<0.0001	0.50	<0.005	0.23	>0.05	0.55	<0.005
Isoxathion	Creek water	7	0.71	<0.05	0.75	<0.05	0.80	<0.05	0.90	<0.05	0.89	<0.05
Lopinavir	Creek water	7	0.40	>0.05	0.97	<0.0001	0.97	<0.0001	0.87	<0.05	0.89	<0.05
Caffeine	Creek Water	5	0.73	>0.05	0.80	<0.05	0.63	>0.05	0.03	>0.05	0.70	>0.05

Table S4. Distribution (%) and loading (RU) of each component in select samples of this study.

Water source and Contaminant	Contaminant Conc (ppm)	C1 % Loading (RU)	C2 % Loading (RU)	C3 % Loading (RU)	C4 % Loading (RU)	C5 % Loading (RU)
Tertiary effluent						
Ibuprofen	128	32	0	21	31	17
Gasoline	153	14	52	17	17	0
Diesel	178	14	0	77	9	0
Post-Ozone						
Ibuprofen	158	12	0	0	8	80
Gasoline	153	12	72	8	9	0
Diesel	178	11	18	67	3	0
UV-AOP						
Ibuprofen	165	1	0	0	0	98
Gasoline	153	6	87	5	2	0
Diesel	178	1	70	21	2	6
Creek Water						
Ibuprofen	150	13	7	1	4	75
Gasoline	148	12	62	3	17	4
Diesel	146	16	15	63	5	0
Isoxathion	333	45	3	52	0	0
Lopinavir	167	21	31	18	20	9

Table S5. Summary of regression analysis results for CDOM and Region T intensities from both a submersible fluorometer and 3D Aqualog bench top fluorometer. The subscript AQ, means results from a 3D benchtop aqaulog fluorometer while portable means results from portable submersible fluorometer.

Contaminant	**Water source**	**No. of samples**	**CDOM $_{portable}$**		**Region T $_{portable}$**		**CDOM$_{AQ}$**		**Region T $_{AQ}$**	
			R^2	**p-value**	**R^2**	**p-value**	**R^2**	**p-value**	**R^2**	**p-value**
Diesel	Tertiary effluent	8	0.19	>0.05	0.99	<0.0001	0.76	<0.05	0.87	<0.005
	Post Ozone	9	0.63	<0.05	0.92	<0.0001	0.25	>0.05	0.15	>0.05
	UV-AOP	11	0.87	<0.0001	0.99	<0.0001	0.96	<0.0001	0.96	<0.0001
	Creek Water	9	0.42	>0.05	0.95	<0.0001	0.19	>0.05	0.88	<0.005
Gasoline	Tertiary effluent	12	0.27	>0.05	0.99	<0.0001	0.34	<0.05	0.84	<0.0001
	Post Ozone	12	0.27	>0.05	0.99	<0.0001	0.34	<0.05	0.84	<0.0001
	UV-AOP	12	0.99	<0.0001	0.97	<0.0001	0.82	<0.0001	0.80	<0.0001
	Creek Water	11	0.00	>0.05	0.97	<0.0001	0.58	<0.05	0.96	<0.0001
Ibuprofen	Tertiary effluent	11	0.88	<0.005	0.90	<0.0001	0.26	>0.05	0.10	>0.05
	Post Ozone	11	0.00	>0.05	0.85	<0.0001	0.74	<0.005	0.52	<0.05
	UV-AOP	9	0.62	<0.05	0.91	<0.0001	0.72	<0.005	na	na
	Creek water	15	0.17	>0.05	0.90	<0.0001	0.45	<0.05	0.00	>0.05
Isoxathion	Creek water	4	0.76	>0.05	0.98	<0.01	0.92	<0.005	0.84	<0.005
Lopinavir	Creek water	5	0.55	>0.05	0.86	<0.05	0.89	<0.05	0.97	<0.0001
Caffeine	Creek water	5	0.46	>0.05	0.50	>0.05	0.51	>0.05	0.30	>0.05

na = no analysis was done because there were no detectable peak intensities in the sample.

Table S6. Instrument detection limits (IDL) for a 3D benchtop fluorometer for Region T

Sample	Tertiary effluent (RU)	Post-ozone (RU)	UV-AOP (RU)	Creek water (RU)
1	0.410	0.000	0.000	0.503
2	0.806	0.000	0.000	0.242
3	0.736	0.152	0.000	0.503
4	0.836	0.052	0.006	0.242
5	0.410	0.067	0.006	0.242
6	0.736	0.000	0.006	0.503
7	0.806	0.052	0.000	0.503
MEAN	**0.677**	**0.046**	**0.003**	**0.391**
STDEV	**0.187**	**0.055**	**0.003**	**0.139**
IDL	**1.237**	**0.211**	**0.013**	**0.809**

The Instrument detection limits were calculated as “Mean+3*STDEV” (From standard methods).
STDEV = standard deviation

Table S7. Instrument detection limits for a submersible fluorometer for Region T and CDOM

Sample	UV-AOP water		Post-ozone water		Tertiary effluent		Creek water	
	CDOM (RFU)	**Region T (RFU)**	**CDOM (RFU)**	**Region T (RFU)**	**CDOM (RFU)**	**Region T (RFU)**	**CDOM (RFU)**	**Region T (RFU)**
1	9.45	110.63	543.00	121.00	3673.00	222.00	3020.00	200.00
2	24.00	154.34	685.76	116.84	3586.40	215.48	3340.73	203.15
3	4.45	125.62	723.96	151.58	3512.16	216.70	3349.61	199.64
4	4.78	126.14	475.44	130.08	3307.90	218.88	3368.03	198.89
5	5.44	118.69	335.07	124.61	3646.48	220.67	3308.38	196.05
6	6.95	133.00	480.51	124.80	3677.49	216.05	3329.27	199.10
7	6.80	126.72	395.22	126.63	3671.88	222.39	3367.85	202.15
8	5.18	123.61	487.78	122.28	2936.14	214.20	3343.80	198.61
9	7.04	151.92	490.99	128.30	3740.42	219.51	3369.85	195.80
Mean	**8.23**	**130.07**	**513.08**	**127.35**	**3527.99**	**218.43**	**3310.84**	**199.27**
STDEV	**6.11**	**14.45**	**124.68**	**9.91**	**256.33**	**2.96**	**110.92**	**2.43**
IDL	**26.56**	**173.43**	**887.13**	**157.09**	**4296.97**	**227.31**	**3643.58**	**206.54**

RFU = Relative Fluorescence Units

The Instrument detection limits were calculated as “Mean+3*STDEV” (From standard methods).
STDEV = standard deviation

Table S8: Regression analysis and level of significance between Region T fluorescence intensity (RFU) on the submersible fluorometer and Peak T (RU) on the benchtop fluorometer.

Contaminant	Water source	R^2	p-value
Gasoline	UV-AOP	0.841	<0.0001
Gasoline	Post-ozone	0.927	<0.0001
Gasoline	Tertiary effluent	0.807	<0.0001
Gasoline	Creek Water	0.908	<0.0001
Diesel	UV-AOP	0.949	<0.0001
Diesel	Post-ozone	0.927	<0.0001
Diesel	Tertiary effluent	0.882	<0.005
Diesel	Creek Water	0.738	<0.005
Ibuprofen	UV-AOP	0.916	<0.0001
Ibuprofen	Post-ozone	0.827	<0.0001
Ibuprofen	Tertiary effluent	0.888	<0.0001
Ibuprofen	Creek Water	0.579	<0.005