

Using Synchronous Fluorescence Technique as a Water Quality Monitoring Tool for an Urban River

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Abstract The development of a monitoring tool for predicting water quality and tracing pollution sources are important for the management of sustainable aquatic ecosystems in urban areas. In this study, synchronous fluorescence technique was applied to 18 sampling sites of a typical urban watershed in Korea, some of which are directly affected by the effluent from a wastewater treatment plant (WWTP), to investigate the capability of the technique for biochemical oxygen demand (BOD) prediction and source discrimination. Sampling was conducted three times at the same sites during the low flow period between October and November, 2005. Protein-like fluorescence intensities of the samples showed a positive linear relationship with the BOD values (Spearman's $\rho=0.90$, $p<0.0001$). The BOD prediction capability was superior to other monitoring tools such as UV absorption and conductivity measure-

ments particularly for the upstream sites from the WWTP, which ranged from 0.0 to 5.0 mg/l as BOD. The protein-like fluorescence and a ratio of protein-like/fulvic-like fluorescence were suggested as good fluorescence signatures to discriminate different sources of dissolved organic matter (DOM). The samples collected from four different DOM source regions including upstream sites from the WWTP, downstream sites, discharge from a reservoir, and headwater were distinguished from one another by varying ranges of the two selected fluorescence signatures. Our results suggest that the synchronous fluorescence technique has the potential to be developed into a real-time water quality management tool for the comprehensive monitoring of urban rivers.

Keywords Water quality monitoring · BOD · Source discrimination · Fluorescence measurement · Urban rivers

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1 Introduction

The development of real-time in-situ monitoring techniques is needed to cope with the abrupt deterioration of water quality of natural waters as well as to manage sustainable aquatic ecosystems in a timely and proper manner. Although the traditional water quality parameter, biochemical oxygen demand (BOD), has been widely accepted as an indicator for water pollution, the problem lies in the difficulty

applying such a parameter for real-time monitoring of natural and waste water quality. This is because of a series of processes are involved and from several hours to days are required for the completion of the analyses. To resolve this problem, spectroscopic techniques based on the UV absorption and fluorescence properties of natural and waste waters have been explored for environmental monitoring. From UV-visible absorption spectroscopy, UV absorbance at 254 nm or at 280 nm has been widely used to monitor the BOD value (Muzio et al. 2001; Nataraja et al. 2006). However, this method has certain limits for gaining accurate estimations because the absorption intensity of natural and wastewater are proportional to the aromatic organic carbon rather than the biodegradable organic carbon. As a result, the BOD prediction technique based on the UV absorption measurement is prone to fail particularly when the environmental samples being monitored contain a wide range of biodegradable organic carbon content at a limited range of total organic carbon concentration.

Recently, optical techniques based on fluorescence spectroscopy have been utilized for a variety of environmental applications, raising the hope that the fluorescence spectroscopy will be a promising real-time monitoring technique for the multi-purposes of water quality management. The three dimensional excitation-emission matrix (EEM) technique has been widely applied for such purposes (Baker 2001; Baker and Inverarity 2004; Lee and Ahn 2004). It was reported that three different fluorescence peaks were identified from the EEM and each of them were assigned to protein-like, fulvic-like, and humic-like fluorescence. While the capability of the fluorescence EEM to capture many spectral features in a wide range of excitation/emission wavelength is an advantage, the series of scanning is required for the completion of the measurement. In contrast, synchronous fluorescence measurement is a two-dimensional fluorescence technique requiring a single scan. As with the EEM, the synchronous fluorescence spectra of natural and wastewater exhibit three or four broadly-defined peak regions (Jaffé et al. 2004). It is known that fluorescence peaks from a synchronous spectrum at a short excitation wavelength between 200 to 300 nm have the fluorescence characteristics associated with the presence of fluorescent amino acids and proteins, which are analogous to the protein-like fluorescence characteristic of the EEM.

In general, the two types of fluorescence measurement applications have been made in the field of water quality management: (1) the prediction of water quality parameters of natural and waste waters and (2) the detection of pollution sources in watersheds. The protein-like fluorescence intensity defined from the fluorescence data has been suggested as the BOD or the chemical oxygen demand (COD) predictor. For example, Reynolds and Ahmad (1997) suggested one well-defined fluorescence peak (excitation 280 nm, emission 340 nm) as the BOD index of wastewater based on reasonable correlations between the two values. Baker and Inverarity (2004) applied fluorescence EEM techniques to demonstrate a positive correlation between the protein-like fluorescence intensity and the BOD values of river water in England. Lee and Ahn (2004) applied a multi-regression equation based on a protein-like fluorescence peak from the EEM and a light scattering intensity at the 633/633 nm excitation/emission wavelengths to predict the COD values of untreated and treated sewage samples. More attempts have been made using the fluorescence techniques to trace the sources of water contamination and/or dissolved organic matter (DOM) in rivers, reservoir and groundwater (Galapate et al. 1998; Baker 2001; Hur et al. 2006, 2007). For example, Baker (2001) applied the protein-like intensity and the ratio of the protein-like and the fulvic-like fluorescence peaks from the EEM data to detect the pollution of sewage, pulp industrial wastes, and farm wastes. Recently, Hur et al. (2007) demonstrated that DOM samples collected from a middle layer of a dam reservoir, which was previously impacted by turbid storm water runoff, exhibited very different fluorescence characteristics from those of the surrounding layers.

Although a number of studies have demonstrated the usefulness of the fluorescence techniques as a monitoring tool for the management of watersheds, it is still doubtful whether the techniques are feasible for multi-applications in a single defined watershed. Little to no effort has gone into applying the synchronous fluorescence technique simultaneously for BOD prediction and pollution source discrimination. In addition, we have only a limited number of application cases in urban watersheds for BOD prediction. The objective of this study was to examine the potential use of the synchronous fluorescence technique in the two monitoring applications for a

typical urban watershed, in which a municipal wastewater treatment is located along the river and the sewage effluents have a major impact on water quality immediately downstream from the developed urban areas.

2 Materials and Methods

2.1 Study Area

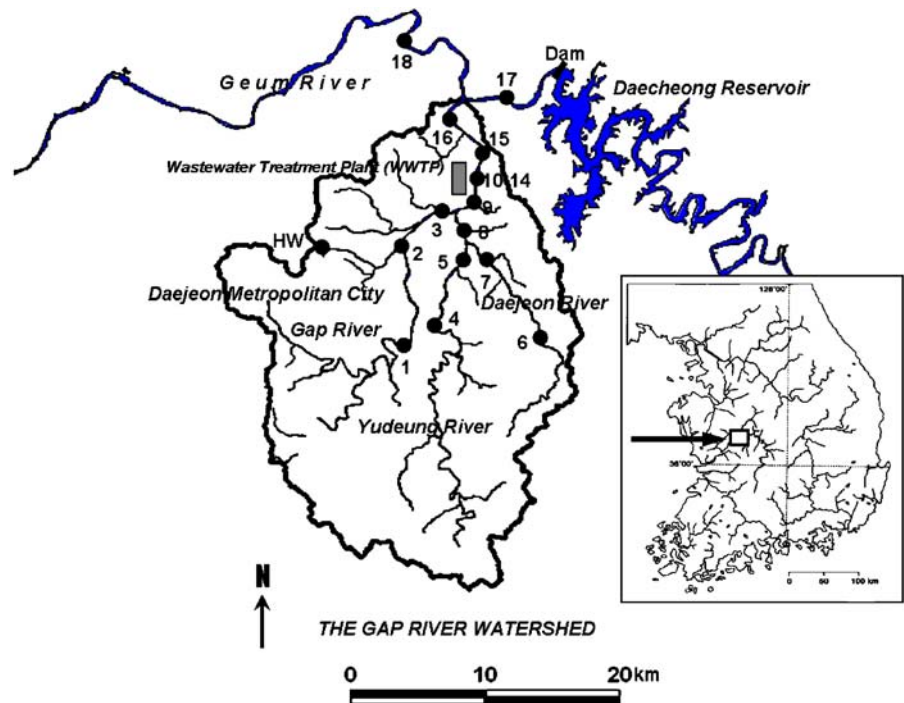
The Gap River is a typical urban river flowing south to north through the city of Daejeon, the sixth largest city in Korea, located in a central region of Korea ($127^{\circ}26'E$, $36^{\circ}20'N$). The Gap River watershed has a catchment area of 662 km^2 and a length of 73.7 km , and the population within the catchment is $920,600$. The overall land use/cover in the Gap River watershed is 58% forest, 22% agriculture, and 20% urban. The Gap River comprises two major tributaries, the Yudeung River and the Daejeon River (Fig. 1). The Yudeung River is the primary tributary of the Gap River, comprising 43% of the catchment area of the Gap River, flowing through the downtown of Daejeon city. The river meets the Gap River after joining the Daejeon River in the southern part of the watershed.

The Daejeon River is the secondary tributary of the Gap River and the primary tributary of the Yudeung River, having the catchment area and the length of 87.8 km^2 and 24.0 km , respectively. The two major tributaries and the upstream Gap River are influenced by the urban area and the corresponding urban activities. The effluent of a large-scale municipal wastewater treatment plant (WWTP), located at the downstream from the confluence point of the Gap River and the Yudeung River, has had an impact on the water quality immediately downstream of the developed urban area. The Gap River finally meets the Geum River after receiving the discharge of the Daecheong dam reservoir (Fig. 1).

2.2 Sample Collection and Preparation

Eighteen locations were sampled from the major rivers, the WWTP effluent, and the discharge of the Daecheong Reservoir (Fig. 1). A headwater sample was also collected from an upstream area of a small tributary of the Gap River to represent the forest water unaffected by anthropogenic activities. Five samples were taken at sampling locations (sites 10–14) within 100 m of the WWTP including the effluent from two outlets of the WWTP. Sampling was conducted at

Fig. 1 Map of the Gap River watershed, showing the locations of a wastewater treatment plant, two major tributaries (the Yudeung River and the Daejeon River), and grab sample sites. The *inlet* represents the location of the watershed in Korea



each location three times during the low flow condition between September and November, 2005. No precipitation occurred for at least 5 days prior to the sampling dates.

The temperature, pH, dissolved oxygen (DO) and electrical conductivity were measured on the sites. Water samples were collected in 2 l sterile polyethylene bottles. The samples were kept refrigerated upon return from the field. For general water quality assessment, the samples were analyzed in the laboratory for total suspended solids (TSS), BOD, total nitrogen, total phosphorous, ammonia nitrogen, nitrate nitrogen, phosphate phosphorous and chloride concentrations. The water quality parameters were determined following the standard protocol (APHA 2005). An aliquot (50 ml) of all the collected samples was filtered (pre-ashed Whatman GF/F microfiber filter paper, Whatman, West Chester, PA) and acidified with 1 N HCl to pH 3 for the measurements of dissolved organic carbon (DOC), ultraviolet (UV) absorbance, and fluorescence. It was previously recommended that natural and wastewater samples should be acidified to ~pH 3 for fluorescence analysis because the low pH can minimize potential interferences of metals (Westerhoff et al. 2001). No precipitates were observed with the samples at the pH.

Suwannee River humic acid (SRHA) and Suwannee River fulvic acid (SRFA) were obtained from the International Humic Substances Society (IHSS) and used without further purification as reference materials for comparison of the fluorescence spectra.

2.3 Analytical Methods

DOC concentrations in sample filtrates were measured by total organic carbon (TOC) analyzer (Tekmar Dohrman, Phoemix 8000). The relative precision of the DOC analysis was less than 3% as determined by repeated measurements. Absorption at the wavelength from 200 to 600 nm was undertaken by a UV-vis spectrophotometer (Cary 300 Conc, Varian) using a 1 cm quartz cuvette. When the UV absorbance of the sample filtrate at 254 nm was above 0.05/cm, the corresponding samples were diluted prior to the fluorescence measurement to avoid inner-filter correction (Baker 2001; Hur et al. 2007). The synchronous fluorescence spectra were measured within 24 h after the return from the field using a luminescence spectrometer (Perkin-Elmer LS-55) equipped with a

20 kW xenon arc lamp. Excitation and emission slits were adjusted to 10 and 5 nm, respectively. Synchronous fluorescence spectra for excitation wavelengths ranging from 250 to 600 nm were recorded using two constant offsets ($\Delta\lambda=30$ nm, 60 nm) between excitation and emission wavelengths. Preliminary studies showed synchronous fluorescence spectra with the selected offsets successfully captured various fluorescence characteristics (Hur et al. 2007). To limit second-order Rayleigh scattering, a 290-nm cutoff filter was used for all the samples. The fluorescence response to a blank solution (Milli-Q water) was subtracted from the spectra of each sample (McKnight et al. 2001; Chen et al. 2003). The measured fluorescence intensities were then standardized to a Raman peak at 395 nm emission following a suggestion by Baker (2001). The measured Raman peak intensity was 94.2 ± 2.4 (arbitrary unit). An inner-filter correction was made for some randomly chosen samples using a method of Gauthier et al. (1986). However, no shift of fluorescence peaks was observed by comparing the synchronous fluorescence spectra with and without inner-filter correction for this study. It is noteworthy that such inner-filter correction may impair the advantages of the fluorescence measurements as a monitoring tool because of additional calculations and measurements involved. Relative precisions of <2% were routinely obtained by three-times repeated absorption and fluorescence measurements of randomly chosen field samples.

The total nitrogen was analyzed using the persulfate oxidation method followed by detection of nitrate with the cadmium reduction method (APHA 2005). Ammonia was measured using the colorimetric phenate-method (APHA 2005). Dissolved organic nitrogen was calculated by subtracting the nitrate and ammonia concentration from the total nitrogen concentration. Chloride and phosphorous were measured using mercuric nitrate method and ascorbic acid method, respectively (APHA 2005).

3 Results and Discussion

3.1 Variations in Chemical Water Quality Parameters in the Gap River

Table 1 summarizes chemical water quality data for all the sampling sites. The headwater sample showed

Table 1 Summary of water quality at different locations of the Gap River watershed

Sites	Conductivity ($\mu\text{S}/\text{cm}$)	DO (%)	SS (mg/l)	BOD (mg/l)	TN (mg/l)	$\text{NH}_3\text{-N}$ (mg/l)	$\text{NO}_3\text{-N}$ (mg/l)	TP (mg/l)	$\text{PO}_4\text{-P}$ (mg/l)	Cl^- (mg/l)	DOC (mg/l)
St. 1	166 (16) ^a	94.1 (2.8)	1.88 (0.19)	1.2 (0.3)	2.0 (0.1)	0.02 (0.01)	1.16 (0.50)	0.041 (0.005)	0.029 (0.006)	9.6 (1.9)	1.1 (0.1)
St. 2	171 (15)	123.2 (4.0)	1.33 (0.18)	1.2 (0.2)	2.0 (0.1)	0.04 (0.01)	1.06 (0.44)	0.045 (0.015)	0.023 (0.009)	11.6 (2.3)	1.0 (0.1)
St. 3	201 (19)	108.3 (0.6)	4.47 (0.79)	1.7 (0.3)	2.5 (0.1)	0.31 (0.10)	1.14 (0.49)	0.082 (0.003)	0.041 (0.006)	14.7 (2.6)	1.3 (0.2)
St. 4	214 (4)	98.0 (3.3)	1.20 (0.20)	0.9 (0.2)	1.8 (0.1)	0.01 (0.00)	0.99 (0.46)	0.008 (0.001)	0.004 (0.003)	7.1 (0.6)	0.5 (0.1)
St. 5	237 (8)	110.9 (4.1)	2.23 (1.23)	1.2 (0.2)	1.8 (0.1)	0.03 (0.01)	0.99 (0.45)	0.019 (0.005)	0.004 (0.004)	9.3 (0.9)	0.7 (0.1)
St. 6	314 (21)	116.4 (2.6)	0.92 (0.08)	1.3 (0.3)	4.6 (0.4)	0.06 (0.02)	1.67 (0.51)	0.020 (0.002)	0.016 (0.004)	13.1 (4.8)	0.7 (0.1)
St. 7	358 (30)	108.7 (4.6)	3.25 (1.78)	1.4 (0.2)	4.8 (0.3)	0.24 (0.10)	1.66 (0.50)	0.075 (0.017)	0.036 (0.007)	17.8 (6.0)	1.0 (0.2)
St. 8	250 (10)	108.5 (6.7)	3.38 (1.15)	1.8 (0.4)	2.2 (0.2)	0.12 (0.02)	1.09 (0.40)	0.033 (0.006)	0.007 (0.004)	9.5 (1.3)	0.8 (0.2)
St. 9	224 (19)	114.3 (4.2)	4.63 (0.48)	2.0 (0.3)	2.3 (0.1)	0.23 (0.06)	1.10 (0.42)	0.064 (0.001)	0.024 (0.005)	13.3 (2.9)	1.2 (0.2)
St. 10	675 (36)	76.3 (1.2)	4.35 (0.31)	24.5 (0.5)	18.1 (1.8)	11.10 (2.74)	1.98 (0.59)	0.993 (0.520)	0.605 (0.327)	70.4 (11.0)	5.0 (1.0)
St. 11	600 (53)	86.5 (2.6)	5.17 (0.44)	19.7 (1.3)	15.7 (2.5)	9.80 (2.35)	1.77 (0.56)	0.819 (0.458)	0.534 (0.320)	60.9 (13.6)	4.4 (1.1)
St. 12	414 (72)	107.0 (5.7)	4.17 (0.34)	10.1 (1.4)	9.9 (2.2)	5.50 (1.69)	1.32 (0.47)	0.440 (0.248)	0.323 (0.205)	39.3 (12.0)	2.9 (0.8)
St. 13	598 (37)	92.1 (1.7)	3.23 (0.50)	15.8 (2.5)	18.6 (0.8)	3.80 (1.80)	1.94 (0.63)	1.296 (0.087)	0.957 (0.021)	75.0 (10.3)	4.4 (0.8)
St. 14	495 (43)	92.1 (1.8)	4.70 (0.40)	11.5 (1.0)	14.0 (0.8)	4.70 (10.8)	1.75 (0.58)	0.931 (0.074)	0.707 (0.041)	57.6 (8.4)	3.6 (0.6)
St. 15	464 (45)	91.4 (1.0)	7.05 (0.88)	13.8 (3.1)	12.3 (1.0)	4.80 (1.13)	1.63 (0.48)	0.827 (0.097)	0.616 (0.061)	50.5 (8.9)	2.9 (0.4)
St. 16	429 (66)	103.4 (7.0)	7.40 (0.72)	11.5 (2.7)	11.7 (2.1)	3.50 (1.18)	1.95 (0.57)	0.683 (0.176)	0.493 (0.127)	46.8 (12.6)	2.5 (0.5)
St. 17	119 (3)	117.8 (7.3)	3.43 (0.30)	1.5 (0.1)	1.5 (0.1)	0.04 (0.01)	0.32 (0.09)	0.022 (0.003)	0.004 (0.003)	5.9 (0.3)	1.6 (0.1)
St. 18	234 (8)	100.9 (1.7)	5.02 (0.42)	2.9 (1.1)	4.5 (0.1)	0.56 (0.19)	1.13 (0.31)	0.225 (0.016)	0.171 (0.018)	21.5 (1.9)	1.9 (0.2)
Headwater	45 (6)	106.6 (1.2)	0.30 (0.10)	0.6 (0.1)	1.0 (0.2)	0.01 (0.01)	0.26 (0.02)	0.001 (0.001)	0.004 (0.003)	3.2 (0.2)	1.4 (0.1)

^a The values in the parentheses represent the standard errors based on three-time sampling events.

the lowest value for most of the measured water quality parameters, reflecting little anthropogenic impact on its water quality. A comparison of the data for the upstream sampling sites of the WWTP (sites 1–9) versus the headwater demonstrated that the urban river water quality has been partially deteriorated by urban activities. For example, mean BOD values for the upstream sampling sites ranged from 0.9 to 2.0 mg/l, which are three to seven times higher

than that of the headwater site (Table 1). A visible trend within the urban area upstream of the WWTP was a consistent deterioration of the general water quality with each downstream distance of the Gap River, the Yudeung River, and the Daejeon River as demonstrated by the increasing values of the water quality parameters with increasing downstream distance of each river. In particular, an increasing trend of chloride concentration is notable. Since chloride is

typically assumed to be a conservative tracer as well as an urban signal in many aquatic systems (Chang and Carlson 2005), the trend may indicate that the Gap River is continuously receiving a significant amount of various urban activities-related contaminants overriding the physical dilution effects by tributaries and groundwater inputs that likely contain low chloride concentration. For example, septic systems, leaky sewer pipes, abandoned wastes and lawn fertilizer in urban residential areas may contribute the contaminant inputs (Ellis and Revitt 2002).

Compared to the upstream sites, the deterioration trend of water quality was more dramatic at the downstream sites of the WWTP. For example, mean BOD value for the site 16 located about 6 km downstream of the WWTP site was 7.40 mg/l, 5.8 times higher than the value for the site 9, which is just upstream of the WWTP. Other water quality parameters, such as total nitrogen and total phosphorous that may contribute eutrophication in lakes and oceans, were 5.1 times and 10.7 times enhanced by the WWTP effluent, respectively.

Ammonia and chloride ions, typically enriched in sewage, have been used as indices to evaluate the impact of sewage in surface waters (Holzer and Krebs 1998; Kim et al. 2002). In this study, both species concentrations at the site on the Geum River (i.e., site 18) were still higher than those for the urban site (site 9) just upstream of the WWTP, demonstrating that even the Guem River experiences the effluent impacts of the WWTP on the Gap-River.

3.2 Synchronous Fluorescence Characteristics of the Gap River

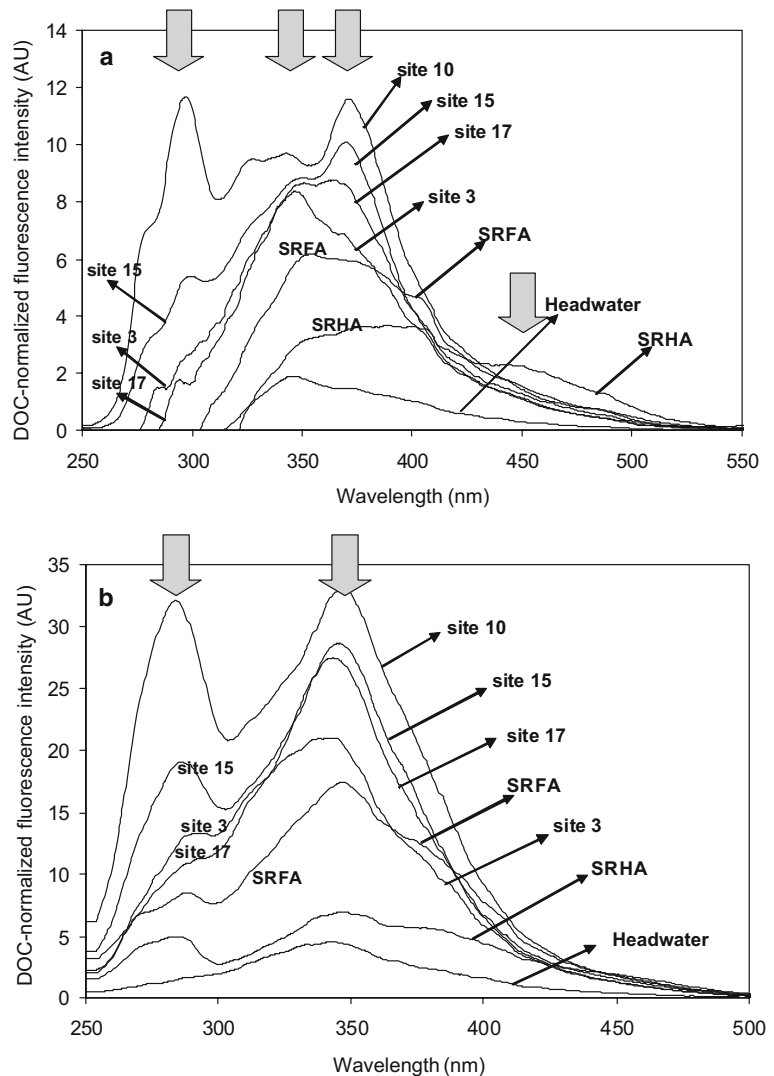
Figure 2 shows the synchronous spectra ($\Delta\lambda=30$ and 60 nm) of the samples collected from five representative sampling sites of the Gap River watershed including an upstream urban area (site 3), the WWTP effluent (site 10), a downstream site affected by the WWTP effluent (site 15), a discharge of the Daecheong Reservoir (site 17), and headwater. Two standard humic substances were also included in the spectra for comparison. The original synchronous spectra were normalized by the DOC concentration of the samples to compare the fluorescence characteristics at the same concentration level for this comparison.

In the synchronous spectra at a $\Delta\lambda$ value of 30 nm, four defined peaks could be identified (Fig. 2a). The

first peak was present at the wavelength between 280 to 300 nm, which was assigned to Peak I for this study. Peak I was not observed for the IHSS humic substances and the headwater samples. It was previously reported that the peak is associated with fluorescent proteins and amino acids and that the peak intensity is correlated to microbial and algal activities (Baker 2001; Westerhoff et al. 2001; Chen et al. 2003; Jaffé et al. 2004; Hur et al. 2007). The second peak (peak II) and the third peak (peak III) were found near the wavelengths of 340–350 nm and 370 nm, respectively, both of which appear to relate to fulvic acid and/or two condensed ring structures according to Jaffé et al. (2004). The association of peak II with the presence of fulvic acid is also supported by the spectral features of SRFA showing the presence of a broad peak at 350 nm. The SRHA sample showed a broad peak (peak IV) at the wavelength of 450 nm, suggesting that the peak is associated with the presence of humic acid. However, peak IV was not clearly seen in our field samples.

The shape of the spectra and the locations of each peak were very consistent with other prior reports (Westerhoff et al. 2001; Jaffé et al. 2004). Jaffé et al. (2004) used the percentage of the first peak intensity relative to the four peaks (% peak) to differentiate between mangrove- and terrestrial-affected locations from an estuarine region. In this study, peak II was commonly observed in the synchronous spectra irrespective of the collection locations and the sources of the samples. Peak I and peak III appear to be associated with some particular fluorescent compounds of treated sewage and algal activities because the two peaks were more pronounced for the sampling sites affected by the WWTP effluent (e.g., sites 10–16) and the discharge of the Daecheong reservoir (i.e., site 17). In contrast, the two peaks were not present or less pronounced for headwater and the upstream urban sites. This observation suggests that the two peaks could be applied to differentiate the sites between downstream and upstream of the WWTP. As expected, the two peaks were the most prominent at site 10 (i.e. the treated sewage). It was previously reported that a significant amount of extracellular polymeric substances were produced from activated sludge processes and also that some soluble fluorescent compounds may be present in the treated sewage. Esparaza-Soto and Westerhoff (2001) and Shen and Yu (2006) have characterized the

Fig. 2 Comparison of synchronous fluorescence spectra. **a** $\Delta\lambda=30$ nm. The shaded arrows represent the fluorescence peaks I, II, III, and IV, respectively (from the left to the right). **b** $\Delta\lambda=60$ nm. The shaded arrows represent the fluorescence peaks A and B, respectively (from the left to the right)



changes of fluorescence signals from extracted activated sludge.

Peak I was observed for the upstream urban sites unaffected by the WWTP effluent as well although it was not prominent. The result may be attributed to degradable organic matter supplied by the non-point sources from the densely populated catchments such as leakage from the municipal sewer systems, abandoned landfills, and waste dumps (Kini et al. 2001; Ellis and Revitt 2002) and by the production of labile DOM compounds (i.e., fast-decomposing organic matter) from the decomposition of aquatic macrophytes and benthic algae in the streambed (Thomas 1997).

Two different peaks were typically observed for the synchronous spectra at a $\Delta\lambda$ of 60 nm (Fig. 2b).

The first peak appearing near the wavelength of 285 nm was assigned to peak A, which is analogous to tryptophan-like fluorescence (Reynolds 2003). The IHSS humic substance and headwater samples exhibited positive fluorescence intensities at the wavelength corresponding to peak A, which contrasts with no positive intensities observed at the peak I wavelength for the spectra at a $\Delta\lambda$ value of 30 nm. The second peak was commonly found near the wavelength 350 nm for all the samples investigated, which was assigned to peak B for this study.

Table 2 presents a summary of the selected fluorescence characteristics of the synchronous spectra for this study. As for the previous water quality data, the selected fluorescence characteristics in-

Table 2 Selected fluorescence characteristics of the synchronous spectra for this study

Sites	Peak I ^a	% Peak I ^a	Peak I/peak II ^a	Peak I/peak III ^a	Peak A ^b	Peak A /peak B ^b
St. 1	1.7 (0.4) ^c	7.00 (0.01)	0.22 (0.06)	0.26 (0.07)	11.1 (1.3)	0.61 (0.10)
St. 2	1.9 (0.4)	8.61 (0.02)	0.24 (0.06)	0.29 (0.07)	12.0 (1.4)	0.65 (0.10)
St. 3	3.3 (0.9)	10.19 (0.03)	0.31 (0.08)	0.37 (0.09)	17.0 (2.8)	0.66 (0.10)
St. 4	0.1 (0.1)	0.00 (0.00)	0.03 (0.02)	0.04 (0.03)	5.4 (0.4)	0.51 (0.08)
St. 5	0.7 (0.3)	5.89 (0.02)	0.13 (0.05)	0.15 (0.07)	8.0 (0.9)	0.63 (0.10)
St. 6	1.5 (0.4)	5.65 (0.00)	0.19 (0.06)	0.25 (0.07)	10.5 (1.8)	0.59 (0.12)
St. 7	2.5 (0.4)	15.16 (0.05)	0.59 (0.36)	0.34 (0.06)	14.2 (2.4)	0.65 (0.11)
St. 8	1.5 (0.4)	10.50 (0.05)	0.21 (0.06)	0.26 (0.07)	12.4 (3.5)	0.76 (0.20)
St. 9	2.9 (0.3)	10.16 (0.01)	0.29 (0.03)	0.35 (0.04)	15.5 (1.8)	0.67 (0.09)
St. 10	72.8 (7.1)	34.41 (0.06)	1.53 (0.16)	1.29 (0.24)	167.7 (5.1)	1.16 (0.09)
St. 11	54.9 (9.1)	33.32 (0.05)	1.44 (0.14)	1.24 (0.20)	134.1 (18.8)	1.12 (0.11)
St. 12	33.7 (6.4)	15.19 (0.09)	1.16 (0.28)	1.14 (0.18)	76.9 (20.9)	1.11 (0.16)
St. 13	43.9 (0.9)	28.19 (0.02)	0.98 (0.04)	0.93 (0.01)	123.6 (20.5)	0.93 (0.16)
St. 14	32.9 (6.2)	27.32 (0.01)	0.91 (0.16)	0.97 (0.10)	101.4 (20.0)	0.68 (0.35)
St. 15	27.7 (7.2)	23.68 (0.05)	0.90 (0.16)	0.93 (0.11)	81.4 (17.6)	0.89 (0.16)
St. 16	23.2 (9.1)	21.52 (0.05)	0.82 (0.21)	0.95 (0.15)	69.0 (21.4)	0.89 (0.19)
St. 17	2.1 (0.4)	2.93 (0.03)	0.18 (0.02)	0.59 (0.43)	14.5 (1.1)	0.41 (0.03)
St. 18	7.7 (1.6)	13.05 (0.02)	0.49 (0.10)	0.71 (0.20)	30.4 (3.8)	0.72 (0.11)
HW	0.0 (0.0)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	5.0 (0.1)	0.36 (0.01)

^aThe values were calculated from the synchronous spectra at a $\Delta\lambda$ value of 30 nm.

^bThe values were calculated from the synchronous spectra at a $\Delta\lambda$ value of 60 nm.

^cThe values in the parentheses represent the standard errors based on two- or three-time sampling events.

creased further downstream within the urban area upstream of the WWTP, implying that the fluorescence characteristics may be positively correlated to the deterioration of the water quality. Baker and Inverarity (2004) have proposed a protein-like fluorescence intensity from the fluorescence EEM data (ex. 275 nm, em. 350 nm) of a watershed in England as an alternative water quality index, which was based on statistically significant relationships between the protein-like fluorescence peak and some general water quality parameters such as BOD, DO, nitrate and phosphate.

3.3 Using Protein-Like Fluorescence Intensity to Estimate BOD

Numerous studies have suggested that peak I and/or peak A may reflect microbial-derived, labile and biodegradable DOM (Baker 2001; Westerhoff et al. 2001; Chen et al. 2003). They demonstrated that such protein-like fluorescence characteristics are positively correlated with the BOD value (Reynolds and Ahmad 1997; Galapate et al. 1998; Baker and Inverarity 2004). Consistent with those prior studies, the BOD

values of our field samples exhibited excellent positive relationships with both peak I and peak A intensities of the synchronous spectra. The correlation coefficients (Spearman's rho) were 0.892 and 0.901 for peak I and peak A, respectively, based on the linear correlation analyses (Table 3 and Fig. 3). Although both selected fluorescence characteristics can be easily applied to the in-situ monitoring tool for the BOD value, it is worthy to compare the correlation coefficients with those obtained from other monitoring parameters available on sites such as conductivity and ultraviolet (UV) absorbance for more rigorous evaluation of the effectiveness of the fluorescence characteristics for BOD prediction. For the overall BOD data, the results demonstrated that our selected fluorescence characteristics are better capable of predicting the BOD values over the other parameters (Table 3 and Fig. 3).

It is notable that the fluorescence data showed little deviation from the regression line in the BOD range of <5 mg/l, which corresponds to the upstream region unaffected by treated sewage. In contrast, such a deviation was more pronounced for UV absorbance and the conductivity data in the low BOD range. Our

Table 3 Spearman's rho, linear regression fits, and correlation significant levels between BOD values and various monitoring parameters and associated statistics

All sites, $n=55$	Peak I	Peak A	UV ₂₅₄	Conductivity	EEM peak
Spearman's rho ^a	0.892	0.901	0.778	0.684	0.910
Regression fit	2.79x–2.03	6.52x+2.93	0.004x+0.025	21.4x+187	13.5x+10.5
Sig. level (p value)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Upstream sites (unaffected by treated sewage), $n=31$					
Spearman's rho ^a	0.615	0.591	0.249	0.102	0.654
Regression fit	1.67x+1.80	6.77x+1.80	0.001x+0.016	47.8x+146	16.5x+3.79
Sig. level (p -value)	<0.0001	<0.0001	0.0050	0.8570	<0.0001
Downstream sites (affected by treated sewage), $n=24$					
Spearman's rho ^a	0.627	0.755	0.545	0.706	0.737
Regression fit	2.73x–0.840	6.21x+8.20	0.003x+0.043	12.5x+26.9	19.8x+212
Sig. level (p value)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^a Spearman's rho, instead of Pearson's r value, was used in this study because the data is highly skewed, with most data having low BOD (see Fig. 3).

results appears to contradict those of Reynolds and Ahmad (1997) who reported that UV absorbance data at 254 nm of untreated and treated sewage samples exhibited higher correlations with the BOD values than did the measured fluorescence peak intensity selected for their study. The better capability of fluorescence versus UV absorption measurements for BOD prediction demonstrated in our study may be explained by the simple pretreatments (i.e., filtration and lowering pH) made in this study to avoid the interferences from the presence of suspended solids and metals. In contrast, no attempt was made in the prior study of Reynolds and Ahmad (1997) to remove the interferences. In addition, different BOD ranges used for the two studies may also cause inconsistent results. In other words, a wider BOD range (0–400 mg/l) was used in Reynolds and Ahmad (1997) whereas a relatively low concentration range was tested in this study since untreated sewage was not included in this study. An examine of various pretreatment and correction methods for improving the correlation between peak I and/or the protein-like fluorescence intensity and BOD values is currently being undertaken for further publication.

To compare the effectiveness of the synchronous fluorescence techniques versus the fluorescence EEM in predicting the BOD value, the protein-like fluorescence intensities previously defined from the EEM of our samples (i.e., the peak intensity at the excitation/emission wavelengths of 275 and 350 nm; Baker 2001) were also plotted against the corresponding BOD data and the correlation coefficient was calcu-

lated (Fig. 3e). The correlation (Spearman's $r=0.910$) was similar to those obtained from the synchronous spectra, indicating that the fluorescence synchronous spectra are comparable to the EEM technique in predicting BOD values at least for our limited number of the samples (i.e., low flow condition) and for our limited scope of the purpose. In addition, it should be kept in mind that a single scan of synchronous spectrum may provide more benefits than EEM when it is developed for real-time monitoring applications in the field.

To further examine the BOD predictability of the selected fluorescence peaks, the BOD data were separated into two regions—the sites affected (upstream) and unaffected (downstream) by the WWTP effluent—and the corresponding statistics were compared (Table 3). The superiority of fluorescence peaks versus other monitoring tools for BOD prediction was more pronounced in the upstream sites by the higher correlation coefficients and the lower correlation significant level of fluorescence peaks versus UV absorbance and conductivity. In contrast, both UV absorbance and conductivity showed much higher positive correlations (p values<0.0001) with BOD values for the downstream sites. For particular, the correlation coefficient (Spearman's $r=0.706$) between conductivity and BOD data was comparable to those of the protein-like fluorescence peaks (Table 3). This suggests that conductivity itself could be a potential BOD predictor for river sites that are directly affected by treated sewage, although the correlation coefficients may be lower than those obtained with

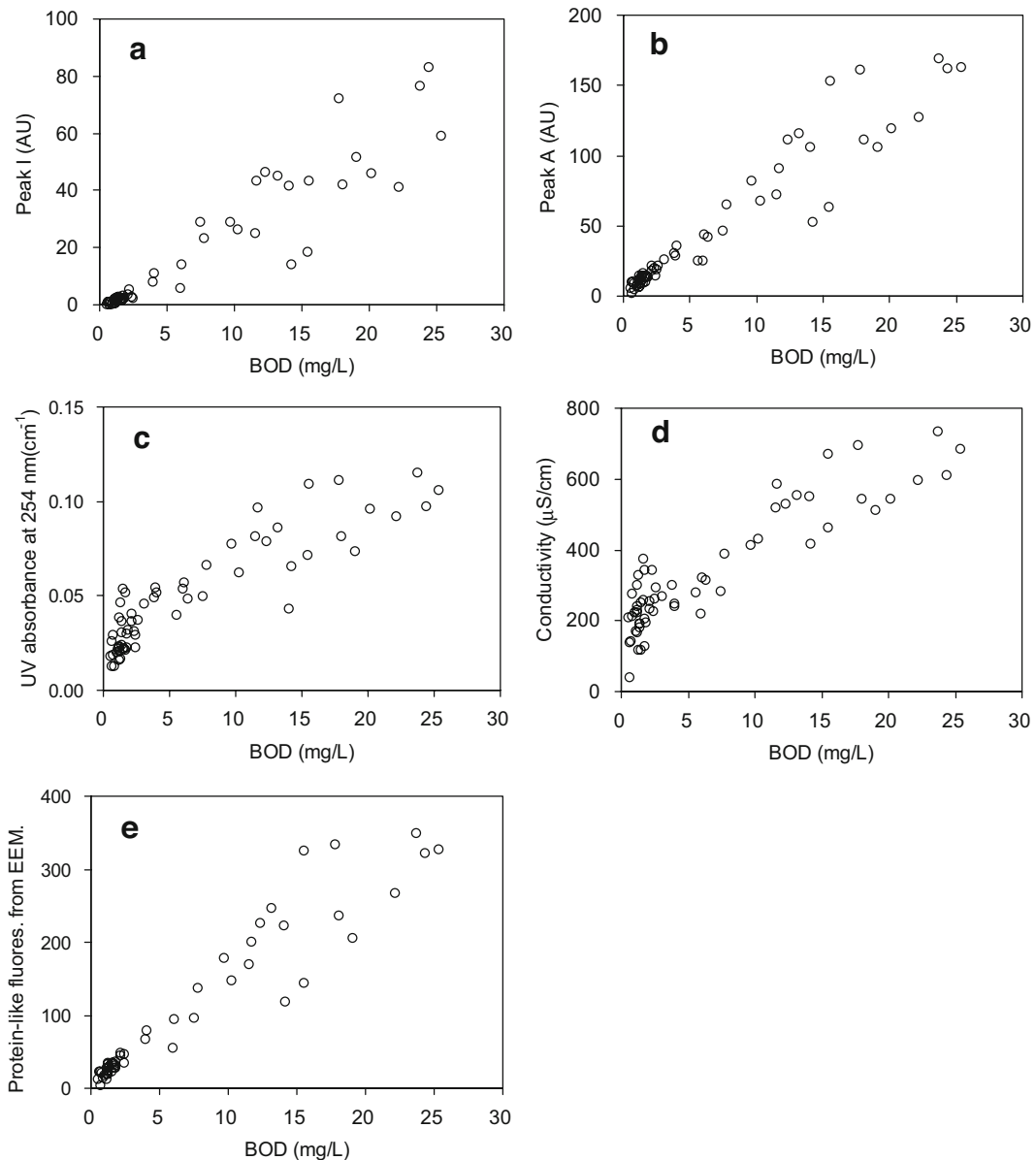


Fig. 3 Correlations between the overall BOD values and the selected parameters **a** peak I, **b** peak A, **c** UV absorbance at 254 nm, **d** conductivity, and **e** protein-like fluorescence intensity at the excitation and the emission wavelengths of 275 and 350 nm

fluorescence peaks. Irrespective of the sewage impact, peak I and peak A had their correlation coefficients higher or comparable to those obtained with the protein-like fluorescence peak from EEM.

3.4 Using the Synchronous Fluorescence Technique to Differentiate DOM and Water Pollution Sources

Various fluorescence signatures have been used to track water pollution and to distinguish the source of

DOM. For example, Baker (2001) investigated the impact of sewage on small streams using a distinct spectroscopic feature from fluorescence EEMs of stream samples. Sierra et al. (2005) showed that fluorescence EEMs, single-scan excitations, and synchronous fluorescence spectra together can provide a good picture of DOM sources and aging for a series of extracted humic and fulvic acids.

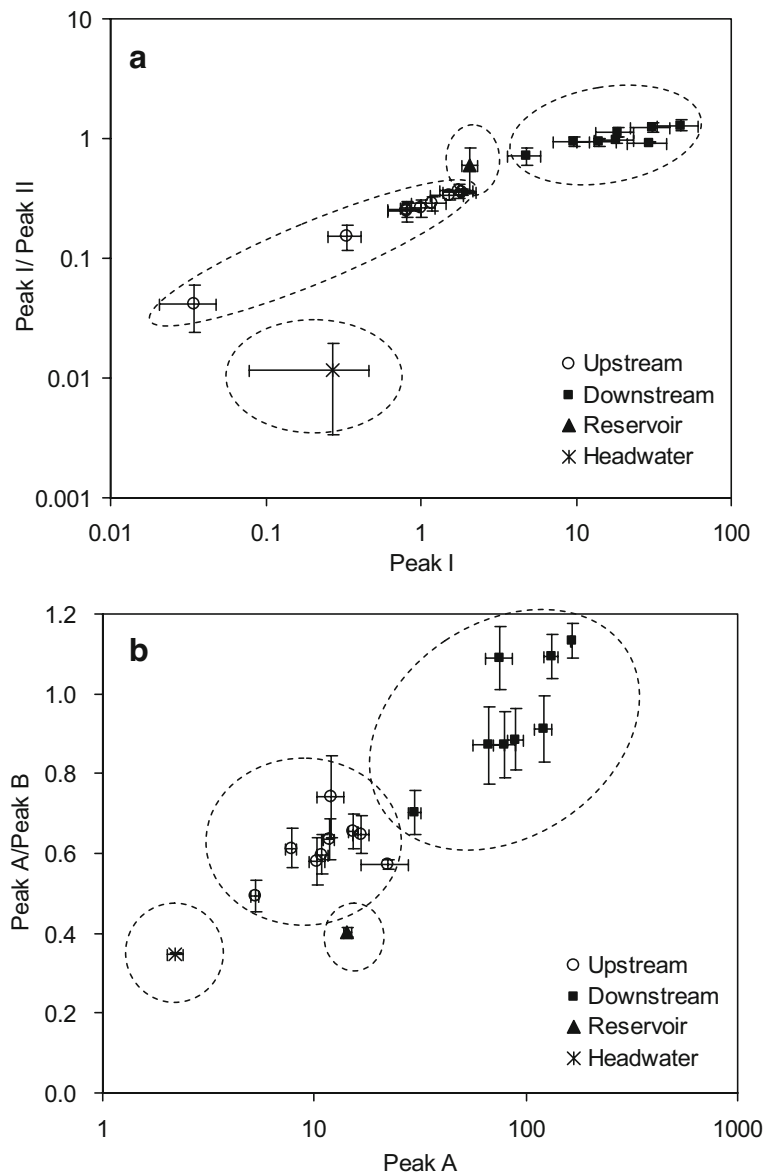
For this study, the sampling sites of the Gap River watershed were classified into four different regions

based on land use and the corresponding DOM sources—the urban region upstream from the WWTP, the downstream region including the effluent from the WWTP, the discharge of the Daecheong Reservoir, and the headwater region. The upstream urban region may be influenced by diverse urban activities although it is not directly affected by the WWTP effluent. In contrast, water quality of the downstream region is primarily impacted by the treated WWTP effluent. The discharge of the Daecheong Reservoir exhibits relatively good water quality because the reservoir serves as a major drinking water supply for

the city of Daejeon. Therefore, labile organic compounds produced by algal/microbial activities likely constitute a significant proportion of DOM components in the reservoir (McKnight et al. 2001; Wetzel 2001). Headwater may represent unpolluted natural water and its DOM components are likely to be primarily derived from humified terrestrial materials originating from the decay of plants and soil (Hood et al. 2005).

In this study, various fluorescence intensities and their relative ratios previously selected from the synchronous spectra were applied as discrimination

Fig. 4 Discrimination of water sources using fluorescence characteristics selected from the synchronous spectra. **a** Peak I and peak I/peak II ratio. **b** Peak A and peak A/peak B ratio. The horizontal and the vertical lines around the symbols represent the standard errors of the samples based on three-times sampling events



indices to differentiate different regions among the samples. The discrimination efficacy was tested by plotting all the samples with two selected fluorescence characteristics. The indices successfully used for the discrimination included peak I and the peak I/peak III ratio from the spectra at a $\Delta\lambda$ of 30 nm, and peak A and the peak A/peak B ratio from the spectra at a $\Delta\lambda$ of 60 nm (Table 2). Figure 4a illustrates peak I and the peak I/peak III ratio for all the sampling locations. The samples collected from the downstream region were characterized by the high intensity of peak I and high peak I/peak III ratio so that they could be easily distinguished from the samples for the upstream urban region exhibiting low values of the two indices. The headwater region was distinguished from the upstream sites by its lower peak I/peak III ratio with respect to the upstream sites. However, the peak I/peak III ratio of the Daecheong reservoir was located intermediately between the upstream and the downstream regions with the error bars overlapping with some samples from the upstream region.

Peak A and the peak A/peak B ratio was proven to be a better combination of discrimination indices as demonstrated by no overlapping of the error bars between the four different regions (Fig. 4b). The upstream sites, the downstream sites and the headwater could be distinguished from one another as illustrated by decreasing both fluorescence characteristics in the order of the upstream sites > the downstream sites > headwater. The discharge from the Daecheong reservoir was differentiated from the upstream region by the lower peak A/peak B ratio.

4 Conclusions

Fluorescence characteristics selected from the synchronous spectra were proven to be potential tools for monitoring water quality of an urban river that is affected by treated sewage and diverse urban activities. Peak A (protein-like fluorescence) selected from the synchronous spectra at a $\Delta\lambda$ of 60 nm showed an excellent positive relationship with the BOD values of the Gap River watershed samples. Its superior capability for the BOD prediction was more pronounced for the upstream sites, which are unaffected by the WWTP effluent, compared to other on-site monitoring methods such as conductivity and UV absorption measurements. Four different regions of

the samples based on the DOM sources could be distinguished in a watershed system by comparing selected fluorescence intensities and their relative ratios. The combined results suggest that synchronous fluorescence technique can be successfully applied to two monitoring tools for urban rivers—the BOD prediction and source discrimination although such applications may be limited to a low flow condition during a limited time period (i.e. dry season).

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